

EthoVision® XT

Reference Manual

Version 8.5

Noldus
Information Technology

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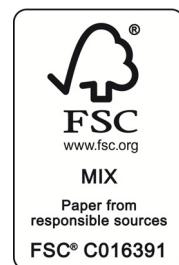
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1

Introduction

1.1 About this manual

Who should read this manual?

This manual is written for all users of EthoVision XT:

- Information for users upgrading from previous versions can be found in the following sections:
 - **In EthoVision 3.1 but not (yet) in EthoVision XT 8.5** (page 28).
 - **Differences between EthoVision XT 6.x and 8.5** (page 28).
 - **Differences between EthoVision XT 7.x and 8.5** (page 29).
- A basic overview of the program for new users can be found in the **Introduction to EthoVision XT** section in this chapter.

More detailed information can be found in the remaining chapters. The manual is written so that it is understandable without the program in front of you (but it is probably more rewarding to try out the procedures as they are described).

Although you need a hardware key to carry out data acquisition and use the extra modules, you can install the program on another computer to create and set up your experiment and perform analysis.

Chapter 1

How to use this manual

The manual is also provided as a PDF file. You can read this file and print it using Adobe Acrobat® Reader. If you did not install Adobe Acrobat Reader as part of your EthoVision installation, you can still install it from your EthoVision installation DVD. You can either copy the PDF file to your hard disk, or open it directly from the DVD.

You can use this manual in one of two ways:

- Read through the chapters pertaining to particular functions to get background information before using the program.
- Consult the manual as a reference document to find out particular information. You can find a topic either by consulting the table of contents (at the front of the manual), or the index (at the end). If you just want a short explanation of a concept, try the glossary (Appendix E).

This manual can also be opened directly from EthoVision XT. Press **F1**, or from the **Help** menu select **Help Topics** to open the PDF.

On the EthoVision XT installation DVD and on the downloads section of the home page of Noldus IT (<http://www.noldus.com/downloads>) you can also find:

- EthoVision in a nutshell – A 2-page document explaining the EthoVision workflow.
- A general Quick Start Guide – A small document with a summary on how to use EthoVision XT.
- Manuals on how to use EthoVision XT in combination with additional hardware, like a DanioVision Observation Chamber, or a PhenoTyper.
- Quick Start Guides about using EthoVision XT with different standard tests.
- Technical Notes that contain specific technical details or instructions. More technical information can also be found on the downloads section of the home page of Noldus IT (<http://www.noldus.com/downloads>) and the technical support knowledge base (<http://www.noldus.com/knowledge-base/search>).



These documents can also be opened directly from the Windows Start Menu after you have installed EthoVision XT. Open the folder **Noldus** and then **EthoVision XT and Documentation**.

1.2 Typographical conventions

- Software elements (such as menus or commands) are shown in **bold**.
- Keys such as **F1** are also shown in bold. If you have to press two or more keys at once, they are joined with a plus sign, for example, **Ctrl+N** creates a new experiment.
- Functions that have tool bar icons are indicated by the icon in the margin.
- Check box options are indicated by a square.
- Radio buttons (only one item in a group can be selected at once) are shown by a circle.



Warnings are indicated by an exclamation mark icon and text in bold.
Ignoring warnings can lead to data loss, wrong analysis results or damage.



Important information is shown by an information button.



Tips and tricks are shown by a light-bulb and text in italics.



A reference to other documentation than this manual is indicated by this icon.



Details about hardware are shown using this icon.

1.3 How to get additional support

Help menu

The EthoVision **Help** menu contains the following options:

- **Help Topics** – Opens a PDF of this Reference Manual.
- **Video Tutorial** – The Video Tutorial explains in a few minutes how to (1) set up an experiment, (2) acquire data and (3) analyze data in EthoVision XT.

Chapter 1



The Video Tutorial can also be opened from the Windows Start Menu after you have installed EthoVision XT. Open the folder **Noldus** and then **EthoVision XT and Documentation**.

- **Noldus Online** – If your computer is connected to the Internet, you can choose this option to go to the EthoVision XT home page, check for updates, contact the help desk or consult the EthoVision knowledge base. If you encounter a problem with EthoVision XT, you can inform Noldus IT with the **Report an Issue** option. The EthoVision XT home page contains all kinds of information about the program as well as examples of how EthoVision XT is used.
- **Upgrade** – If you have purchased an upgrade of EthoVision or an extra module, choose this option to type the new **Upgrade Key** number that you have received from Noldus.
- **About EthoVision XT** – Choose this option to see details of exactly which version of EthoVision you are using. You can click **User Info** to see the registered user and your license number of the software.

Noldus Knowledge base

The Noldus Knowledge base contains hundreds of entries with questions submitted by our customers and answers by our support staff. To access this database, browse to our web site (www.noldus.com) and click **Services**. Under **Customer Support**, click **Knowledge base**.

Known problems & solutions

On the **Known problems** page of our web site (www.noldus.com) you can find a list of known issues in EthoVision XT and their solution. The list is regularly updated. On the top of the window, click **Login**. To be able to login, you need to register (<http://www.noldus.com/form/request-access-download-section>). Click **Downloads** and then **Software and Documentation**. Click **EthoVision** in the list on the right and select the version you have. Then click **Known problems**.

Noldus Help Desk

If you have any problems, questions, remarks or comments, please let us know.

Introduction

You can contact us via our web site (www.noldus.com) and fill out a Support Request Form (preferred), phone during working hours in two time zones or fax. See Appendix D to get details on how to contact our Support department.

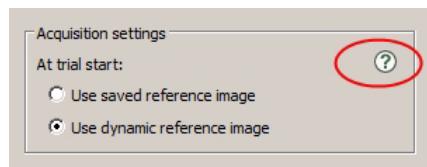
Before contacting our support department, please check this manual. Press **F1** or select **Help Topics** in the **Help** menu to open this Reference Manual, then enter a keyword related to your problem in the search field or use the index or table of contents.

Before you contact Technical Support, please have the following information available. To find this information, go to the **Help** menu and select **About EthoVision XT**:

- The version number of your copy of EthoVision.
- The name of the registered user of EthoVision (click **User Info**).
- The license number of your copy of EthoVision (click **User Info**).
- Please refer to our web site (www.noldus.com) for other contact information (choose **Contact**).
- Our Technical Support department may request the EthoVision log file with error messages to be able to answer your support question. See below under Error messages for its location. They may also ask you to make a backup of your experiment and send it by e-mail. See page 565 for the instructions to make a backup.

Information messages

In some parts of the program you see a question mark next to specific features. Clicking a question mark opens a window with more information about this feature.



Chapter 1

Error messages

When an error occurs, a message is shown and the error is written in the EthoVision log file, **EthoVision.log**. Our Technical Support department may request this file when answering your support question. You can locate the file in:

- For **Windows XP** – C:\Documents and Settings\All Users\Application Data\Noldus\EthoVision\XT 8\Log.
- For **Windows 7** – C:\ProgramData\Noldus\EthoVision\XT 8\Log.



It is possible that this folder is hidden. To view hidden folders: in Windows XP, from the **Tools** menu of the Windows Explorer, choose **Folder Options**, then **View**, and select **Show hidden files and folders**. In Windows 7, in the **Control Panel**, click **Appearance and Personalization**, click **Show Hidden Files and Folders**, and in the **Hidden files and folders** section, select the radio button **Show hidden files and folders**.

1.4 Introduction to EthoVision XT

This section gives a general overview of what the EthoVision program does. It describes why you have to carry out certain steps before you see the results. We recommend reading this section to all first-time users of EthoVision. For detailed descriptions listing all EthoVision's functions, please see the next chapters in this manual.

What is EthoVision?

EthoVision is an automated video tracking and motion analysis system. It offers a wide range of video tracking options, and extensive analysis of locomotor tracks. With the **Multiple Arena** module, you can track up to 100 arenas with a single subject at the same time. With the **Multiple body points** module, you can not only track the center-point of your animal, but also the nose-point and tail-base (see page 258 for more information). The **Social Interaction** module allows you to analyze interactions between two or more animals in an arena (see page 480 for more information). With the **Physiology Integration** module you can co-acquire external (physiological) data that you can visualize together with your track data (see Chapter 10 for more information). The **Trial & Hardware Control** module

Introduction

allows you to automate the start and/or stop of data recording, and to operate hardware devices like a pellet dispenser or a light (see the Trial and Hardware Control Manual on your installation DVD for more information). The **GLP** module supports you in making GLP-compliant experiments (see Chapter 4 for more information).

The following sections are a general introduction to *digital image processing* (the technique by which an analog video image is translated into digital information), in which the various components of a video tracking system are reviewed.

Overview

The entire process carried out by EthoVision may be summarized as follows: a video camera observes the movement of one or more subjects, and passes images of the subjects to your computer. There they are transformed into a digital signal and optionally encoded as a digital video file. From this digital signal or file, EthoVision first detects the subjects and then extracts the size of the subjects and position of one or more body points of the subjects in each image. That data is then transformed into a series of dependent variables quantifying the behavior of the subjects.

Image sensing

The starting point of an imaging system is a video camera. A video camera transforms a scene (the area in front of the lens), into an image (a picture taken by the camera). If you have an analog camera the image must be converted into a digital image consisting of pixels. With FireWire or USB cameras digitization occurs within the camera. You can plug them directly into your computer. Instead of using live video from a camera, you can also make a digital video file and use that for tracking.

One video image from a camera or digital video file is called a frame. The frame is made by a point which scans the scene in a series of horizontal lines (called fields), starting at the top and working its way down to the bottom. The fields are interlaced, that is, the camera first scans the odd lines, then the even lines. As the scene is scanned, the brightness (or color) of the scene is transformed into an analog signal describing the intensity of the image at each point of the scan.

The number of frames scanned in each second is called the frame rate, and this determines the maximum possible sample rate for EthoVision. The frame rate

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differs according to which TV standard your camera uses – 29.97 frames/second for NTSC (America) and 25 frames per second for PAL or SECAM (Europe).

Image digitizing for EthoVision

Analog cameras

A computer can only make sense of discrete numbers expressed as binary code. EthoVision comes with a frame grabber and encoder board that converts the analog signal to digital and subsequently creates a video file. There are two types of frame grabber and encoder boards:

- **Picolo Diligent frame grabber and encoder board** – This board creates MPEG-4 video files.
- **Picolo U4 H.264 frame grabber and encoder board** – This board creates H.264 SD files.



H.264 compression is often used to create High Definition (HD) files on Blue Ray Disks. However, H.264 High Definition (HD) files are very highly compressed and your computer processor has to do a lot of work in order to play an HD file back. The high demands that EthoVision places on video are more than computers can currently handle with the H.264 codec. Therefore, the H.264 encoder board that is sold with EthoVision only creates H.264 Standard Definition (SD) files.

Both boards can be used in three ways:

- **Track live** – To acquire data from the live image. See page 274 for details. The board functions as a frame grabber and converts each analog image into a digital image.
- **Track live and save video simultaneously** – To acquire data from the live image and create a video file simultaneously. See page 274 for details. The board functions as a frame grabber and an encoder. It converts each analog image into a digital image that can be used in EthoVision. Subsequently, EthoVision creates a video file.
- **Track offline** – Either acquire data from a video file created by another program (see page 275) or create a video file with EthoVision and acquire data later (see page 274). The board functions as an encoder. It converts the analog output of a camera into a digital video file.

Noldus offers two standalone programs to create video files that can be used in EthoVision for offline tracking. These are:

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- **Noldus MPEG Recorder 2.1** – This recorder comes with an IVC-4300 Video Capture Card that creates MPEG-2 video files from the output of analog cameras. This video capture card, however, only works under Windows XP. EthoVision can accurately acquire data from video files created by the Noldus MPEG Recorder 2. The IVC-4300 Video Capture Card is an encoder only. It cannot be used directly in EthoVision as a frame grabber for Live tracking.
- **Media Recorder 1** – This program creates MPEG-4 files from input from many different types of camera output. If you want to create digital video files from analog video input, you can buy the Picolo U4 H.264 encoder board together with the Media Recorder. This encoder board creates H.264 SD video files from analog input. EthoVision has predefined settings for tracking from MPEG-4 and H.264 video files created with the Media Recorder (see page 137).



If you have upgraded from previous versions of EthoVision, you may have older versions of the Noldus MPEG Recorder that (also) create MPEG-4 files. EthoVision has predefined settings for tracking from these video files (see page 137).

Digital cameras

With digital cameras, digitization occurs within the camera. You can plug them directly into your computer. However, for most types of digital cameras you cannot directly use the output in EthoVision.

The output of FireWire cameras can be used directly in EthoVision to track live. However, for acquisition the quality of the input signal is crucial. EthoVision has extensively been tested with two types of industrial FireWire cameras. These are ImagingSource DFK21AF04 and Med Associates Basler Camera (Type A602f). You cannot use these FireWire cameras to let EthoVision create video files. You need the Media Recorder to create video files with these cameras.

The use of other cameras directly in EthoVision is not supported.

Subject detection

When EthoVision receives the digital image from, for instance, the frame grabber, it does not 'see' a rat in an open field (for instance), but a bitmap composed of pixels, each of which has a particular gray value. The first thing that EthoVision has to do is to distinguish between the subjects to be tracked, and the background. You can select one of four methods to detect the subject:

- **Gray scaling** – All pixels which have gray values between two threshold values (a dark threshold value and a light threshold value) are identified as subject pixels.

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- **Static subtraction** – EthoVision compares a reference image that contains no subject, with the live image containing your subject. All pixels that differ between the two images are identified as subject pixels.
- **Dynamic subtraction** – When you use Dynamic subtraction, EthoVision also compares a reference image with the live image. Instead of a static reference image as in 'Static subtraction', the dynamic method uses a dynamic reference image that is updated for each sample.
- **Differencing** – Just like **Dynamic subtraction**, **Differencing** makes a reference image made of pixels which have an average value of previous images. The difference is that whereas **Dynamic subtraction** simply compares the absolute contrast between each pixel in the reference image with the current image, **Differencing** makes a statistical (probabilistic) comparison between each pixel in the reference image and the pixels of the current image. The statistical comparison uses the variance in the contrast between the current and reference image to calculate the probability that each pixel is the subject.



See page 232 for more information about the detection methods.

Identifying multiple animals

To track more than one animal per arena and identify the animals, you must use color markers. Each subject that has to be tracked by the system has to be marked with a distinctive color. If you are only interested in the animals center-point, you can use color marker tracking (see page 216 for more information). If you want to use nose-tail tracking with multiple animals, you must choose marker assisted tracking (see page 215 for more information). If it is not necessary to identify the animals, you can also track multiple animals without using color markers. For instance, track a group of beetles to study their walking patterns on different substrates (wet soil, dry soil, grass, etc.).

Excluding noise

Whichever detection method you choose, it is possible that some pixels will be identified as the subject, that are in fact just system noise or reflections. You can exclude these as follows:

Before tracking

- Define an arena (pixels outside the arena are ignored) – see page 133.

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- Make various settings to exclude noise – see page 212.

After tracking

- Smooth the track to filter out system noise, outliers and small movements – see page 378.
- Use the Minimal distance moved filter to correct for small movements – see page 380.

Feature extraction

When EthoVision has distinguished a group of adjacent pixels from the background, and so identified it as a subject, it then needs to turn that into useful information – feature extraction. EthoVision extracts two features from the raw data:

- **Subject size** – The number of pixels of the surface area composing the subject.
- **Subject position** – The x,y coordinates of the body points that you choose to track (either the center-point only, that is the point mathematically in the center of the shape considered to be the subject) or modelled on a contour of the body for determining the center-point, the nose-point and the tail-base). See page 258 for more information about nose-tail detection.

EthoVision records the coordinates in terms of the pixels of the image, but you can calibrate the image (see page 160) so that the position is displayed in real distances (e.g. millimeters or inches).

Quantifying behavior

The process described above converts each video frame of your moving subject into a series of numbers representing the x,y coordinates and size of the subject. From these raw data EthoVision can calculate a number of dependent variables describing the behavior of the subject. These include simple calculations such as the speed and direction of the subject's track and more complex variables such as 'Heading'. For all the dependent variables, EthoVision can calculate a full range of descriptive statistics (such as mean, standard deviation, etc.).

If you define an experimental setup with one or more independent variables (such as the subject ID or the treatment that your subjects received), you can select your data by the independent variable of your choice and produce statistics

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such as, e.g., the mean velocity of all the subjects which received 0.1 mg of a given neurotransmitter.

1.5 In EthoVision 3.1 but not (yet) in EthoVision XT 8.5

The following features are present in EthoVision 3.1, but not in EthoVision XT. These features will be implemented in EthoVision XT in the future.

Advanced calibration – Advanced calibration is needed when your video image is distorted. When using EthoVision XT make sure that your experimental setup complies with the restrictions as described on page 160.

Grouping of tracks – Grouping of tracks by the values of your independent variables (for visualization and analysis) is not possible in the current version of EthoVision.

1.6 Differences between EthoVision XT 6.x and EthoVision XT 8.5

- **Manual event recorder** – You can also use EthoVision XT 8.5 to record behavior of your subjects that cannot be detected automatically, for example grooming, head dip or biting. You score behaviors and events by pressing a pre-defined key or clicking an appropriate button on the screen as soon as the behavior or event occurs. Data are stored in a special Manual scoring log associated with the track data. See page 120 how to set up manual scoring.
- **General User Interface** – You can now access all common workflow functionality from the Experiment Explorer. Each component (e.g., Experiment Settings, Arena Settings, Trial Control, etc.) in the Working area contains a component caption and a component tool bar. The View Settings are now also component-specific and located in the component tool bar of the Working area under the **Show/Hide** button  .

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- **Trial Control Settings / Data Profiles Workspace** – The trial control and selection boxes now snap to a grid (which can also be made visible), so it is easier to align and organize boxes. You can now move a group of boxes. The workspace is dynamic, which means that the workspace automatically extends if you move a box outside the visible window on the screen. Furthermore, you can zoom in/out and zoom to view all boxes in the workspace, which is useful if you have extensive Trial Control Settings. The Trial Control Settings and Data Profiles can be exported to an image.
- **Hidden zone coordinates** – When an animal enters a hidden zone, the coordinates of its body points are set to the center of the hidden zone. You can now also use the arrow tip of the hidden zone label to set the coordinates of the body points; this is useful when you have a large hidden zone and you do not want the distance moved from the entry to the hidden zone to the center of the hidden zone to contribute too much to the total distance moved.
- **Interpolating points** – The interpolating procedure has been simplified to minimize the chance that interpolation results in data with little biological significance. Moreover, it is no longer possible to interpolate missing points at the beginning or at the end of the track. See page 365 how to interpolate data points.
- **Track Smoothing** – Track smoothing is now also applied to the angle between the center-point and the nose-point and tail-base.

1.7 Differences between EthoVision XT 8.0 and EthoVision XT 8.5

- **Missed samples and Subject not found** - The proportion of samples that are analyzed by EthoVision XT is a measure for the quality of tracking. The proportion of samples in which your animals are detected is a measure for how well your animals are detected. Both measures are now stored automatically for each track in the Trial List. It is possible to make a Data Selection based on these values. This way you can automatically filter out the bad tracks. Also, when you have many missing data points, you can now see in the Data Selection whether this is caused by wrong detection settings (high proportion of subject not found), or low processor capacity (high proportion of missed samples).

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- **Detection Settings** - There are several changes in the detection settings. This gives you more control on how the reference image is used in Static Subtraction, Dynamic Subtraction and Differencing.
- **Track Noise Reduction** - It is now possible to smooth the data during acquisition. This may especially be useful if you use trial and hardware control and the results are affected by flickering of the detected body. You can select track noise reduction in the detection settings.
- **Results per zone** - If you have defined a number of zones in your arena settings it is now easy to analyze the data for each zone separately. Simply select “**Results per zone**” in your data profile and specify which zones to analyze. The analysis output will show separate results for each selected zone. The results per zone are given for all dependent variables you defined in your analysis profile.
- **Help menu** - You can access the EthoVision XT home page via the Help Menu in the program. Via the Help menu you can now directly check for updates, contact the help desk or consult the EthoVision knowledge base. If you encounter a problem with EthoVision XT, you can inform Noldus IT with the **Report an Issue** option in the Help menu.
- **Easy access to all documentation** - When you install EthoVision XT, you can access all technical documentation via the Windows Start Menu, or via a shortcut on your desktop. Besides the Reference Manual, you here find more advanced technical information and the video tutorial.

1.8 Versions of EthoVision XT

The **Base version** of EthoVision XT allows tracking of the center-point of one subject in one arena. You can also record your subjects' behavior manually using the Manual Scoring function.

You can extend the base functionality with six modules:

- **Multiple arenas** module. Allows tracking in more than one arena at the same time. You can track one subject per arena.
- **Multiple body points** module. With this module you can track not only the center-point of a subject (the point mathematically in the center of the subject), but also its nose-point and tail-base and other relevant features. See page 258 for more information on nose-tail detection.

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Tracking the nose-point is, for instance, relevant in a plus maze test to determine the number of times the subject puts its head in one of the open arms of the maze without actually entering the arm. Tracking the center-point is then not so useful.

With the **Multiple body points** module you can also measure **Elongation** on the basis of thresholds that you set yourself. See page 528 for more information on this variable.

- **Physiology Integration** module. With this module, you can import any external data in ASCII format. External data may include physiological data (e.g. EEG, blood pressure, heart rate, body temperature, etc.) or environmental data (e.g. room temperature, humidity, etc.). You can synchronize track data and associated external data and subsequently visualize, select and export these data. See Chapter 10 for more information about acquiring external data.
- **GLP** module. This module supports you in making GLP-compliant experiments. See Chapter 4 for more information about EthoVision XT and GLP.
- **Social Interaction** module. This module allows you to track more than one animal per arena and study social interactions. To be able to identify the animals you need to color mark them. For the statistical analysis a number of variables are available like the distance between the animals, and the relative movement. See Chapter 8 for information about the settings you need to make to track multiple animals.
- **Trial & Hardware Control** module. With this module, you can make advanced rules to start and stop the trial. You can also control hardware, like, e.g., a pellet feeder and make settings in such a way that the pellet feeder drops a pellet when the animal is in the trigger zone for 30 seconds. See the Trial & Hardware Control Manual for more information about advanced trial control.

1.9 The EthoVision XT interface

By default, the main application window of EthoVision XT consists of two views:

- The **Experiment Explorer** (on the left) displays the content of the currently opened experiment. See below.
- In the **Working area** (on the right) you can edit the items of the **Experiment Explorer**. See page 34.

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By default, the Standard Tool bar is hidden. To display the Standard Tool bar, from the **View** menu, select **Standard Tool bar**.

Experiment Explorer

The **Experiment Explorer** displays all the components belonging to the currently opened experiment. Figure 1.1. shows the Experiment Explorer of the sample experiment called 'Elevated plus maze XT 80'. The experiment details have been made visible by expanding the main items (Setup, Acquisition and Analysis), Profiles and Settings.



Figure 1.1. The Experiment Explorer with all folders and items expanded.

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The look and feel of the Experiment Explorer

- **Expand and Collapse** – One of the main characteristics of the **Experiment Explorer**, like any tree view, is that its elements are expandable and collapsible.
- **View or hide** – By default, the **Experiment Explorer** is visible. You can hide it by going to the **View** menu and clearing the check mark in front of **Explorer View**. With the **View** menu you can also hide the **Status Bar** to create a larger **Working area**.
- **Right-click menus** – Most items in the Experiment Explorer have right-click menus to access key functionality.

View Settings

The View Settings pane has been replaced by a **Show/Hide** button  . This Show/Hide button can be found in the component-specific tool bar and only lists component-specific windows and options. For example, during acquisition, the **Show/Hide** menu shows the option to display the **Feedback** window, the **Track Features**, the **Arena Features**, the **Video Source** in the video window, the **Playback Control** and the **Analysis Results and Scoring** tabs (see Figure 1.2.). Clearing the check box in front of **Track Features**, for instance, clears the detection status information (the name of the arena and the number of samples not found and missing) in the video window.

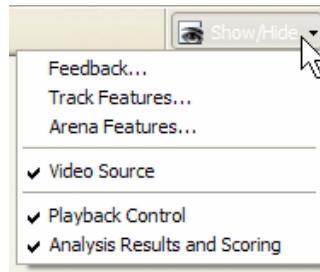


Figure 1.2. The View Settings under the Show/Hide button in the top-right corner of the Working area.

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Working area

The **Working area** is where all experiment procedures are carried out. When you click on an item in the **Experiment Explorer**, the **Working area** shows the required windows and views to carry out the procedure that goes with this item. For instance, if you click the item **Trial List** in the **Experiment Explorer**, the **Trial List** appears in the **Working area**. In this view, you can list the trials that you want to carry out in the experiment or enter your independent variables.

2

Installation

This chapter is about:

- **Installing the EthoVision XT software**
→ See page 49
- **Installing the Picolo Diligent frame grabber and encoder board** – You can use the Picolo Diligent board to do live tracking and to record your live video to a video file in MPEG-4 format.
→ See page 39
- **Installing the Picolo U4 H.264 frame grabber and encoder board** – You can use the Picolo U4 H.264 board to do live tracking and to record your live video file in H.264 SD format
→ See page 40
- **Installing and upgrading the hardware key**
→ See page 52
- **Setting up your arena** – What considerations to bear in mind when choosing your arena and setting it up.
→ See page 54
- **Camera setup** – What settings to make to your camera before you start data acquisition
→ See page 58
- **Lighting setup** – What are the criteria for good lighting and what measures can you take to prevent reflections.
→ See page 60

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If you ordered a computer from Noldus Information Technology when you purchased EthoVision XT, the software and any internal hardware has already been installed and tested.

2.1 Before you install

Please note: To be able to install EthoVision XT you must have administrator rights, in other words: either you are the system administrator or you are a member of the Windows group Administrators and have been assigned administrator rights.

Prior to installation, please check the packing list to make sure all the components are present. If any of the components listed is missing or damaged, please report this to us immediately.

The contents of your package differ for new and existing users:

- **If you are a new user of EthoVision** – You received the EthoVision XT installation DVD, this Reference Manual and a hardware key.
- **If you are upgrading from older versions of EthoVision** – You received the EthoVision XT installation DVD and this Reference Manual. You also received a number to upgrade your current hardware key. No hardware key is enclosed, because the one you have is upgraded automatically when you start the new version of EthoVision XT for the first time and enter the upgrade number.

System requirements

Operating system

Microsoft Windows XP or Windows 7

EthoVision XT has been thoroughly tested with a U.S. English version of Windows XP Professional 32-bit (with Service Pack 3) and Windows 7 (32-bit and 64-bit Professional edition). Like any software package, it remains possible that minor differences in the operating systems of certain local language versions may affect how well the program runs. If you encounter a problem of this sort, please contact Noldus Technical Support (see Appendix D).



Please note that if you run EthoVision XT on a Windows 7 (or Windows Vista) computer, the performance will considerably increase if you set the Windows Theme to Windows Classic. From the Windows **Start** menu,

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select **Control Panel** and then **Personalization** and **Theme** and if necessary change the setting.



We recommend to turn off automatic updates for device drivers. See section 2.5.

Computer

EthoVision XT has been tested with a Dell Precision™ T3500 PC. If you order a complete solution from Noldus Information Technology, you will obtain this type of computer with the EthoVision XT software installed and ready to use.

Technical specifications Dell Precision T3500:

- Processor: Intel Xeon W3530, 2.8 GHz.
- Internal memory: 6 GB.
- Hard disk: 1 TB free space.
- Graphics card: 256 MB NVIDIA Quadro NVS 295, resolution 1920x1080.

With this system you can do live tracking of up to four animals in one arena at half the maximum sample rate with color marker tracking and the image resolution set to medium. If you choose to order a PC from another supplier, you can use the above specifications as a guideline.

We recommend that you use a professional workstation. It is possible to buy consumer-range computers with a high processor speed and plenty of memory, but in order to remain competitive regarding price, the manufacturers often economize on the underlying system architecture. That means those computers are suitable for home use, but not for running professional scientific software. You should select a computer which is intended for professional use or labeled by the manufacturer as a workstation.

Keep the following factors in mind when deciding what PC to purchase:

- **Number of animals and arenas** – The more animals and arenas you track, the more processor load you require.
- **Number of body points** – Nose-tail tracking requires more processor load than tracking the center-point only.
- **Sample rate** – The optimal sample rate depends on the species you track. Mice, e.g., require a higher sample rate than rats. See page 224 for more information about the optimal sample rate.



When tracking multiple animals with nose-tail tracking (using marker assisted tracking), we strongly recommend to first create a video file and then acquire data from this video file. Select the 'Detection

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determines speed' option in the Acquisition Control window to make sure that no samples are missed.



See page 227 for an overview of the measures you can take to prevent the processor load from being too high.

Please note that your monitor should be set to at least 24 bit. If you use a dual-monitor set-up, both monitors should be at least 24 bit.

Camera

EthoVision XT has been tested successfully with Panasonic PAL and NTSC analog cameras and with the following FireWire cameras: Med Associates Basler camera (type **A602f**) and ImagingSource **DFK 21AF04**. If you would like to use another model of FireWire camera, please check with your nearest Noldus support office first.

Disk space to store media files

- If you are tracking from video files on the hard disk, you need sufficient free space to store them. The MPEG-4 video files that the Picolo Diligent board creates are approximately 400 MB/hour - 1.5 GB/hour. The H.264 video files that the Picolo U4 H.264 encoder board creates are approximately 350 MB/hour.
- If your video files are on a DVD or network drive, first copy them to your hard disk drive before tracking from them.

Using EthoVision XT across a network

Please note that it is not possible to install EthoVision XT on one computer and access it from another across a network. The program must be installed on the computer where it will be used. It is possible to access video files located at other computers in your network (depending on your network's bandwidth), although we recommend to copy the file to your hard disk before tracking. From the **File** menu, select **Preferences** and select your alternative media location. You can also save export files elsewhere.

Working with EthoVision XT as a restricted user

The user rights for folders and files are different for System administrators, Power users, and restricted (normal) users. Users who have Administrator rights and Power users have enough access rights to use EthoVision XT and its files without any limitations. For restricted users there are the following limitations:

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- 1 As a restricted user you can create new experiments and view, edit and delete your own experiments.
- 2 If you want to make it possible for other users to edit or delete your experiments, your system administrator must change the Windows security rights for the folder that contains the experiments. The default location of this folder is C:\Documents and Settings\All Users\Shared\Documents\Noldus\EthoVision XT (Windows XP) and C:\Users\Public\Documents\Noldus\EthoVision XT (Windows 7). For instance, to set the **Modify** rights to **Everyone**: in the Windows Explorer, select the folder mentioned above, right-click it, select **Properties** and then the **Security** tab and set the rights.

2.2 Installing the frame grabber and encoder board

In order to do live tracking from an analog camera you need a frame grabber installed in your computer. If you want to create video files with EthoVision you need an encoder board. If you ordered a computer from Noldus Information Technology when you purchased EthoVision, it came with either a Picolo Diligent board or a Picolo U4 H.264 board. Both boards work as a frame grabber as well as an encoder board. The board has already been installed and tested. If you bought your computer somewhere else, you will have to install the frame grabber yourself. The drivers for the frame grabber and encoder boards are on the EthoVision XT installation DVD. During installation of the EthoVision XT software you will be asked whether you want to install the drivers for these boards.

Installing the Picolo Diligent Board

- 1 Turn off your computer and all connected peripherals, such as the monitor and printer.
- 2 Unplug the computer, IO box (if present) and its peripherals.



The IO box is used to control hardware like, for instance, a pellet feeder.

- 3 Remove the PC's enclosure according to the instructions provided in the PC's user manual.



When touching the board, its electronic components can be damaged by static electricity. To avoid any such risk, make sure that you are

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grounded. You can ground yourself by putting on an earthing wristlet, and attaching its clip to the metal frame of the computer. If an earthing wristlet is not available, you can hold the metal frame with one hand while holding the board in your other hand. Also ensure that your clothing does not touch any components while handling the card.

- 4 Select a free expansion slot (the smaller PCI slot), and remove the corresponding extension cover making sure that the screw is put aside safely.

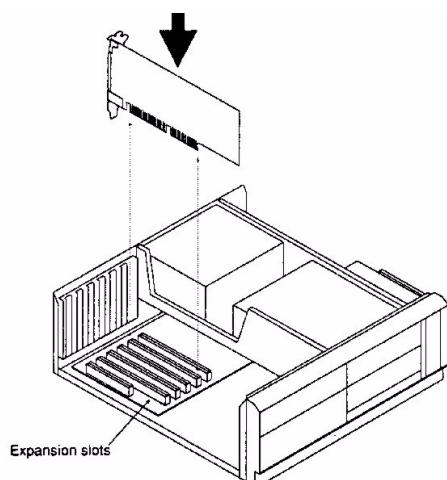


Figure 2.1. Installing the Picolo Diligent Board.

- 5 Unpack the board, place it into the slot, and press it carefully into position (see Figure 2.1). If the card does not fit into place easily, remove it and repeat the operation.
- 6 Fix the board to the chassis using the screw retained earlier and re-fit the computer's cover.

Installing the Picolo U4 H.264 Board

The following steps are required to install the Picolo U4 H.264 board. Please pay particular attention to step 7.

- 1 Make sure your computer is turned off and the power cable is disconnected.

Installation

- 2 Open the computer and gently but firmly insert the Picolo U4 H.264 board into a free PCI express slot. Avoid touching the contacts or other metal parts of the card.



- 3 Close the computer.
- 4 With the Picolo U4 H.264 card you received an HD44M (=Male) to BNC breakout cable with six BNC connectors. Connect the HD44M connector to the HD44F (=Female) connector on the Picolo U4 H.264 card.
- 5 Connect at least one video camera to one of the video inputs of the board. Do not use the cables that are marked with **OUT** or **IN CAS**.



- 6 Connect the power cable and turn the computer on.

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7 Windows XP – A “New Hardware Found” window opens.



Do not select one of the options. Click **Cancel** instead.

Windows 7 – Two balloons appear in the right-bottom corner of your screen:



Ignore these messages.



If you use the H.264 board, you need H.264 decoding software to be able to play the videos. This decoding software is present on your installation DVD (see page 49 for details).

2.3 Connecting your analog video camera to the frame grabber and encoder board

Connecting your camera to the Picolo Diligent board

Once the frame grabber and encoder board is installed, you have to connect your video camera to it. The Picolo Diligent board has four BNC inputs (see Figure 2.2).

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Figure 2.2. A computer with a Picolo Diligent board installed. The board has four BNC inputs and four dip switches.

Connecting your camera to the Picolo U4 H.264 board

Once the frame grabber and encoder board is installed, you have to connect your video camera to it. The Picolo U4 H.264 board has a HD44F connector that can be connected to a breakout cable with six BNC inputs (see Figure 2.2).

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Figure 2.3. A computer with a Picolo U4 H.264 board installed. The board has a HD44F connector that can be connected to a breakout cable with six BNC inputs.

Making connections for live tracking without video recording

If you want to do live tracking without recording your video to a digital video file, you have to connect your camera to one of the video inputs of the frame grabber.

If you have the Picolo Diligent board, make sure that the dip switch for this connector is on. You can find the dip switches next to the BNC connectors on the frame grabber board (see Figure 2.2). Dip switch 1 belongs to BNC connector 1, dip switch 2 to BNC connector 2, etc. The Picolo U4 H.264 board does not have dip switches. This card comes with six cables with BNC connectors. Do not use the connectors labelled with **IN CAS** or **OUT**.

Installation



BNC

If your camera has a BNC connection, you can connect a coaxial cable, with BNC connectors (Figure 2.4) on both sides, between **Video Out** of the camera and one of the **BNC** inputs of the frame grabber.



Figure 2.4. A BNC connector.

If your camera has a **Cinch** connection (Figure 2.5), you need a Cinch-to-BNC converter.

- 1 Plug the **Cinch** side of the Cinch-to-BNC converter into **Video Out** of your camera.
- 2 Connect a coaxial cable, with **BNC** connectors on both sides, to the **BNC** side of the converter.
- 3 Connect the other end of the coaxial cable to one of the **BNC** connectors of the frame grabber.



Figure 2.5. A cinch (phono) connector.

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S-video

To connect a camera with **S-Video** output (Figure 2.6) to the frame grabber, you need an S-video-to-BNC cable.

- 1 Plug the **S-Video** side of the S-video-to-BNC cable into **Video Out** of the camera.
- 2 Connect the BNC side of the cable to one of the BNC connectors of the frame grabber.



Figure 2.6. S-Video connection. On the left: an S-video connection; on the right: an S-Video Out Jack.

SCART

To connect a camera or other video device with SCART output (Figure 2.7) to the frame grabber, you need a SCART-to-BNC cable.



Figure 2.7. A SCART connector.

- 1 Connect the **SCART** connector of the cable to the **SCART** output of the camera.
- 2 Connect the other end of the cable to one of the BNC connectors of the frame grabber.

Making connections for live tracking with video recording

When you want to do live tracking and record your video to a digital video file, you have to connect your video camera to two video inputs of the frame grabber and encoder board (see Figure 2.8 for the Picolo Diligent board and Figure 2.9 for the Picolo U4 H.264 board).

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Figure 2.8. Connections for live tracking with video recording using the Picolo Diligent board.

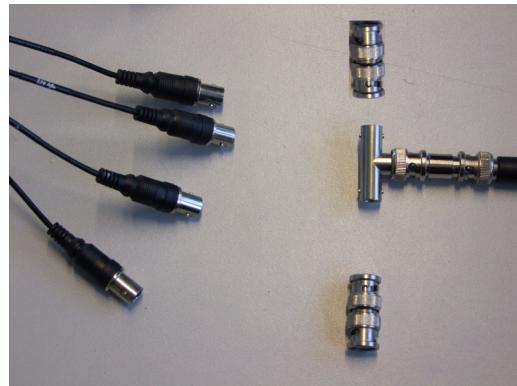


Figure 2.9. Connections for live tracking with video recording using the Picolo U4 H.264 board.

- 1 Connect a **BNC** T-connector to one of the **BNC** inputs of the frame grabber and encoder board.
- 2 Connect a coaxial cable, with **BNC** connectors on both sides, to one side of the T-connector and the other side to one of the other **BNC** inputs of the frame grabber and encoder board.

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- 3 Connect your camera to the other end of the T-connector. See "Making connections for live tracking without video recording" on page 44.
- 4 For the Picolo Diligent board: make sure that the dip switch for one of the two connectors (either one) is on and the other one is off. You can find the dip switches next to the BNC connectors on the frame grabber board (see Figure 2.2). Dip switch 1 belongs to BNC connector 1 (the connector right next to the dip switches), dip switch 2 to BNC connector 2, etc.

2.4 Connecting your FireWire camera to the PC

If you use a digital camera provided with a FireWire port instead of an analog camera, you do not need a frame grabber board. You can connect the camera directly to an available FireWire port on your computer.

Before connecting the camera, install the driver software for the digital camera.



We have tested successfully the following FireWire cameras: Med Associates Basler camera (type **A602f**) and ImagingSource **DFK 21AF04**. If you would like to use another model of FireWire camera, please check with your nearest Noldus support office first.

Using consumer camera models

If you want to use a camera model other than one of the mentioned above, before starting an experiment make sure that the timing of the camera is accurate enough for a frame-level video analysis. To do so, create a live tracking experiment, and carry out a test trial with the video footage of a digital clock for some ten minutes. Just before stopping the trial, check that the trial duration shown on your screen (page 270) does not deviate from that obtained with the clock time. Also check with your Noldus contact person for more information.

2.5 Installing the EthoVision XT software



For Windows 7, turn off automatic updates for device drivers!

Although the general recommendation from Microsoft to use automatic updates is good, especially for security updates, automatic updates of hardware device drivers can sometimes give problems, especially in Windows 7. This procedure describes how to specifically turn off the automatic updates only for device drivers.

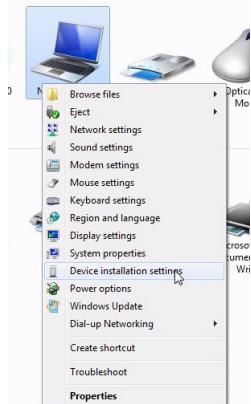
- 1 From the Windows Start menu, go to **Devices and Printers**.



If you do not see **Devices and Printers**, open the Windows **Start** menu and type **Devices and Printers**.

- 2 Right-click on the icon of your computer and select **Device installation settings**.

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- 3 Select No, let me choose what to do.**
- 4 Select Never install driver software from Windows Update.**



- 5 You can now go ahead and install EthoVision XT.**



Physically install the frame grabber and encoder board before you install the EthoVision software!



If you have added EthoVision XT users in User Management (see page 80) and you re-install the software, those users and their rights are deleted.



Close all other Windows programs before installing EthoVision and turn off the screen saver and power save options. Check that programs do not run in the background (look in the system tray at the bottom right of your screen).



We strongly recommend to uninstall all video editing and DVD burning programs before installing EthoVision XT. These programs often include codecs which sometimes conflict with the codecs that EthoVision uses. Note that such software is sometimes pre-installed on a computer when you buy it. Windows 7 includes DVD burning functionality. If you need to install DVD burning software on a Windows XP computer, we recommend CDBurnerXP (<http://cdburnerxp.se/en/home>). This is free and it does not install codecs. CDBurnerXP is installed on all Windows XP computers that Noldus Information Technology delivers. The Observer, Noldus MPEG Recorder or the Media Recorder will not normally interfere with EthoVision (so long as they are recent versions).

- 6 Insert the EthoVision XT installation DVD into your DVD-ROM drive. The setup program should start automatically.
-  If the setup program does not start automatically:
 - i In Windows XP, from the **Start** menu on the Windows task bar, select **Run**.
In Windows 7, from the **Start** menu, select **All Programs, Accessories** and **Run**.
 - ii Click **Browse** and select the file **Setup.exe** from the DVD-ROM drive. Then click **OK**.
- 7 In the **InstallShield Wizard** window, click the **Install** button to start the installation. If necessary, click **Continue** to proceed.
- 8 The **Frame grabber Driver** window opens.
- 9 Select whether you want to install the drivers for the Picolo Diligent board or the Picolo U4 H.264 board. Select **Do not install** if you do not want to track live or if you track with a FireWire camera.
- 10 Installing the frame grabber drivers involves a reboot of your system. After reboot, the installation continues automatically. A **Welcome** screen appears. Click **Next**.
- 11 Read the **Software License Agreement**, select **I accept the terms in the license agreement** then click **Next**.
- 12 On the **Destination Folder** screen, select where to install the EthoVision XT program files. We recommend that you accept the default location: C:\Program files\Noldus\EthoVision XT. Then click **Next**.
- 13 On the **Experiments Folder** screen, accept the default directory (Windows XP: C:\Documents and Settings\All Users\Documents\Noldus\EthoVision XT\Experiments; Windows 7: C:\Users\Public\Public Documents\Noldus\

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EthoVision XT\Experiments) or select another directory to which you have write access. Then click **Next**.

14 In the **Setup Type** window, select **Complete** and click **Next**.

15 The installation program now has all the information it needs. On the **Ready to Install the Program** screen, click **Install**. The installation will start. If necessary, click **Continue** to proceed. When the installation is complete, you will get a message. Click **Finish**.



The installation DVD contains Acrobat Reader (version 8) for you to install. You can use Acrobat Reader to display and print the documentation (PDF) files.

You are now ready to start using EthoVision XT 8.5. To register EthoVision XT 8.5, follow the link on the enclosed card and register your software license online. Doing so entitles you to technical support and product announcements. So, register online as soon as possible!



EthoVision 3.1 does not work with the new Picolo Diligent frame grabber board or the Picolo U4 H.264 encoder board. It works with an "old" Picolo frame grabber. If you have both boards in the same computer, EthoVision may sometimes take the video signal from the wrong board. Therefore the use of EthoVision 3.1 and EthoVision XT 8 on the same computer is not supported.



Experiments created in EthoVision 3 cannot be imported into EthoVision XT, neither can experiments created in EthoVision XT be imported into EthoVision 3.

2.6 Installing and upgrading the hardware key



First install the EthoVision software and then connect the hardware key to your computer, not the other way around!

Each license for EthoVision XT comes with a hardware key, which determines which setup is available to you (see page 30 for the extra modules that are available). This is a very important piece of equipment, as it is your license and will not be replaced free of charge.

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Please keep this in mind and make sure that you do not lose the key! You will need to pay for a new license if you do so.

Please be careful with the hardware key. It is sensitive and can be easily damaged.

Upgrading the hardware key

When you upgrade from a previous version and start EthoVision XT for the first time, the system automatically detects the hardware key and asks you to type the new **Upgrade Key** number in the **Upgrade Key** dialog box. This number is normally sent to you by e-mail or in your welcome letter.

2.7 Restoring your computer to factory settings

The computer that you received with Noldus software has extensively been tested for correct functioning. However, new programs that you install may interfere with the Noldus software. For example, viewers for playing back video files may contain codecs that interfere with the codecs used by EthoVision XT.

If you encounter a problem with our software, there are several options to solve this. You can consult the online help of the program, or browse through the known problem page of the product on www.noldus.com. Furthermore, you can contact our support department. Make sure you have your licence number available if you do so. To get your licence number, open the Noldus product, go to the **Help** menu, choose about **EthoVision XT** and click **User Info....**

It is not always possible to determine the exact cause of a computer problem. For this purpose, Noldus IT has created a backup of your c-drive on your d-drive. Furthermore, the system is delivered with a **System Restore Disc**. If all other solutions fail, you can restore the c-drive on your computer to factory settings. For more information, see the Technical Note 'Restoring your computer to factory settings' on the EthoVision XT installation DVD.

2.8 Setting up your arena

The EthoVision software can interpret your data best if it gets a good clean video signal. To achieve that you must set up the hardware (enclosure, camera, lighting, etc.) correctly. This is particularly important when you want to do nose-tail tracking or track multiple subjects. For particular problems with your hardware setup, see the Troubleshooting section (page 66).

Choosing your arena

Mazes and open fields

Noldus offers a wide range of mazes and open fields suited for most commonly used behavioral tests. Mazes and open fields are available in different sizes suitable for rats and mice. Please see our web site (www.noldus.com) for more information.

PhenoTyper

PhenoTyper is an observation system for continuous, automated monitoring of rodent behavior with minimal human interference. The video output from a PhenoTyper can be fed into a computer with EthoVision XT running to measure and analyze the activity and movement displayed by a mouse or rat.

PhenoTyper is available in two sizes: a small cage for monitoring a single, small rodent (e.g. a mouse) and a large cage for monitoring single or multiple rodents of any size. Both the small sized and the large sized PhenoTyper are available in two versions: the basic cage (see Figure 2.10) and the home cage version

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(identical to the basic version, but with one or more water bottles, a feeder and a shelter).



Figure 2.10. A small basic PhenoTyper cage for monitoring a single mouse.

A PhenoTyper cage has multiple infrared light sources, an infra-red sensitive video camera, and an IR high-pass filter, which ensure robust monitoring regardless of ambient light conditions. This allows you to carry out studies of circadian rhythms without having to change settings when light conditions change. PhenoTyper can even monitor an animal in complete darkness.

With the **Trial & Hardware Control** module you can control the hardware that is integrated in the PhenoTyper Top Unit (white light, yellow light, buzzer). For instance, in an anxiety test, switch on a light when the rat or mouse enters a certain zone of the cage.



Please see the PhenoTyper Reference Manual for more information.

DanioVision Observation Chamber

DanioVision™ is a system for zebrafish tracking. The DanioVision Observation Chamber (see Figure 2.11) contains a back-lit holder that fits an ANSI SBS compatible multi-well microtiter plate, infrared (IR) and white light sources (underneath the microtiter plate), an IR-sensitive camera and an extra internal LED light. The optical design provides a steady and equal view of each well and a

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controlled and constant environment within the observation chamber. You can set up a water-flow through the basin around the well-plate holder.



Figure 2.11. The DanioVision Observation Chamber.

Number of Arenas

With the Base version of EthoVision XT you can track in one arena only. With the **Multiple arenas** module, you can track single subjects in up to 100 arenas simultaneously. If you want to use more than four arenas or more animals per arena, first check that no samples are missed or work with video files.

Arena properties

You should think carefully about the kind of arena to use. You need to consider both the arena background, as well as its size and position. There are three main considerations to bear in mind when deciding on the background of the arena:

- 1 The animals you are tracking should be able to move freely and naturally.
- 2 The background must not interfere with EthoVision's detection of the image:

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- The background must be fixed in relation to the camera. If the arena moves, you will have to redefine it (though you can copy and paste an existing arena). Make sure you firmly anchor the entire setup when it is completed.
- The background should be made of non-reflective surfaces. If this is unavoidable (e.g. the water in a Morris water maze) use indirect illumination (e.g. bounce light of the ceiling) and non-reflective surfaces (e.g. black pool sides). Please see **Lighting setup** on page 60 for more details.
- There should be maximum contrast between the background and the animal. If you use dark rats in a Morris water maze, you can color the water with milk powder (400 g, pre-dissolved in 2 l). It is also possible to track white rats or mice with dark coloring in the water: add 300 g of tempera black nontoxic powdered paint to a 45 liter pool.



Preventing reflections and ensuring maximum contrast is especially important when you want to do nose-tail tracking. Please note that nose-tail tracking is virtually impossible in a water maze because the tail base is always under water and it is not possible to get a full body contour.

3 The arena must have the correct dimensions:

- The area where the animal can move should have no 'depth' – one camera can only 'see' in two dimensions. If the animal can move towards or away from the camera, its apparent size will vary, which will interfere with subject detection and identification. If you want to track fish in an aquarium, then make sure the aquarium is as shallow as possible or only calculate variables like **In zone**, not **Distance moved** or **Distance to zone/point**. The arena must be in a plane at right angles to the camera's axis.
- The maximum arena size (in pixels) is limited by the image resolution of the digital video file you are using, your camera, frame grabber, or the image resolution setting in the **Experiment Settings** (see page 96; this only applies when you are using a frame grabber). Your subject must be at least three pixels wide in order for EthoVision to distinguish it from system noise. Therefore, the ratio of your maximum subject size to the maximum arena size is given by the limiting resolution divided by three. If you use the Picolo Diligent frame grabber at maximum resolution, this is approximately 200 times the animals' size.

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Example: Parasitic wasp

When you select medium resolution (like we recommend) (PAL: 384 x 288 pixels) to track a parasitic wasp of 7 x 4 mm (length x width):

- Ratio subject size to arena size = $288/3 = 96$.
- Maximum arena size = $4 \times 96 = 384$ mm.

If the camera was further away, the arena could be bigger but then the wasp would be less than three pixels wide and EthoVision would not be able to distinguish it from random noise. If the camera was closer the wasp's image would have more pixels but the arena would have to be smaller.



If you use multiple arenas, the calculation applies to the total distance across all arenas, thus if you have four arenas in a square, the maximum width of each one is half what it would be if you had only one arena.

2.9 Camera setup

In order to be able to track your subjects effectively, you must select and set up your camera correctly.

Camera type

You should select the video camera most suitable for your needs:

- **Camcorders** – Although in principle it is possible to use a camcorder with a FireWire connection together with EthoVision, we recommend you use a professional FireWire camera instead.
- **CCD Camera** – All modern video cameras are based on a CCD chip. You should ensure that the chip has sufficient resolution for your needs.
- **Color or monochrome** – Monochrome cameras are cheaper, more compact and usually with a greater light sensitivity. If you want to film in near darkness, you must use a monochrome (or specialist IR) camera. If you want to track multiple animals and distinguish them on the basis of color markers, you (of course) need a color camera.
- **Digital (FireWire) or analog** – FireWire cameras used to be less light sensitive than analog cameras, but nowadays that is not the case anymore. Noldus can

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supply a monochrome FireWire camera that is sensitive to 0.5 lux, and to near IR light. A plus-point when using FireWire cameras is that you do not need a frame grabber, but on the other hand, you cannot make any video files (unless you have the Noldus Media Recorder software). An advantage of analog cameras is that they can be placed at a considerable distance from the computer. FireWire cameras often have to be relatively close which can be a problem because of ultrasound emissions from computers which stress rodents. Some industrial FireWire cameras require an external power supply when used in combination with certain laptops.

Camera settings

You must make the following settings to your camera before you start data acquisition:

- Ensure that the camera is positioned at a straight angle (perpendicular) to the plane in which the animal moves. If this is not the case, the shape of the arena and subject inside will appear distorted on the screen and calculated distances, velocities and spatial statistics will be incorrect.
- Adjust the zoom setting (or select the appropriate lens) and focus the lens until the entire experimental arena is visible on the screen and is displayed in focus.
- Turn off all automatic camera settings: focus, gain, exposure (auto-iris), anti-shake.
- Adjust the camera aperture until the image shows maximal contrast.
- Position the illumination in such a way that no light reflection are visible on the screen (please see **Lighting setup** on page 60).

Anchor the entire setup. Secure the position of the arena, camera and illumination relative to each other, and fasten the camera zoom, focus control and aperture settings.

Close-up photography

If you need to get higher magnification images than is available with a standard lens (get 'closer'), then you can use one of the following techniques:

- **Macro lens** – A true macro lens (that is: one of a magnification of at least 1:1, 'real world':CCD chip) will usually give good quality pictures, but it is expensive

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and will probably give a lower magnification than either a high-powered close-up filter, or an extension ring.

- **Extension rings** – A ring inserted between the camera body and the lens to increase the distance between the CCD chip and the lens. This enables you to focus on subjects closer to the camera, and so provides a greater magnification (like with back-focusing). An extension ring is usually cheaper than a true macro lens, but more expensive than a close-up filter. It may give a reduction in optical quality, and it will reduce the light intensity of the signal, but you can use it to get very high magnifications.
- **Close-up filter** – A lens attached in front of the normal lens, to give greater magnification. Also known as a close-up lens. It works by shortening the focal length of the lens while keeping the lens-to-camera distance constant, which increases the magnification of the lens. The strength of a close-up filter is measured in diopters, which is the fraction by which the lens' focal length is shortened. Thus, a +3 lens reduces the focal length of the lens to which it is attached by 1/3. A close-up filter is usually cheaper than a macro lens or extension ring, but it gives lower magnification, and poorer optical quality.
- **Microscope** – EthoVision tracks well from cameras attached to microscopes. Make sure you have back-lighting to maximize contrast.

2.10 Lighting setup

A good lighting setup is vital to get a good image that EthoVision can use to accurately track your animal. This is particularly important when you want to do nose-tail tracking or track multiple animals with color markers. To produce a good image, the lighting must satisfy three criteria:

- **It must be bright enough** – Stand-alone video cameras (which we recommend) can sometimes work at 0.1 lux or darker. At higher light levels you can work with a smaller aperture. See also [Using EthoVision in near darkness](#) on page 63.
- **It must be even** – That is, a diffuse light source, such as a shaded fluorescent tube or globe-type incandescent bulb. Spotlights can produce changing shadows and reflections.
- **There must be no reflection** of the light source visible in the image (see below).

Eliminating reflections

If you have reflections in your image, EthoVision may confuse those reflections with your subject, and track the reflections rather than your animal. Preventing reflections is particularly important when you want to do nose-tail tracking. There are a number of measures that you can take to reduce this problem:

- Surfaces within the arena should be (whenever possible) a dark color and matt texture.
- Lighting should be from a diffuse source, such as a shaded fluorescent tube or globe-type incandescent bulb.
- The light source should not be in direct line-of-sight of the camera (that is, it should be indirect lighting). You can achieve this by bouncing the light off the ceiling or walls (which should be a pale color and matt texture) and, if necessary, placing a shield in between the light source and lens.
- If your arena is transparent (for example, a glass olfactometer), you can use backlighting. This will eliminate reflections, but you must take special care that the lighting is even and diffuse. Place the light source underneath the setup and attach a sheet of white paper to the bottom to create diffuse back light. It is important to avoid light coming from above as much as possible, otherwise there is no effect of back light.
- You could also try a polarizing filter in front of the camera lens. The filter will remove reflections that are incident to the reflective surface at 32° - 37°.
- If possible, draw your arena in such a way that bright reflecting rims of mazes are excluded.
- To remove reflections brighter or darker than your animal, set appropriate thresholds in the Detection Settings (see page 212).
- To remove reflections larger than your animal, enter an appropriate value for the **Maximum Subject size** in the Detection Settings (see page 212). Note that if your animal enters the area of the reflection, EthoVision might no longer be able to track it if you use this setting.
- To ensure that EthoVision ignores parts of the arena not immediately next to the animal (which may contain reflections), define a scan window in the **Detection Settings** (see page 212).

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Lighting a water maze

Lighting a Morris water maze needs to be carried out particularly carefully because the water can give large and variable reflections. Some of these can be excluded by the software settings listed above, and the following lighting setup should give good results:

Place four (or more) bulbs round the pool, below the level of the water surface (Figure 2.12). 'Globe' type bulbs are ideal (twice the diameter as standard incandescent light bulbs). They should be close enough to the pool wall so that there is no direct line of sight between the bulbs and the camera lens. The light is reflected off the walls and ceiling, so that it only reaches both the lens and water surface indirectly.



Figure 2.12. Lighting a water maze. Left: view from side. Right: view from above.



Take special care when dealing with water and electricity in the same room. The lights should be placed so that water cannot splash on them, ideally they should be suitable for outdoor use (double-insulated) and if connected to an electrical outlet in the same room as the water maze they should be connected through a circuit-breaker.



Ask a photographer for advice on the lighting of your water maze.

Lighting water tanks

When using water tanks like in the Porsolt forced swim test, a problem is often that there is a lot of reflection, especially just above the water surface. Furthermore, detection of the animal can be hindered by the water's meniscus.

Try to make a set-up with as much contrast between animals and background as possible. For instance, when you have white rats, preferably use a completely black background. Back light (light from behind the subjects so you only see the animals' contour) generally gives very good results. A solution to remove the water's meniscus in the video image is also to place the test cylinders in a

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separate tank with water (for example an aquarium) and then make recordings. The water level in the tank should be higher than in the cylinders.



Browse to our web site www.noldus.com and download an application note on using EthoVision with the Porsolt swim test.

See also the Dependent Variable **Mobility** on page 530.

Using EthoVision XT in near darkness

Nocturnal animals like rats and mice are active in the dark. It is, therefore, a logical choice to measure their behavior in the dark. You can use infra-red (IR) lights to illuminate the arena and a camera that is sensitive in the infra-red. Most animals, including rats and mice, do not see infra-red or near-infrared light. IR-illumination is perceived by them (and also by humans) as being total darkness.

Camera

Infra-red light is light of a longer wavelength than visible light. Generally, to track with EthoVision, near-infrared light is used, with a wavelength of 700-900 nm ("near" the visible range of 400-700 nm). Color video cameras are insensitive to infra-red but standard monochrome cameras have reasonable sensitivity to near IR (see Figure 2.13). However specialized frame transfer monochrome CCD cameras are especially sensitive in the IR, and are the preferred (though more costly) option.

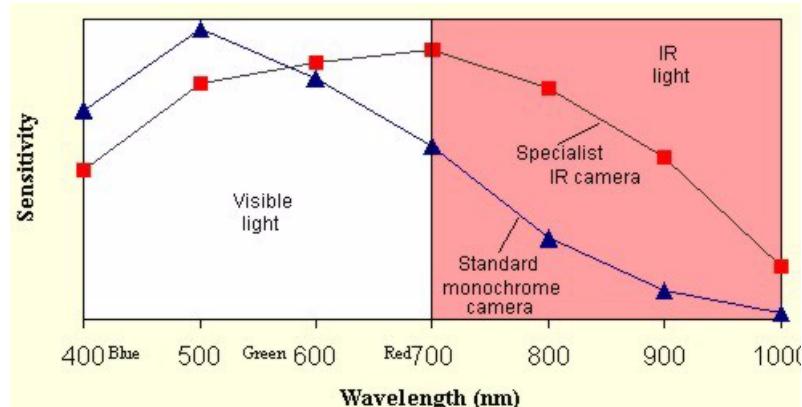


Figure 2.13. Sensitivity to infrared light of a standard monochrome camera and a specialist IR camera.

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Light source

As your light source, you can use IR illuminators, which basically are LED lamps that produce infrared rather than visible light. Using an IR illuminator is just like using a lamp, except that neither you nor your animals can see its light. Check the image from your camera to see what the illumination actually looks like. Just as with visible light, you want uniform lighting throughout your arenas. You may find that objects that look light in visible light, appear dark in the IR image, and vice versa. Likewise, objects that appear transparent may be opaque, or opaque objects (especially some kinds of acrylic) may be transparent in the IR image. You can record through acrylic, unless it has been colored or coated with pigments that absorb IR light. You can use, for example, a sheet of acrylic to cover the top of a cage or arena. If you use an IR illuminator from overhead, you may get spots of reflective glare, just as with normal lighting. Moving the illuminators to the sides, rather than overhead, can eliminate the glare. To eliminate shadows, you may get the best results by using two or more illuminators.



You can obtain an IR LED array directly from Noldus Information Technology.

You can also use an IR lightbox as your light source. An IR lightbox provides a uniform source of IR illumination from beneath your animal. Unlike the case with an IR illuminator, the camera will see a dark silhouette of the animal against a light background, which is easy to track in EthoVision. An IR lightbox is also an excellent solution if your animals are highly variable or mottled in color (e.g. Long Evans rats), and eliminates any concern about glare.

24-hour experiments

If you want to go from light to dark conditions (or vice versa) during a single trial (e.g., recording across a 24-hour circadian cycle), EthoVision needs consistent lighting. This can be accomplished by using an IR high-pass filter on your camera and by preventing any variation in the amount of IR illumination. The filter prevents the camera from seeing visible light, only IR light gets through. The filter is not necessary under constant lighting conditions, but you need it if the visible light level changes during the trial. The IR illumination must remain on for the entire duration of the trial. Some IR illuminators have the feature of automatically turning off when there is visible light (for example, IR illuminators designed for security applications usually turn off during the day). If your illuminator has this function, it needs to be disabled. Consult the documentation for your illuminator to see how this is done. If it is not possible to prevent the automatic turning off, you can cover the illuminator's light sensor with tape so that it will not detect light.

The normal light in the room should not affect the detection setting unless it also emits IR light. Many light fixtures will give off some IR illumination in addition to visible light. Incandescent and halogen lights especially will produce a great deal of infra-red light. Preferably use fluorescent lights which produce much less infra-

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red light. If there is an intensity adjustment on your IR illuminator, turn it up as high as possible. Increasing the amount of IR illumination will minimize the impact of any infra-red light from your visible lights. Sunlight also has an IR component, so if you have windows, they should be covered. When watching the camera image, ideally you should see no change in the image when the visible lights turn on or off. It might be that your image is slightly out of focus, but that is no problem for tracking in EthoVision.

If you have light colored animals, use the darkest non-reflective bedding possible for improved contrast. This kind of bedding can be ordered at http://www.ssponline.com/cellu_dri.htm.



If you have not eliminated all infra-red light from your visible light, and you are using '**Static**' subtraction' as your detection method, the entire arena may be detected as the animal after the visible lighting changes. Setting a '**Maximum subject size**' will prevent this. You can also choose '**Dynamic** subtraction' instead of 'Static subtraction' and the animal will be detected after a few frames.

2.11 Video Adjustment settings

You should ensure that the best possible signal reaches your computer by setting up the camera and lighting correctly (see above). Once you have done that, you can fine-tune the image with the **Video Adjustment** settings that are part of the **Detection Settings**. You can alter the contrast and brightness. See page 229 for more information about the **Video Adjustment** settings.

If you use a FireWire camera, it is advisable to set the frame rate and other camera features within the appropriate software that controls the camera (see page 48).

If you are tracking from a digital video file, the **Video Adjustment** settings are not available. You can make such settings in the encoding software you use for making digital video files.

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2.12 Troubleshooting hardware problems

The list of problems below is derived from the questions that our support department most frequently deal with. If you are unable to find the answer to your problem here (or the rest of the Reference Manual), please contact Technical Support (see Appendix D of this manual).



If your EthoVision computer is connected to hardware devices such as a pellet dispenser, a Noldus lickometer or a PhenoTyper, see the EthoVision XT Trial and Hardware Control Manual on your installation DVD.

I see no image in my Video window

This might be due to one of the following factors:

- There is a broken cable or loose connection between your camera and the frame grabber. Try replacing it.
- In the View Settings under the **Show/Hide** button on the component tool bar, you have not selected the **Video Source** checkbox.
- You have not installed your frame grabber correctly. Try re-installing, following the instructions on page 39.

I am having problems with my hardware key

If you have problems with your hardware key, it may be due to one of the following causes:

- A virus has infected your computer. Run a virus scanner program with a recently upgraded virus database. Make sure that it checks all drives and all types of files.
- Your hardware key may be physically damaged. Please contact our support department.

Ethovision is very slow in carrying out tasks

Please see page 36 for the requirements of your computer. To find out your computer's speed, check the manual of your computer or look in the **Control Panel** (click **Start** on the Windows task bar and then choose **Control Panel** and **System**). Close all other programs so there are more system resources available for EthoVision.

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My computer or monitor makes a noise which affects my animals

Place the computer and monitor in a separate room and use one of the following two options:

- Define a **Trial Control** profile with rules for start and stop recording, for instance, 'Start recording 0.1 second after the subject is first detected in the water maze' and 'Stop recording when the rat has reached the platform'. Start the trial on the computer with EthoVision running. This starts the trial, but not acquisition. Then go to the room with your arena and put your animal in the arena. EthoVision will detect your animal and tracking will start (after 0.1 s). Instead of a **Stop recording** rule, you can also define a maximum trial duration in your **Trial Control profile**.
- Use a remote control (which you can obtain from Noldus). Double-click the file `remote_control.reg` in the Utilities folder on your installation DVD. This way the page-up and page-down keys on your remote control give the commands **Start Acquisition (Ctrl+F5)** and **Stop Acquisition (Ctrl+F6)**. After you have placed your animal in the arena, you can click the appropriate button on the remote control to start and stop acquiring data without having to walk over to your computer.

My animals are spotted, which confuses EthoVision

Tracking a spotted animal on a background in one of the colors of the animal works well with **Differencing** as the detection method. For experiments with spotted animals we recommend a background that contrasts as much as possible with all colors of the animal. For instance, when tracking black and white Long Evans rats, a gray arena works well.

How can I run EthoVision on multiple computers?

You must plug the hardware key in your PC in order to use EthoVision. However, only data acquisition and the use of extra modules (**Multiple body points**, etc.) are blocked when this device is not present and not the visualization and analysis functions. You can view tracks and analyze data on another computer without the hardware key.

The computer used for acquiring data (in the animal house) should contain the frame grabber and the hardware key. You (and other users) can install EthoVision on another computer (in your office) to visualize your data and analyze the tracks.



If you are using EthoVision XT with an extra module (for example, the **Multiple body points module**), your hardware key must always be connected to your PC, whether you are acquiring data or analyzing them.

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Procedure:

- 1 After a day of measurements, make a backup of the current experiment on the computer where data acquisition took place (see page 565).
- 2 Restore this backup on another computer (see page 566).
- 3 Carry out your data analysis on the computer with the restored data.

How do I run EthoVision with more than one video camera?

It is possible to connect more than one video camera to the frame grabber and encoder board (the board has four video inputs). This allows you to run experiments at different locations without the need to move the equipment from one setup to the other. In the **Experiment Settings** you can select which video camera you want to use (see page 96).



If you use more than one video camera, you must define separate arenas for each video camera.

My FireWire camcorder goes to standby after a few minutes

Remove the tape from your camera.

3

Getting Started

This chapter aims to take you step by step through the key stages of using EthoVision XT, so that you can get up and running as quickly as possible. For more details, please refer to Chapters 4-15.

A typical EthoVision XT procedure involves the following steps:

- 1 Creating a new experiment or opening an existing one**, see the next page.
- 2 Setting up your experiment** – See page 72.
- 3 Acquiring data (tracking)** – See page 74.
- 4 Co-acquiring external data** – See page 75.
- 5 Selecting data** – See page 76.
- 6 Visualizing data** – See page 77.
- 7 Analyzing data** – See page 77.
- 8 Exporting data** – See page 78.

Creating a new experiment or opening an existing one

When you start EthoVision XT, the EthoVision XT 8 Startup window appears. From this window, you can open an existing experiment, restore a backup experiment or create a new experiment.

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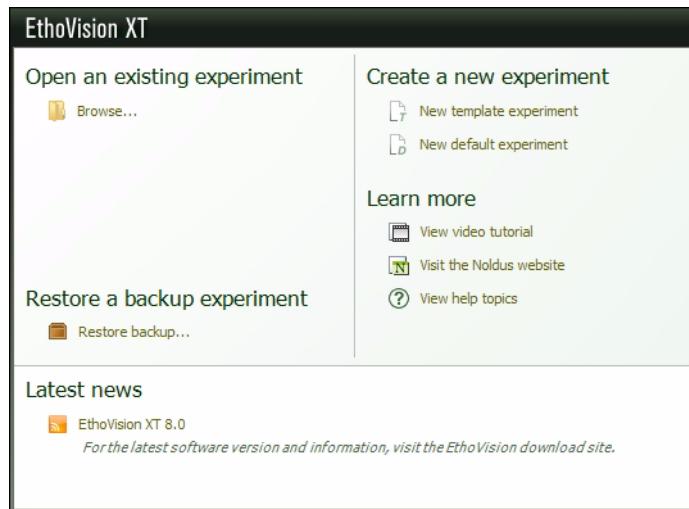


Figure 3.1. The EthoVision XT Startup window.

Creating a new experiment

When you want to create a new experiment, you have two options:

- **New template experiment** - Selecting this option guides you through the steps necessary to set up an experiment for a number of standard tests, such as, the Morris Water Maze test or a Radial Maze test. Based on a number of choices you make (for example, video source, type of animal, type of arena, number of subjects per arena, etc), EthoVision XT creates an experiment that you can use as a basis for your specific test. Furthermore, you can use an existing experiment as a template.
- **New default experiment** - This is the way you created an experiment in previous versions of EthoVision XT.

1 To create a new experiment, do one of the following:

- In the EthoVision Startup window, under **Create a new experiment**, click **New template experiment** or **New default experiment**.
- From the **File** menu, choose **New Template Experiment** (or press <Ctrl+T>) or **New Default Experiment** (or press <Ctrl+N>).

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2 If you have chosen to create a new template experiment, select one of the template options:

- **Apply a pre-defined template** - Follow the instructions in the setup guide. After you have entered an experiment name, check the settings created in the guided setup.
- **Use a custom template** - Open an existing experiment and use it as a template for your new experiment.

3 In the **Name** field, enter a name for your experiment.



Select **GLP Experiment** if you want EthoVision to help you making a GLP-compliant experiment (see Chapter 4 for more information).

4 Browse to the location in which you want to store your experiment. Then click **OK**.

5 The **New Experiment** window closes and the experiment is created in a folder with the same name as the experiment.

6 You are now ready to (continue to) set up your experiment.

Opening an existing experiment

1 To open an existing experiment, do one of the following:

- In the EthoVision XT Startup window, under **Open an existing experiment**, click on an one of the experiments in the list.
- In the EthoVision XT Startup window, under **Open an existing experiment**, click **Browse** to locate an existing experiment.
- To open an existing experiment and use it as a template for a new experiment, in the EthoVision XT Startup window, under **Create a new experiment**, click **New template experiment** and **Use a custom template**.
- From the **File** menu, choose **Open Experiment** (or press <Ctrl+O>).

2 Browse to the location where your experiment is stored, select the experiment file name (*.evxt, or *.evx for experiment from previous versions of EthoVision XT) and click **Open**. Your experiment loads.



You can also open an experiment from the list of previously opened experiments. in the **File** menu.

Chapter 3

Setting up your experiment

When you have created a new default experiment, the EthoVision Overview window opens.

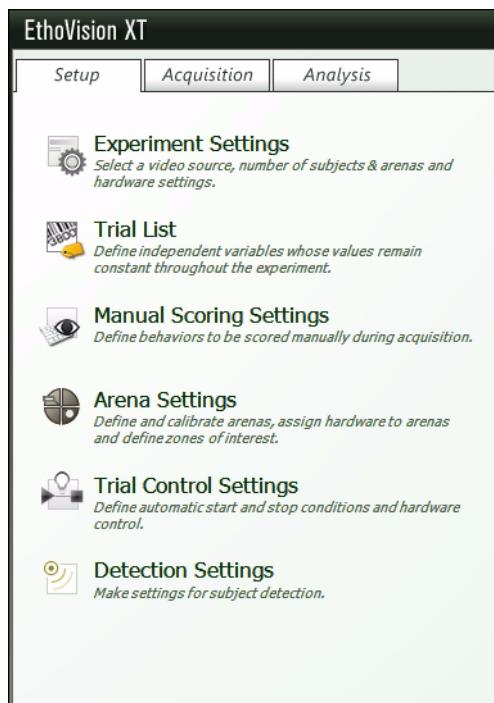


Figure 3.2. The EthoVision Overview window.

The Setup tab shows, from top to bottom, the different for setting up your experiment:

- **Experiment Settings** - Specify those aspects of your experiment that remain constant for the duration of the whole experiment, e.g., the number of arenas in which you are going to track, the number of subjects in each arena, the body points to be detected (the center-point only or the center-point with nose-point and tail-base), and the video source that you are going to use (video file or live).

Getting Started



To track in multiple arenas you need the **Multiple arenas** module. To track multiple subject in one arena you need the **Social interaction** module. To detect nose-point and tail-base, the **Multiple body points** module.

- **Trial List** - In the Trial List, you can define Independent variables which are either simple descriptive categories (such as the genotype or age of your rats or mice), or conditions manipulated by the researcher (such as the substance being tested and the dose). In the **Trial List**, you can also specify the number of trials that you are going to carry out. For each trial you can enter the values of the independent variables. For instance, add 10 trials and for each trial, specify the genotype of the rat that you are going to track and the treatment that it will receive.
- **Manual Scoring Settings** - Here you can define behaviors (start-stop or mutually exclusive) you can manually score during acquisition.
- **Arena Settings** - Before you start data acquisition you should tell EthoVision in what region in the video image on the screen your subject moves. This region, the arena, can be a water maze, an open field, a petri dish or any other enclosure.



With the Base version of EthoVision you can track in one arena. With the **Multiple arenas** module, you can define up to 100 arenas in one field of view.

If you like you can divide your arenas into a number of zones. For instance, when you carry out an open field test, you can divide your arena into a center zone and a border zone and use the time spent in the center zone or the latency to enter the center zone as a measure of the fearfulness of your rat or mouse. In a water maze test you can define the hidden platform as a zone and calculate how long it takes before your subject finds the platform, i.e. enters the zone. If you define a **Trial Control** profile with the rule that recording must stop when the subject is inside the zone, the trial is automatically ended as soon as the subject reaches the platform.

When you have defined your arenas and zones, you must calibrate your arenas. EthoVision measures the distance between two points on the screen in pixels. To convert this distance in pixels to a 'real world' distance (in, e.g., centimeters or inches), you must calibrate. See Chapter 6 for more information about defining your arenas and zones.

- **Trial Control Settings** - Specify rules that control the start and stop of data acquisition. For instance, when performing a water maze test: start recording 2 sec. after the rat has been detected in the water maze and stop recording when the rat has reached the platform.

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With the **Trial and Hardware Control** module, you can create complex trial control, for example control a pellet dispenser or automate repeated tasks.

- **Detection Settings** – Select the detection method of your choice: **Gray scaling**, **Static subtraction**, **Dynamic subtraction** or **Differencing** (see page 212).

To open and define each one of the settings mentioned above, do one of the following:

- In the EthoVision XT **Overview** window, click the appropriate item.
- In the Experiment Explorer on the left, in the **Setup** folder, click the appropriate item.
- From the **Setup** menu, choose the appropriate item.



For more information:

- Chapter 5 for setting up your experiment.
- Chapter 6 for defining arenas and zones.
- Chapter 7 for controlling trials.
- Chapter 8 for detection settings.

Acquiring data

You can acquire data in one of the following four ways:

- Track from a live video signal (directly from your camera).
- Track live and record video at the same time with the frame grabber and encoder board that are part of your EthoVision setup.
- First record video to a video file (with the frame grabber and encoder board), and do tracking at a later stage for better performance during tracking.
- Track from a video recorded with software/hardware other than EthoVision in combination with one of the frame grabber and encoding boards.

- 1 To start acquisition, from the **Acquisition** menu, choose **Open Acquisition**.



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- 2 Add a trial or start one of your planned trials by clicking the appropriate buttons in the **Acquisition Control** window.



- 3 Stop the trial by clicking the **Stop** button.

You can also automatically start and stop your trial depending on the behavior of your subject (see Chapter 7).

During data acquisition, EthoVision converts the signal from your camera or video file into useful information. For each sample it extracts the following features:

- **subject position** – The x,y coordinates of the animal's center-point.
- **subject size** – The number of pixels composing the subject.



With the **Multiple body points** module you can also extract the x,y coordinates of the animal's nose-point and tail-base, the direction of the head, the degree of elongation of the animal's body and the change in size of the body.

See Chapter 9 for more information about acquiring data.

Co-acquiring external data

Ethovision XT allows you to co-acquire external data with a separate **Data AcQuisition (DAQ)** system. For example: physiological data (EEG, blood pressure, heart rate, etc) or environmental data (room temperature, humidity, etc).

To import external data, from the **Setup** menu, choose **Import External Data**. EthoVision XT offers import profiles for a number of DAQ systems:

- DSI – DataQuest ART 2.3.
- MiniMitter – VitalView 4.1.
- BIOPAC – AcqKnowledge 3.7.3.
- Polar – Polar Precision Performance 4.0.1.

You can also create your own import profile if there is no predefined import profile for your system. You can synchronize your track data with the associated external data and subsequently select and visualize the combined data.



See Chapter 10 for more information about importing external data.

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Track Smoothing

You can apply **Smoothing** to your tracking data. By using the Lowess smoothing method, you can get rid of outliers or small, erratic movements in the data of your animal.

To smooth your tracks, you can also use the **Minimal Distance Moved** filter.

To apply Track Smoothing to your data, from the **Acquisition** menu, select **Track Smoothing Profile**.

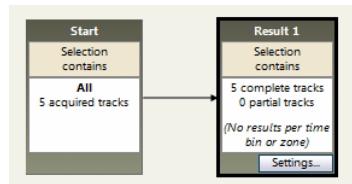
Selecting data

In EthoVision XT, there are three ways to select your data:

- **Filtering** - You can **filter** your data directly by choosing a specific set of tracks or indirectly according to the values of your independent variables (for example, filter the tracks of all the animals which received treatment A).
- **Nesting** – You can **nest** over **time**, **zones**, **behavioral states** or states defined by Trial Control events. For instance, visualize the data of the first 5 minutes of the experiment, calculate the distance moved while your subject was in the center of the open field or visualize all samples when the subject was moving.
- **Time bins** – Time bins allow you to visualize or analyze a series of time intervals within each track. For instance, when you are doing 24-hour measurements, you may want to analyze your tracks using the following time bins: midnight - 6 am, 6 am - noon, noon - 6 pm and 6 pm - midnight.

You can combine these methods with AND/OR Boolean logic.

You can select data by creating a data profile: from the **Analysis** menu, choose **Data Profile**. To select data, you must build a sequence of selection boxes connected to each other. This sequence always starts with a **Start** box, containing all the data in your experiment, and represents the flow of data progressively extracted (filtered or nested over) in each box. The last box, called **Result**, represents the data set used for visualization or analysis.



Getting Started



See Chapter 12 for more information about selecting data.

Visualizing data

Before carrying out quantitative analysis, you can visualize your data to get an impression of the behavior of your subjects and relate that to the physiological data you have co-acquired.

From the **Analysis** menu, select **Results** and choose **Plot Tracks** to view the tracks that you selected in the data selection. This allows you to visually compare tracks. You can play back your tracks to see how your subjects moved.

Choose **Plot Integrated Data** to have a complete dynamic picture of your trials. When you play back your data, you will not only see your tracks, but also the corresponding video, the value of dependent variables (for example the velocity of the animal), events of Trial Control (for example, when a condition becomes true or an action is carried out) and the associated physiological data.

You can have all your tracks displayed in the same color or have color variation at track level, i.e. tracks with different independent variables values are displayed in different colors. A third option is to have color variation at sample level. Choose this option if you want the samples within your tracks displayed with a color according to the value of a dependent variable in your analysis profile. For instance, show samples with different values of velocity in different colors.



See Chapter 13 for more information about visualizing data.

Analyzing data

From the raw data that EthoVision extracts during acquisition (x,y coordinates and surface area), it can calculate an extensive number of dependent variables, like velocity, time spent in a particular zone, distance moved, etc. For each of these variables, you can calculate a series of descriptive statistics, such as mean (average), standard deviation and standard error, total, etc.

You can now also calculate statistics for Hardware devices, for example, the total number of pellets dropped by the Pellet Dispenser or the mean duration of licks at the Noldus lickometer.

You can choose your dependent variables and statistics by making an **Analysis Profile**: from the **Analysis** menu, choose **Analysis Profile**. Click the **Add** button next to the dependent variable that you want to use and choose the statistics that

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you want to calculate. Subsequently, from the **Analysis** menu, select **Analysis Output** and click the **Calculate** button in the Analysis screen.



See Chapter 14 for more information about analyzing data.

Exporting data

To find out if your independent variables (like treatment, dose, etc.) have a significant effect on the dependent variables, you can export your data to a statistical package. You can either export the raw track data or the analysis results, in one of two formats (as a text file or an Excel file). From the **Export** menu, select **Track data** or **Analysis Output**.



See page 496 for more information about exporting data.

4

EthoVision XT and GLP

4.1 What is GLP?

GLP (Good Laboratory Practice) is a set of rules for conducting non-clinical laboratory studies that support or are intended to support applications for research or marketing permits for products regulated by the U.S. Food and Drug Administration. These products include animal food additives, medical devices for human use, biological products and human and animal drugs. The principles of and regulations for GLP are described under the Code of Federal Regulations Title 21, Part 58 (CFR21/58).

For Europe, the GLP rules have been compiled and adapted by the Organization of Economic Cooperation and Development (OECD).

Subparts of the CFR21/58 GLP rules define and describe the following major points:

- Organization and personnel.
- Facilities.
- Equipment.
- Testing Facilities Operations.
- Test and Control Articles.
- Protocol for and Conduct of a Non-clinical Laboratory Study.
- Records and Reports.

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- Disqualification of Testing Facilities.

All these items require to be managed in such a way that GLP-compliance is facilitated and ensured.

CFR21/11 more specifically describes guidelines for the use of electronic records.

4.2 EthoVision XT and GLP

EthoVision XT offers functionality to support the user in making GLP-compliant experiments.



This functionality does not guarantee that experiments made in EthoVision XT with the GLP module are GLP-compliant.



You need the GLP module to carry out GLP experiments in EthoVision XT.

GLP compliance is supported by the following functionality:

- **User management** – You can set up the program so that only authorized users are able to take particular actions, and that certain actions such as editing or deleting data are prevented.
- **GLP Log** – All user actions which can result in changes to the acquired data (for instance altering settings) are logged and every time you leave a part of the program you are prompted to add a comment to the log file. This creates an audit trail suitable for a GLP auditor to review.

4.3 EthoVision XT User Management

With EthoVision XT User Management you can:

- Prevent non-GLP users to access EthoVision XT with the GLP module installed.
- Assign different rights of access to each part of the program by GLP users.

How EthoVision XT User Management works

In EthoVision XT User Management, you can define a list of users that can access EthoVision XT. Furthermore, users can have different rights; these rights can only be assigned by a user with User Management rights in EthoVision XT.

Initially, after installation, only one user has access to EthoVision XT. Which user this is depends on how EthoVision XT was installed:

- EthoVision XT came installed on a computer provided by Noldus Information Technology.
- EthoVision XT was installed by the user.

EthoVision XT came installed on a computer

When you purchased both EthoVision XT and the EthoVision XT computer from Noldus Information Technology, EthoVision XT is already installed on that computer. In that case, a user account **EVXT** has been created to log on to Windows. This user EVXT, with administrator rights, initially is the only user that can access EthoVision XT on that computer.

User EVXT has, by default, the following rights in EthoVision XT: User Management and EthoVision Preferences. Use this user to add new users and assign them rights (see [Adding a user](#) below).

EthoVision XT was installed by user

The user with administrator rights that installed EthoVision XT is automatically added to the list of users in the User Management window in EthoVision XT. Also, a user **EVXT** is automatically added to the list of users.



With this standard user **EVXT**, any administrator can at all times create a user account EVXT through which EthoVision XT can be accessed.

Next, either the user that installed EthoVision XT or user EVXT can add new users and assign them rights (see below).

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Adding a user

The procedure to add a user differs, this depends on whether EthoVision XT came installed on a computer, or was installed by the user, and on whether the computer is on a domain or not. To add a new user to EthoVision XT, follow the procedure according to your situation:

EthoVision XT came installed on a computer that is on a domain

Make sure that user EVXT has also been added to the domain by your network administrator.

- 1 Log on to Windows as user EVXT and open EthoVision XT.
- 2 From the **File** menu, select **Users**.
- 3 In the **User Management** window, click **Add**.
- 4 In the **Add New User** window, click **Network**.
- 5 A list of users on the domain appears. Select a user from the list.



At this point, you have the option to select an initial role (Administrator, Researcher or Technician) for the new user. See also **Assigning rights to users** below.

- 6 Click **Add** to add a user to the list of users that can access EthoVision XT.

Next, you can assign rights to the new user (see page 84).

EthoVision XT came installed on a computer that is not on a domain

When EthoVision XT was installed by Noldus Information Technology on an EthoVision XT computer, initially there is only one user, EVXT, on that computer. Log on to Windows as this user.



You can find the password in the welcome letter you received with the software.

You first need to define new users in Windows before you can add them to the list of authorized users in EthoVision XT. You must have a local computer administrator account to add a new user to the computer.

To add a new user in Windows:

- 1 In Windows, click **Start**, Select **Control Panel** and next **User Accounts**.
- 2 Under Windows XP click **Add**, under Windows 7, first click **Manage User Accounts** and then click **Add**.
- 3 Type in the name of the new user, and click **Next**.

- 4 Select an account type and next click **Finish**.

Next, you can add this user in EthoVision XT:

- 1 Open EthoVision XT.
- 2 From the **File** menu, select **Users**.
- 3 In the **User Management** window, click **Add**.
- 4 Local users on the computer are shown. Select one of the users in the list.



At this point, you have the option to select an initial role (Administrator, Researcher or Technician) for the new user. See also **Assigning rights to users** below.

- 5 Click **Add** to add this user to the list of users that can access EthoVision XT.

Next, you can assign rights to the new user (see page 84).

EthoVision XT was installed by user on a computer that is on a domain

The user that installed EthoVision XT can add new users to the list of authorized users in EthoVision XT.

- 1 Open EthoVision XT.
- 2 From the **File** menu, select **Users**.
- 3 In the **User Management** window, click **Add**.
- 4 In the **Add New User** window, click **Network**.
- 5 A list of users on the domain appears. Select a user from the list.



At this point, you have the option to select an initial role (Administrator, Researcher or Technician) for the new user. See also **Assigning rights to users** on the next page.

- 6 Click **Add** to add a user to the list of users that can access EthoVision XT.

Next, you can assign rights to the new user (see page 84).

EthoVision XT was installed by user on a computer that is not on a domain

First, you might need to add local users to the computer (see page 82 for a description of how to do this).

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You must have a local computer administrator account to add a new user to the computer.

To add a new user to EthoVision XT:

- 1 Open EthoVision XT.
- 2 From the **File** menu, select **Users**.
- 3 In the **User Management** window, click **Add**.
- 4 Local users on the computer are shown. Select one of the users in the list.



At this point, you have the option to select an initial role (Administrator, Researcher or Technician) for the new user. See also **Assigning rights to users** below.

- 5 Click **Add** to add this user to the list of users that can access EthoVision XT.

Next, you can assign rights to the new user (see below).

Assigning rights to users

You can only assign rights to users when you have been assigned User Management rights in EthoVision XT.



You can only assign or modify user right, when there is no experiment open.

When you add a new user, you have the option to select one of three predefined roles, each representing a set of rights.

However, these rights can be changed at any time. The three initial roles are:

- **Administrator** – with default rights User Management and EthoVision Preferences.
- **Researcher** – with default rights Experiment Management, Experiment Settings, Trial Control Settings, Arena / Trial Control / Detection Settings, Acquisition, Import External Data, Edit Tracks, Edit Data Profile / Analysis Profile.
- **Technician** – with default rights Arena / Trial Control / Detection Settings, Acquisition, Import External Data.

The following rights can be assigned to users (Figure 4.1):

- **User Management** – gives you the right to add and remove users, and change user rights.
- **EthoVision Preferences** – gives you the right to change the default experiment folder, the warnings displayed and autosave.
- **Experiment Management** – gives the right to create new experiments, make and restore backups.
- **Experiment Settings** – gives the right to set and change Experiment Settings.
- **Trial Planning** – gives the right to add Trials and Variables to the Trial list. With this right you can also set tracks to To Skip or Redo tracks.

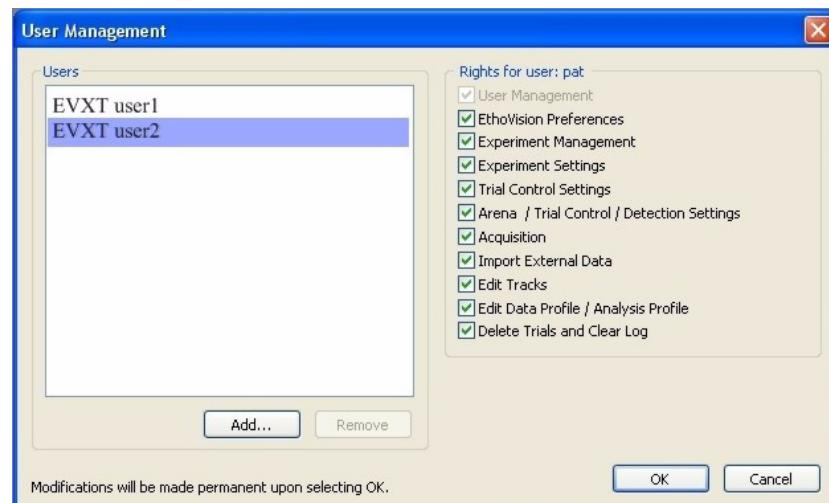


Figure 4.1 The **User Management** window. The pane on the left shows the list of authorized users. The pane on the right the associated user rights.

- **Arena Settings / Trial Control / Detection Settings** – gives you the right to create and or edit Arena Settings, Trial Control Settings and Detection Settings.
- **Acquisition** – gives the right to change Acquisition settings and carry out Acquisition.
- **Import External Data** – gives the right to import external data files.
- **Edit Tracks** – gives the right to edit tracks.



It is likely that your Standard Operating Procedure forbids modifying acquired data. If this is the case, no user should have this right.

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The original data is always stored in your experiment.

- **Edit Track Smoothing, Data, Analysis Profiles** – gives the right to smooth tracks, create and edit Data profiles and Analysis profiles.
- **Delete Trials and Clear Log** – gives you the right to delete trials and delete the Experiment log file.



It is likely that your Standard Operating Procedure forbids modifying acquired data. If this is the case, no user should have this right. By default, this right is disabled for all users.



The User Management right to delete trials and the Experiment log file is useful when you want to create a template experiment. This template experiment, for example, contains default settings as defined in a Standard Operating Procedure, but should not contain test trials and an already filled log file. Also see [Creating an Experiment template](#).



It is advisable to create a special user for this right. In normal operation under GLP no user should be able to perform these actions.

4.4 EthoVision XT Logging

Aim of the logging functionality in EthoVision XT is to enable auditing of (changes to) settings that were made during an experiment. EthoVision XT creates two types of log files:

- a **General log file** – in this Application log the usage of EthoVision XT by authorized users is registered.
- an **Experiment log file** – one for each experiment, to register the settings made and actions carried out during that experiment. The user, date and time are logged for each setting and action.

Furthermore, the user can at any time add additional user comments to the Experiment log file.



The log file is tamper-proof. As soon as someone edits it outside EthoVision XT, that is detected and it is logged that the file has been tampered with.

The General Log file

The General log file is created the moment EthoVision XT is installed. The following actions are logged:

- Start and stop of an experiment.
- Creation of a new experiment.
- Users that are added / user rights that are assigned / user rights that are modified.
- Errors that occurred when opening or closing an experiment.

For every action, the user name, date and time are logged.

To view the **General log file** in EthoVision XT:

- 1 From the **File** menu, select **GLP Log Files**.
- 2 In the **GLP log** window, select the **General log file** radio button.

The Experiment log file

The Experiment log file is started at the moment a GLP experiment is created. Changes made by the user that affect the data and results of an experiment can be traced back in the log file.

These changes are logged from the moment you actually start acquiring data. At that moment a snapshot is taken of the various settings in your experiment and this snapshot is logged. Thereafter, all changes that can affect your data are logged.

When a users makes changes to settings (for example, in the Trial List or Trail Control Settings) and/or leaving a Setting, the user is prompted to add a comment to the Experiment log file. A new snapshot is then taken when new data are acquired.



When the user cancels this automatic pop-up or does not enter a comment, a line appears in the Experiment log file with user name, date and time but without a comment.

At any time, in the current experiment, a user can add a comment to the **Experiment log file** by doing the following:

- 1 From the **File** menu, select **Add log comment** or press **Ctrl+L**.
- 2 Type in comments and click **OK**.

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Experiment Settings, Arena Settings, Trial list, Trial Control Settings and **Detection Settings** are not stored in the log file until the first data are acquired. After that, only changes are logged.

Logged **Arena Settings** include a screenshot of Arenas and their Calibrations. It also contains a screenshot of each **Zone Group** and its **Zones**.

Viewing the Experiment log file

Before you can view an **Experiment log file**, you need to open the corresponding experiment. To view the **Experiment log file** in EthoVision XT:

- 1 From the **File** menu, select **GLP Log Files**.
- 2 In the **GLP log** window, select the **Experiment log file** radio button. If necessary, click on one of the html links to go to a specific part of the **Experiment log file**.



See **General and Experiment log files** (see page 580) in the chapter on File Management to see how to export and print these log files and how to delete the Experiment log file.

4.5 Creating an GLP experiment template

It can be useful to create an experiment with all the right default settings, but without tracks and an Experiment log file, and use this as a template for further GLP-experiments.



In order to create such a template you need to have the user management right to **Delete Trials and Clear Log**.

In EthoVision XT 8, you can use an existing GLP experiment as a template for a new experiment:

- 1 Do one of the following:

- In the EthoVision XT 8 startup window, under Create a new experiment, select **New template experiment**.

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- From the **File** menu, select **New template experiment** or press <**Ctrl+T**>.
- 2 In the Template option window, select **Use a custom template**.
- 3 Click **Browse** and select the existing GLP experiment you want to use as a template. Click **OK**.
- 4 Type in the name of the new experiment and click **OK**.



It is advised to create a backup of the template and store it in a safe place.



You need a hardware key with a GLP module to open a GLP experiment.

Additional remarks

- Other than in non-GLP experiments, in a GLP experiment, in Arena Settings, you cannot change/edit Zones that were already used in Acquisition.
- With a GLP module in your hardware key you can still create or open non-GLP experiments. In that case user management does not apply and no log file is kept.
- In the case of large experiments, when the log file exceeds a size of 1 Mb, it is split in multiple log files.
- Multiple copies of the same version of EthoVision XT installed on the same computer share the General log file. Different versions of EthoVision XT installed on the same computer each have their own General log file.
- When EthoVision XT is uninstalled or upgraded, the corresponding **General log file is not removed**.

5

Setting up an experiment

This chapter is about:

- **Defining experiment settings** – Specify the number of arenas you want to use, the body points to be detected (center-point only or center-point with nose-point and tail-base), and the video source that you are going to use (live tracking or from video file).
→ See page 96
- **Trial List** – In the **Trial List** you can define your independent variables, view the system variables and list the trials that you plan to carry out.
→ See page 103 and page 117
- **Setting preferences** – Specify the location of your experiment files, select in what situations you want to get a warning and enable or disable auto-recovery.
→ See page 126



Unlike most settings in EthoVision XT, you cannot make multiple profiles of your Experiment Settings. However, you can use an existing experiment as a template for a new one, so without any tracks, so that you can easily adjust only these settings in a new experiment if you need to.

5.1 Creating a new experiment

When you start EthoVision, the EthoVision Startup window appears and the Experiment Explorer indicates that no experiment is loaded. The first step is to either create a new experiment (see below) or open an existing experiment (see page 563).

When you want to create a new experiment, you have two options:

- **New template experiment** - Selecting this option guides you through the steps necessary to set up an experiment for a number of standard tests, such as, the Morris Water Maze test or a Radial Maze test. Based on a number of choices you make (for example, video source, type of animal, type of arena, number of subjects per arena, etc), EthoVision XT creates an experiment that you can use as a basis for your specific test. Furthermore, you can use an existing experiment as a template.
- **New default experiment** - This is the way you created an experiment in previous versions of EthoVision XT (see page 562 how to do this).

Creating a new experiment based on a pre-defined template



After you have followed the steps below to create the new experiment, you must still adjust the Arena Settings and Detection Settings before you can track any animal correctly.

- 1 In the EthoVision Startup window, under **Create a new experiment**, click **New template experiment** or from the **File** menu, choose **New Template Experiment** (or press <Ctrl+T>).
- 2 In the **Select a template option** window, select **Apply a pre-defined template** and, next, follow the instructions in the guided setup.
- 3 **Step 1- Select the video source:**
 - **From video file** - If you select this option, you can click **Browse** to select the video file. Optionally, you can also open the video file later.
 - **Live tracking** - Select this option and click **Settings** to select a channel of the Picolo frame grabber board for **Tracking**.

Setting up an experiment

- **Live tracking and saving video file** - Select this option and click **Settings** to select a channel of the Picolo frame grabber board for **Tracking** and another channel for **Recording**.

In the Device Activity list, you can also select DAQ Co-Acquisition. See page 98 for more information.



You can only select live tracking if a frame grabber is installed in your computer or if you use a FireWire or USB camera. However, we do not advise to use a USB camera.

4 Step 2 - Select a subject.

If you select the option **Rodent**, **Fish** or **Arthropods**, you can select a specific species from the list. If your animal is not in one of these lists, select **Other**.



Based on the subject you select, different arena configurations are available in the next step of the guided setup, a suitable sample rate is selected and the nose-tail detection is made available (provided you have the Multiple Body Points module).

5 Step 3 - Select an Arena configuration:

- **Arena template** - Select a type of arena from the list (a picture is shown upon selection).
- **Zone template** - Here you have the choice to select predefined zones for the type of arena you select in the **Arena template** list.
- **Number of Arenas** - Select the number of arenas in the list.



You can only select multiple arenas, if you have the **Multiple Arenas** module.

- **Rows and columns** - If you have multiple arenas, select the layout of the arenas.

6 Step 4 - Select the number of subjects per arena.



You can only select multiple animals, if you have the Social Interaction module.

See page 101 for more information.

7 Step 5 - Select the tracked features.



See page 102 for more information.

Also select whether the animal is **Darker**, **Brighter** or **Brighter and darker** than the background.



See page 212 for more information.

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- 8 **Step 6 - Recommended sample rate.** Here the recommended sample rate is displayed which is based on the settings in the previous steps. The applied sample rate is the sample rate that is used
- 9 **Step 7 - Initialize template experiment.** This window gives an overview of the selected settings. Click **Previous** if you want to change settings, click **Finish** to create the experiment.
- 10 In the **Name** field, enter a name for your experiment. Make sure that the name does not contain any of the following characters: / : * ? \ " < > | . ;.
-  Select **GLP Experiment** if you want EthoVision to help you making a GLP-compliant experiment (see Chapter 4 for more information).
- 11 Browse to the location in which you want to store your experiment. Then click **OK**. The default location is the location that you specified during installation (see page 586).
- 12 The **New Experiment** window closes and the experiment is created in a folder with the same name as the experiment.
The EthoVision XT Overview window appears (see Figure 5.1).
- 13 You are now ready to (continue to) set up your experiment.

What next?

- Check the **Arena Settings** to make sure the pre-defined Arenas and Zones have the correct size and shape for your setup (see Chapter 6 Arena Settings on page 131 for more information).
- Check the **Detection Settings** to make sure it has the correct settings for your setup (see Chapter 8 Configuring Detection Settings on page 209 for more information).



You should also check the **Experiment Settings** and Trial Control Settings to make sure they contain the appropriate settings (see Chapter 5 **Setting up an Experiment** on page 91 and Chapter 7 **Trial Control** on page 173 for more information).

Creating a new experiment based on an existing experiment



When you try to open an experiment from an older version a warning appears, informing you that you cannot open upgraded experiments

Setting up an experiment

in the old version of the software. This is particularly import when you try to open an EthoVision XT 7 experiment in EthoVision XT 8 because:

- **Hardware logs are irrecoverably lost.**
- **Statistics results must be recalculated.**
- **The Minimum Distance Moved nesting criterion is removed from the Data Selection. You can select the Minimal Distance Moved method in your Track Smoothing profile. See page 380 for more information.**



We strongly recommend to make a backup of your experiment in EthoVision XT 7 before opening it in EthoVision XT 8. To make a backup, from the **File** menu, select **Make Backup**. If you uninstalled EthoVision XT 7, you can also make a copy of your experiment using Windows Explorer before you open it in EthoVision XT 8. Always copy the complete experiment folder and not only the *.evxt file otherwise your experiment may get corrupt!

To use an existing experiment as a template for your new experiment:

1 Do one of the following:

- In the EthoVlsion Startup window, under **Create a new experiment**, click **New template experiment** or from the **File** menu, choose **New Template Experiment** (or press <Ctrl+T>).
- **2** In the **Select a template option** window, select **Use a custom template**.
- **3** Browse to the experiment folder of the existing experiment, open the experiment and click **OK**.
- **4** In the **Name** field, enter a name for your experiment. Make sure that the name does not contain any of the following characters: / : * ? \ " < > | . ; . The new experiment contains the same settings as the existing experiment but no track data. If necessary, check if the settings are appropriate for the new experiment.



Select **GLP Experiment** if you want EthoVision to help you making a GLP-compliant experiment (see Chapter 4 for more information).

- **5** Browse to the location in which you want to store your experiment. Then click **OK**. The default location is the location that you specified during installation (see page 586).
- **6** The **New Experiment** window closes and the experiment is created in a folder with the same name as the experiment.
The EthoVision XT Overview window appears (see Figure 5.1).
- **7** You are now ready to (continue to) set up your experiment.

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If you open an experiment which used 'Live tracking' and was created on a different computer, you must remake the Video source settings for the new experiment (see page 98).

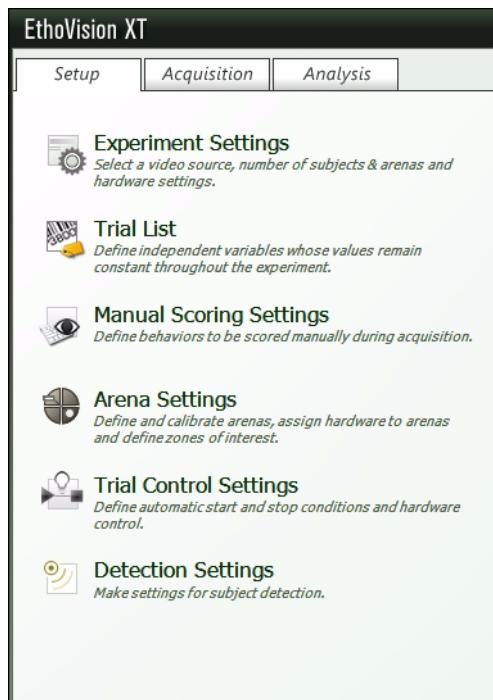


Figure 5.1. The EthoVision XT Overview window.

5.2 Experiment settings

You can open the Experiment Settings by doing one of the following:

- In the EthoVision XT Overview window, click **Experiment Settings**.
- From the **Setup** menu, select **Experiment Settings**.

Setting up an experiment

In the **Experiment Settings** (Figure 5.2) you can specify those aspects of your experiment that remain constant during the entire course of the experiment. You can enter a description of your experiment, specify the number of arenas, the body points to be detected and the video source that you are going to use (video file or live). You can also specify what units you want to use for distance, time and rotation.

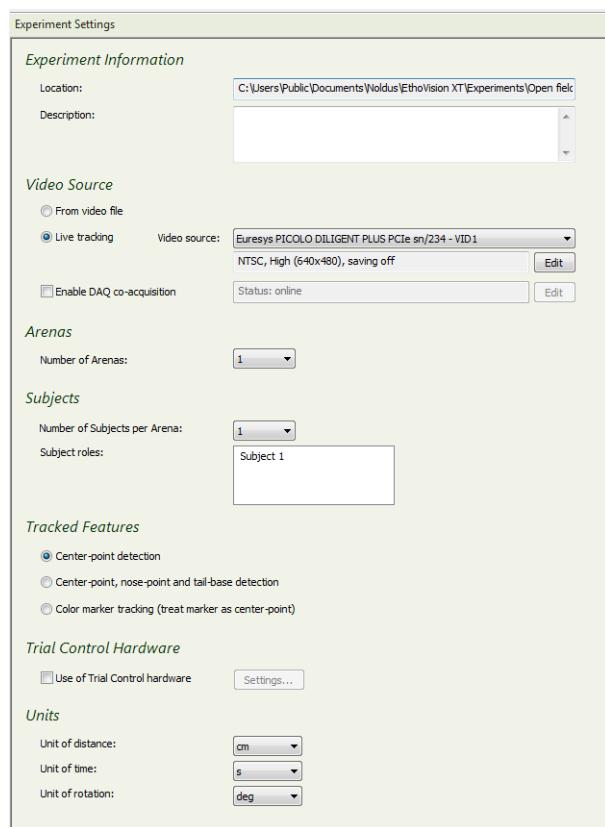


Figure 5.2. The default **Experiment Settings**.

Experiment Information - Location

The location where your experiment is stored on your computer. The default location is (unless you changed it during installation or in the Preferences):

Chapter 5

- For **Windows XP** - C:\Documents and Settings\All Users\Shared Documents\Noldus\EthoVision XT\Experiments.
- For **Windows 7** - C:\Users\Public\Public Documents\Noldus\EthoVision XT\Experiments.



You can change the default location for experiments by selecting **Preferences** from the **File** menu (see page 127).

Experiment Information - Description

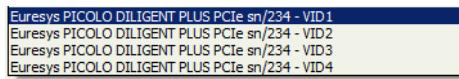
In the **Description** field you can enter information about your experiment such as the date or time of the year of the study, the kind of test that you perform, the scientific research question that you aim to answer with this study, etc. You can edit the description at any time, even after you have acquired tracks.

Limitations - The characters you use must be ASCII characters in the range between 32d and 255d. See page 106 for more information about ASCII characters.

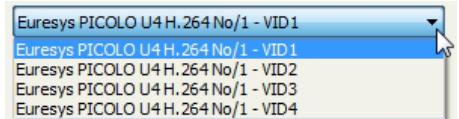
Video Source

- **From video file** - See Appendix B for information about what video file formats you can use in EthoVision XT.
- **Live tracking** - You can only select live tracking if a frame grabber is installed in your computer or if you use a FireWire camera. If you ordered a computer from Noldus Information Technology when you purchased EthoVision, it comes with a Picolo Diligent frame grabber board or a Picolo U4 H264 frame grabber board.
 - i Connect your video camera to the frame grabber. If you want to save your live video to a digital video file, you must connect your camera to two video inputs on the frame grabber board. See page 42 for more information about setting up your hardware. If you use a FireWire camera, connect it to a FireWire port on your computer. When using a FireWire camera, you can only track live, not save your live video to a digital media file. See page 48 for configuring a FireWire camera.
 - ii From the **Video Source** list, select the frame grabber input to which you connected your video camera, or the name of the FireWire camera. If you connected your camera to two video inputs on the frame grabber board, select either one of them.

Setting up an experiment



or



- iii Click **Edit** on the right of the field below the **Video source** field. The **Video Source Settings** window opens (see Figure 5.3 and Figure 5.4).

Analog camera

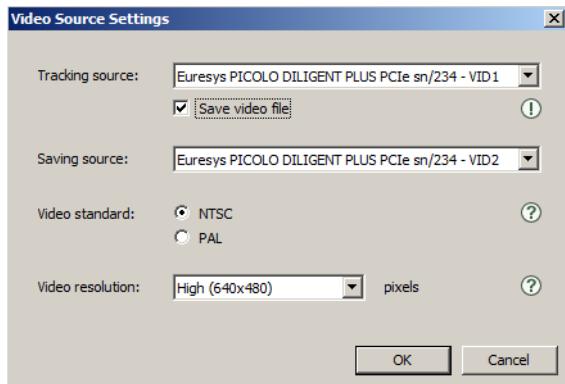


Figure 5.3. The **Video Source Settings** window for an analog camera.

- Save video file** – Select this check box if you want to save your live video to a digital media file. In the **Saving Source** field, select the video input that you want to use for recording. If you connected your camera to BNC connectors 1 and 2 of the frame grabber, and you selected connector 1 in step 2, then you must now select connector 2.

Video standard – Select the TV standard of your camera. You can find this in the specifications section of your equipment's manual. It will probably be the standard used in your country.

- PAL** – Western Europe, China, Indonesia and Australia.
 NTSC – United States, Canada and Japan.

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Video resolution – The number of pixels into which the frame grabber divides the video image.

High – 768x576 (PAL) and 640x480 (NTSC).

Medium – 384x288 (PAL) and 320x240 (NTSC).



For single arenas and subjects more than a couple of centimeters long, Medium resolution is sufficient.



When you choose to save your video to a digital video file and record for a long period of time, the files can get quite big. The higher the video resolution, the bigger the files. In this situation it may be better to choose **Medium** resolution instead of **High** resolution.

FireWire camera

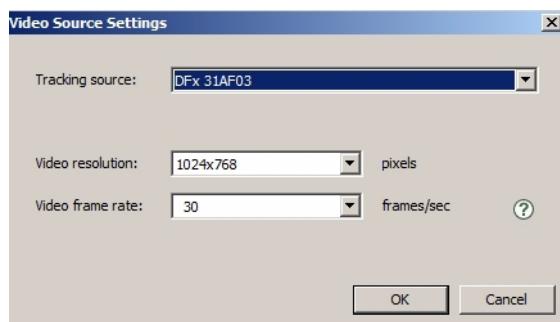


Figure 5.4. The **Video Source Settings** window for a FireWire camera (ImagingSource).

Video resolution – Setting the video resolution is possible if you use a Med Associates Basler camera. If you use an ImagingSource camera there is only one video resolution available. The video resolution determines the maximum arena size that you can use (see page 57).

Video frame rate – High frame rates require more processing power. Select a high frame rate only if your subjects can move fast. We recommend to set the frame rate to the same value as the sample rate in the Detection Settings (see page 223).



Please realize that if you use a high frame rate or resolution, this reduces the number of animals you can track simultaneously.

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Please check the values of the Independent Variables 'Missed samples' and 'Subject not found' to get an indication of the quality of tracking.

When you use a FireWire camera, you can only track live, not save your live video to a digital media file.

- Enable DAQ co-acquisition** – Select this check box if you want to co-acquire external data with a separate Data AcQuisition (DAQ) system. You can co-acquire, for example, physiological data together with the track data. See Chapter 10 for more information about co-acquiring external data and page 299 for the procedure to specify external data co-acquisition settings.



This option is only available when you have selected **Live tracking** as your video source AND you have the **Physiology** module.

Number of Arenas

With the Base version of EthoVision XT you can track one subject in one arena. With the **Multiple Arenas** module you can track in up to 100 arenas simultaneously.



If you want to use more than one arena, first check that no samples are missed during acquisition before deciding to work with live tracking. To display statistics on missing samples during acquisition, see page 270 and page 279.

Number of Subjects per Arena

Select the number of subjects that are present in each arena during a trial. With the Base version of EthoVision XT you can track one subject per arena. With the **Social Interaction** add-on module, you can track up to 16 subjects per arena.

Under **Subject roles**, enter the name of the subjects or accept the default ones (**Subject 1**, **Subject 2**, etc.). To modify the Subject's role name, right-click a name, select **Rename** and type in the new name. Enter generic names (for instance, "Control" and "Treated"), not the ID of the subjects you are going to test.

Control
Subject 2

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Tracked features

- **Center-point detection** – Tracking only the point mathematically in the center of the subject.
- **Center-point, nose-point and tail-base detection** – Tracking the nose-point may be of interest in, e.g., a novel object test to determine how often and how long your subject touches the object. See page 258 for more information about nose-tail detection.
 - To be able to use nose-tail detection, you need the **Multiple body points** module.
 - Select this option also when you track multiple rodents marked with colors.
- **Color marker tracking (treat marker as center point)** – EthoVision will only detect the part of the subject's body marked with color. Use this method for social interaction tests where you do not need to track nose and tail base.
 - This method is equivalent to color tracking in EthoVision 3 and can be applied to all animals.
 - For rodents, select **Center-point, nose-point and tail-base detection** if you use color-marked animals and you want to track the three body points.

Trial Control Hardware

If you plan to control hardware devices with EthoVision XT, select this option and click **Settings**. Select either **Noldus USB-IO Box** or **Noldus Mini USB-IO Box** and click **OK**.



Check the label at the bottom side of the IO-box to see what type of box you have: PTIO-0020 (Noldus USB-IO Box) or PTIO-0030 (Noldus Mini USBIQ Box). If you use a DanioVision system, select Noldus Mini USB-IO Box.



For more information, see **Controlling hardware devices** in the **EthoVision XT Trial and Hardware Control Manual** on your installation DVD.

Units

Specify the units for distance, time and rotation:. Changing the units does not affect the raw data, only their presentation. You can change the displayed units at any time.

- **Unit of distance** – EthoVision measures the distance between two points in pixels. To convert these to real values you must calibrate your arena. Here, you

Setting up an experiment

can choose what unit of distance you prefer: millimeters (mm), centimeters (cm), meters (m) or inches (inch).

- **Unit of time** – Milliseconds (ms), seconds (s), minutes (min) or hours (hr).
- **Unit of rotation** – Degrees ($^{\circ}$), radians (rad), gradians (grad) or rotations (rot).

You can change the units after you have acquired tracks. This only affects the presentation; the data are always correct.



Changing the units only affects the present experiment. If you want to change the default units for future experiments, choose **Preferences** from the **File** menu. Click **Default units** and select the units you want.

Settings of units are saved on your computer. If you open another EthoVision XT experiment from a colleague, numbers will be displayed according to your settings, regardless of the settings your colleague used. For example, your colleague tracked an animal moving one inch; on your computer it is shown as 2.54 cm.

5.3 Trial List



In the **Trial List** you can:

- Define your independent variables – See page 105.
- View the system variables – See page 113.
- List the trials that you plan to carry out – See page 117.

To open the **Trial List**, do one of the following:

- From the **Setup** menu, choose **Trial List**
- In the **Experiment Explorer**, click **Trial List**.

Showing/hiding variables

If you do not want to have all the variables visible in the **Trial List**, you can customize the list in one of the following ways:

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- Click the **Show/Hide** button  in the component tool bar and select **Variables**. The **Show variables** window appears (see Figure 5.5 for an example).
Select the variables of your choice and click **Apply**. By default, only one system variable is visible (**Acquisition status**).
- Right-click the appropriate column header and choose **Hide variable**.

Sorting columns

You can change the order of the columns. Click the column header to select the column of your choice and then drag it to the new position. While you drag it, a red line shows the insertion point of the column.

Showing/Hiding Row Headers

If you do not want to display all Row Headers (Trial, Arena, Subject, row no.) in your Trial List, you can customize the list in one of the following ways:

- 1 Click the **Show/Hide** button  in the component tool bar.
- 2 Select **Row Headers** and the headers you want to display.

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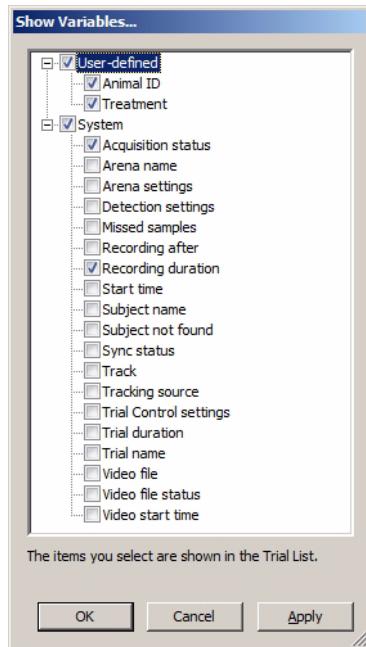


Figure 5.5. The **Show variables** window. In this example, two independent variables have been defined (Animal ID and Treatment).

Defining your independent variables

Independent variables are variables that you define to record specific experiment conditions. For example, you can define the identity of your subjects, name of the treatment or environmental conditions.

An independent variable is a variable that potentially determines the value of a dependent variable (such as speed of movement or distance moved and (in EthoVision) stays constant throughout a data acquisition session.

To define your independent variables:

- 1 Open the **Trial List** (see page 103).

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- 2 Click the **Add Variable** button. A new column is inserted allowing you to specify the label (name), description, type, format, predefined values and scope of the variable. See Figure 5.6 for an example of a **Trial List** with three independent variables.

Trial List			
		Add Trials... Add Variable	
Label		System	System
	Start time	Acquisition status	Rat ID
	The start time of the trial	The current status of acquisition per arena	
	Time stamp		Numerical
	dd-MM-yyyy HH:mm:ss	x	Text
		Unknown; Postponed;	drug; saline
	Trial	Arena	Subject
Trial		Subject	Subject
Trial 1	Arena 1	Subject 1	1
Trial 2	Arena 1	Subject 1	2
Trial 3	Arena 1	Subject 1	3
	13-11-2009 16:29:00	Acquired	1 drug
	13-11-2009 17:11:46	Acquired	2 saline
	16-11-2009 11:38:16	Acquired	3 drug

Figure 5.6. An example of a **Trial List** with thee independent variables (**Rat ID** and **Treatment**).

Variable label

In the **Label** field you can enter the name of the variable. The name must be unique.

Limitations – Names cannot be longer than 64 characters. The characters you use, must be ASCII characters in the range between 32d and 255d.



ASCII characters - How do I know whether I can use a specific character?

If you want to check whether a specific character is allowed, you can look it up in the **Character Map**. From the **Start** menu on the Windows task bar, choose **Accessories**, subsequently **System Tools** and finally **Character Map**. When you click on a character, its ASCII code appears in the lower right corner of the **Character Map**.

For instance, you are a German researcher and want to use 'Ratte Identität' (rat identity) as the name of your independent variable, but you are not sure whether you can use the character ä. When you look in the **Character Map**, you will see that the ASCII code for ä is 228, which means that ä is a valid character in an independent variable name.

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Variable description

In the **Description** field you can enter a short text or any other background information about the variable.

Limitations – The description is limited to 255 characters. The characters you use, must be ASCII characters in the range between 32d and 255d. See above for more information about ASCII characters.

Variable type

Click the **Type** field and from the drop-down list choose one of the following options:

- **Text** – A text variable is denoted by alphanumeric characters, composed of letters, numbers or both. For example, the name of the treatment.
- **Numerical** – A variable represented by numbers only, for example, the dose of the drug that you apply.
- **Time stamp** – A variable represented by a time stamp, for example, the start date and time of the trial.
- **Duration** – A variable represented by a duration, for example the duration of the treatment.
- **Boolean** – A variable that is either 'False' or 'True'. For example, the presence of a novel object.

Variable format

- For **Text** variables you do not need to specify a format.
- For **Numerical** variables:
 - i Double-click in the **Format** field. The **Format** window appears (Figure 5.7).

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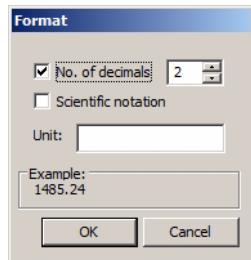


Figure 5.7. The **Format** window for numerical variables.

- ii Select the **No. of decimals** check box and choose the number of decimals (or leave the check box unselected if your values have zero number of decimals). The maximum number of decimals is 9.
 - iii Select the **Scientific notation** check box if you want to write, for example, 1485 as 1.485e+0.03
 - iv In the **Unit** field, enter the unit of your variable, for instance, $\mu\text{g/g}$ for the dose of the drug. Then click **OK**. **Tip:** Use the Characters map (page 106) to copy Greek symbols.
- For variables indicated by a **Time stamp**:
- i Click in the **Format** field. The **Format string** window appears (Figure 5.8).
 - ii Enter your preferred format in the field at the top and click **OK**.

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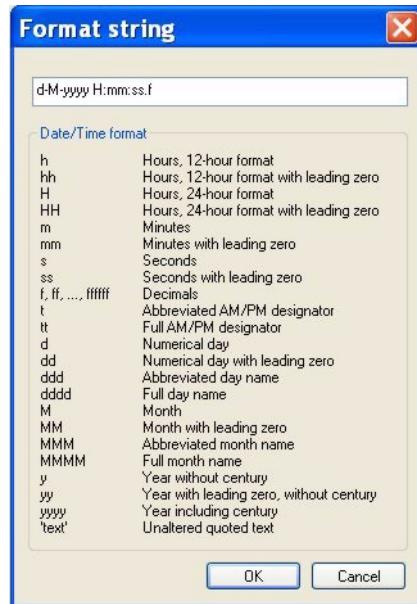


Figure 5.8. The **Format string** window for **Time stamp** variables.

- For variables indicated by a **Duration**:
 - i Click in the **Format** field. The **Format string** window appears.
 - ii Enter your preferred format in the field at the top and click **OK**.

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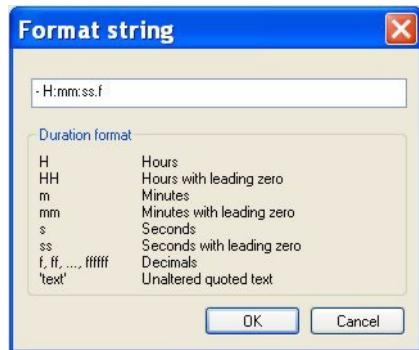


Figure 5.9. The **Format string** window for **Duration** variables.

For **Boolean** variables you do not need to specify a format.

Predefined variable values

- For **Text** variables:

- i Double-click in the **Predefined Values** field. The **Predefined Values** window appears (see Figure 5.10 for an example).
- ii In the **Predefined Value** field enter a value for your independent variable. For instance, if your independent variable is the treatment you apply, you type the name of the drug. The characters you use must be ASCII characters in the range between 32d and 255d (see page 106 for more information about ASCII characters).
- iii Click **Add**. The value that you have entered moves to the **Predefined Values** field. You can now, for instance, enter the name of the substance that the control group receives.

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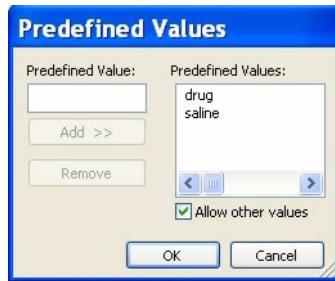


Figure 5.10. The **Predefined Values** window for text variables. The example shows the predefined values for the variable 'Treatment'.

- iv Keep the **Allow other values** check box selected if you are not sure whether the values that you defined are exhaustive. This allows you to add new values if needed. Clear this option if you are sure that you do not want to add new independent variable values.
- v Click **OK** when you have entered all the values of your independent variable.

To delete a predefined value, select it in the **Predefined Values** field and click **Remove**.

- For **Numerical** variables:

- i Double-click in the **Predefined Values** field. The **Predefine Numerical Values** window appears (see Figure 5.11). Select one of the following options:
 - Allow any value** – Select this option if you do not want to pre-define a range of values or individual values and click **OK**.
 - Define a range** – Define a minimum and maximum value for your independent variable. For instance, when the age of the rats in your experiment ranges from 6-18 months, enter '6' as the minimum value and '18' as the maximum. Then click **OK**.
 - Define individual values** – Select this option when you are, for instance, testing a drug in three dosages: 0.05; 0.1 and 0.5 mg/g. Enter '0.05' in the **Predefined Value** field and click **Add**. The value moves to the **Predefined Values** field. Repeat this procedure for the other two values and click **OK**. Keep the **Allow other values**

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check box selected if you are not sure whether the values that you defined are exhaustive. This allows you to add new values if needed. Clear the check box if you are sure that you do not want to add new independent variable values. To delete a predefined value, select it in the **Predefined Values** field and click **Remove**.

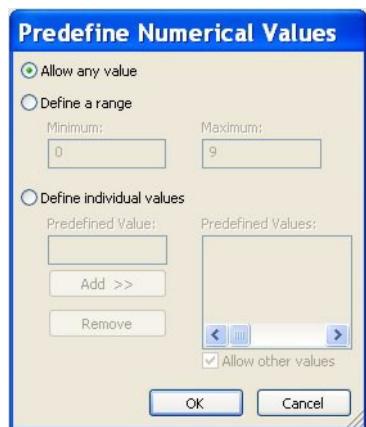


Figure 5.11. The **Predefine Numerical Values** window.

- For **Time stamp** and **Duration** variables you cannot specify any predefined values.
- For **Boolean** variables:
 - i Double-click in the **Predefined Values** field. The **Predefine Boolean** window appears (see Figure 5.12 for an example).
 - ii Enter values for 'True' and 'False'. For instance, if your independent variable is 'presence of novel object', you may define the values 'Yes' (True) and 'No' (False).

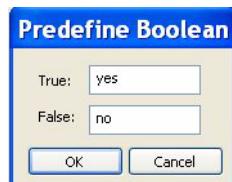


Figure 5.12. The **Predefine Boolean** window.

Variable scope

For the scope of an independent variable you can choose between the following three options:

- **Trial.**
- **Arena.**
- **Subject.**

For example, the scope of the variable 'bedding type' is **Trial** if all the arenas in a trial have the same kind of bedding material. Select **Arena** if the bedding material differs for different arenas. Select **Subject** for those variables that are related to the subject, for instance, subject ID, sex or age.

Deleting independent variables

You can delete an independent variable by right-clicking the column header and choosing **Delete variable**.



You can only delete variables that you defined yourself. It is not possible to delete system variables (although you can hide them).

Viewing system variables

System variables are variables that EthoVision records during acquisition. By default, only one system variable is visible in the **Trial List (Acquisition status)**. To view the other system variables, click the **Show/Hide** button in the components tool bar, select **Variables** and select the variables you want to view. To hide them, clear their check box. EthoVision records the following system variables:

- **Acquisition status** - The current status per arena:
 - **Planned** – When a trial is added to the **Trial List**, all the arenas within the trial get the status **Planned**.
 - **To skip** – You can give an arena the status **To skip** when for some reason you do not (yet) want to carry out acquisition in this arena.
 - **Postponed** – When you have saved your live video to a digital video file, and you did not start acquisition simultaneously, all the arenas in your planned trial get the status **Postponed**.

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- **Waiting** – An arena gets the status **Waiting** when you have started the trial but acquisition did not yet start because you defined a **Trial Control profile** with a **Start recording** rule and the starting condition is not yet met.
- **Acquiring** – An arena gets the status **Acquiring** when acquisition is ongoing.
- **Acquired** – An arena gets the status **Acquired** when acquisition is done.
- **Arena name** – The name of the Arena in which the animal was tracked.
- **Arena Settings** – The name of the **Arena Settings** that you used to acquire your tracks. See page 132 for more information about **Arena Settings**.
- **Detection settings** – The name of the **Detection Settings** that you used to acquire your tracks. See Chapter 8 for more information about Detection Settings.
- **Missed Samples** – Proportion of skipped samples. EthoVision misses samples if the processor load is too high. See also page 227.
- **Recording after** – The length of the interval between the start of the trial and the start of acquisition. This variable has the value 0 unless you defined a **Start recording** rule in your **Trial Control profile**.
- **Recording duration** – The length of the interval from start of acquisition till stop of acquisition. **Recording duration** is equal to **Duration** (see above) unless you defined a **Start** recording rule in your **Trial Control profile**.
- **Start time** – The date/time of the start of the trial.
- **Subject name** – The name of the subject that track refers to. Subject names are specified on page 101.
- **Subject not found** – Proportion of subject not found. If the subject is not found, it means EthoVision processed the sample but did not find anything matching the current Detection Settings. Therefore, this system variable is a measure of the quality of detection.
- **Sync status** – The status of the sync-out file. When no external data co-acquisition has been carried out for a specific trial, the status is **Planned**. As soon as co-acquisition is started on the DAQ system, the status becomes **Acquired**.
- **Track** – The name of the track file.
- **Tracking source** – The video input on the frame grabber to which you connected your video camera (Euresys PICOLO DILIGENT sn/219 - VID1, 2, 3 or 4). If you use a FireWire or USB camera, this is the name of your camera. No tracking source is mentioned when you track from a media file.

Setting up an experiment

- **Trial Duration** – The length of the interval from start of the trial until the stop of the trial.
- **Trial control settings** – The name of the **Trial Control profile** that you used during tracking. See Chapter 7 for more information about defining Trial Control.
- **Trial name** – By default, Trial 1, Trial 2, etc. To change a trial name, double-click it and enter a new name.
- **Video file** – The name and location of the video file that was used for acquisition. **Arena name** – This is the name that you specified when you defined the arena (see page 141).
- **Video file status** – The video file status can be one of the following options:
 - **Generated** – When you save your live video to a digital media file, the video file gets the status **Generated**.
 - **Planned** – This is the video file status when you selected **From video file** as your video source and data acquisition has not yet taken place.
 - **External** – When you track from a video file that was not recorded with EthoVision, the video file status becomes **External**.
- **Video start time** – The date and time the video file that you used for tracking, was created.

System variable format

For the **Time stamp** variables (**Start time** and **Video start time**) and **Duration** variables (**Recording after**, **Recording duration** and **Trial Duration**) you can specify the format.



The **Label**, **Description**, **Type**, **Predefined Values** and **Scope** field are not available for editing.

- For the **Time stamp** variables:
 - i Click in the **Format** field. The **Format string** window appears (Figure 5.13).
 - ii Enter your preferred format in the field at the top and click **OK**.

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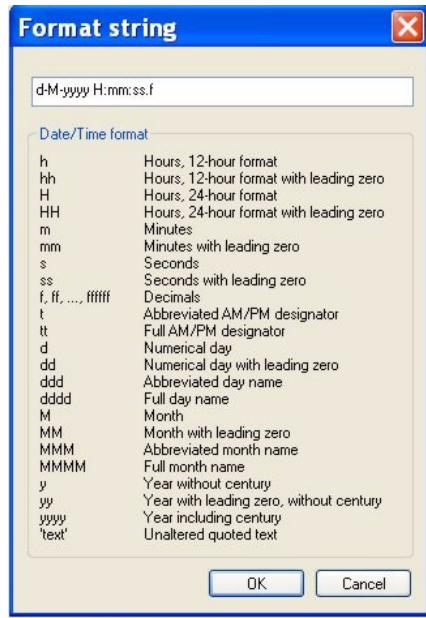


Figure 5.13. The **Format String** window for **Time stamp** variables.

- For the **Duration** variables:

- i Click in the **Format** field. The **Format string** window appears (Figure 5.14).

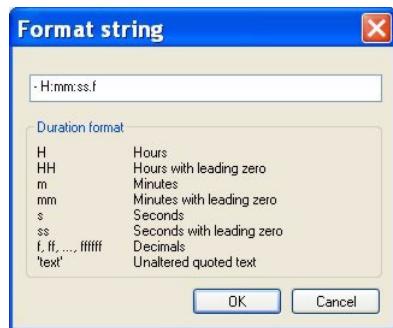


Figure 5.14. The **Format string** window for **Duration** variables.

- ii Enter your preferred format in the field at the top and click **OK**.

5.4 Preparing the list of trials

EthoVision XT gives you the possibility to list your experimental trials with all the important information for your study, for example the start time of the trial, the ID number of the test subject, the amount of drug to be injected, the video file name, etc. This list is called **Trial List**.



We assume at this point of the manual that you have defined all the variables important for your study in the **Trial List** (page 103).

Why use a pre-defined list of trials?

Planning the trials in the **Trial List** allows you to prepare your experimental trials in detail and in advance, so that fewer action have to be taken during their execution, minimizing the chances that mistakes are made. For example, if you fill in the Trial List with the ID number of the test subjects and drug amount planned for each trial, when you start data acquisition the program will inform you that for Trial 1 you need to prepare subject A21034 which has to be injected with 1.0 mg/kg apomorphine.

If you do not pre-define a trial list, it is built up automatically as you carry out the trials.

Planning your trials in the Trial List

1 Make sure that your experiment is open.



2 From the **Setup** menu select **Trial List**, or in the Experiment Explorer click **Trial List**.



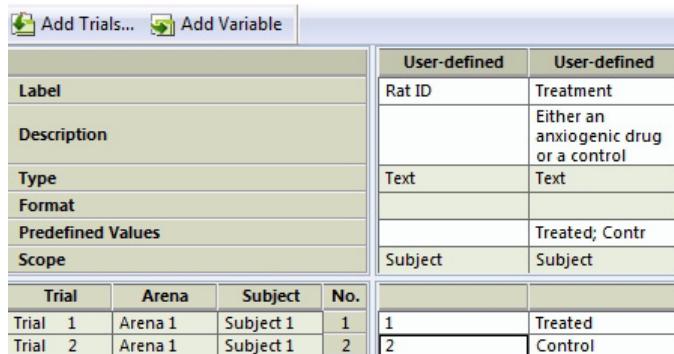
3 To add trials, click the **Add Trials** button in the components tool bar.

4 Type the number of new trials you want to add, then click **OK**.

Result – A number of rows appear in the lower half of the table. Each row represents a trial, that is, an acquisition session to be carried out in one or more arenas. If your setup is made of multiple arenas, each trial is divided in

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multiple rows, each one representing a track, that is, data of one individual subject tracked in a single trial (see the example below).



Label			
Description			
Type			
Format			
Predefined Values			
Scope			
Trial	Arena	Subject	No.
Trial 1	Arena 1	Subject 1	1
Trial 2	Arena 1	Subject 1	2

User-defined	User-defined
Rat ID	Treatment
	Either an anxiogenic drug or a control
Text	Text
	Treated; Contr
Subject	Subject

1	Treated
2	Control

- 5 For each planned trial, enter the value of the variables in the corresponding cells, when required.

You can also enter the variable values immediately before acquiring the data. For more information, see page 283.

Copying the Trial List

You can copy and paste (part of) a Trial List:

- **To/from Excel** – You can copy a Trial List from Excel, for example, when you have made your list of independent variables and its values in Excel.
- **Within the same Trial list** – You can copy and paste part of Trial List to newly added cells, for example, when you have added new trials.

To copy and paste cells:

- 1 Select one or more cells in the Trial List or in the Excel worksheet.
- 2 Right-click one of the selected cells and select **Copy** or press **Ctrl+c**.
- 3 Click a cell in the Trial List or in the Excel worksheet and either right-click and select **Paste** or press **Ctrl+v**.

Important notes



- It is not possible to paste a cell with a non-numerical independent variable to a cell with a numerical variable. If you do that, an empty cell is pasted.
- Check that the copied values of numerical independent variables are either within the predefined range or correspond to individual predefined values of numerical independent variables in the Trial list.
- The upper half of the table shows information on each variable (its name, description, type etc.). For more information, see page 103.
- To show a system variable, click **Show Variables**. To add a new user-defined variable, click **Add Variable**. For more information on showing/adding variables, see page 103.
- All system variables are recorded by the program, regardless of whether you choose to view them in the Trial List.
- If trials are already present in the table, the trials you add are appended.
- You can change the order of variables by selecting a column and dragging it to the new position.
- Filling the **Trial List** table is not mandatory. You can also enter those values immediately before or after acquiring each trial (see page 283).
- The **Acquisition status** variable refers to the status of the tracks, while the **Video file status** variable refers to the video files recorded and used to acquire tracks. When you plan your trials, you cannot change the Video file status. This is done automatically when you record video or track data from that video. For more information on Acquisition and Video file status variables, see page 113.

Tips

- To delete a trial, right-click the trial name in the table and select **Delete Trials**. To delete multiple adjacent trials, click the first trial of the range, hold down **Shift** and click the last row, then right-click and select **Delete Trials**. To delete nonadjacent trials, click the first trial, hold down **Ctrl** and click the other trial names, then right-click and select **Delete Trials**.
- It may happen that you cannot acquire a planned trial because of unforeseen circumstances. You may skip tracks and acquire data for that subject later. If you want to skip one or more tracks, make sure that the **Acquisition status** variable is shown (click **Show Variable** in the table, and select **Acquisition status**), then in the **Acquisition status** column select **To Skip** for the corresponding track.

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Trial 6	Arena 1	Subject 1	21	A21033	Planned
	Arena 2	Subject 1	22	A21034	Planned
	Arena 3	Subject 1	23	A21036	To Skip
	Arena 4	Subject 1	24	A21037	Planned

Note that when you track subjects in multiple arenas it is possible to skip some tracks, not other within the same trial. For example, skip track for subject **A21033** because the animal is sick (see the picture above). The trial starts anyway and the remaining animals will be tracked.

- If a track (or trial) is skipped, you can change its status back to **Planned** in the **Trial List** table until the trial is acquired.

5.5 Manual scoring settings

EthoVision is software for automatic video-tracking, however you can also use it to record behavior of your subjects that cannot be detected automatically, for example, pouncing or fighting. You score behaviors and events by pressing a pre-defined key or clicking an appropriate button on the screen as soon as the behavior or event occurs. Data are stored in a special *Manual scoring log* associated with the track data.

In the **Manual Scoring Settings**, you define the behaviors that you want to record manually. These can be defined independent of each other (in that case they are named **Start-stop**) or grouped in such a way that when you score one of those behaviors, the behavior that is currently active is automatically ended (in that case they are named **Mutually exclusive**).



Notes

- If your setup includes multiple subjects and/or multiple arenas, you can score the behavior of a subject in a specific arena with one key.
- You can only define one key for each behavior. For example, "G", not "Gr" for grooming.
- If you record video and track later (see page 290), you can only record events when you track data, not during video recording.
- If you are familiar with The Observer XT software, the manual scoring settings in EthoVision XT is a simplified Observer **coding scheme**. In EthoVision XT you can only define *state behaviors*, that is, behaviors that have a duration

Setting up an experiment

(Mutually-exclusive or Start-stop). You can import manually scored data from EthoVision XT to The Observer XT for further analysis and editing (see page 495).

- It is not possible in EthoVision XT to score behaviors using the “press-and-hold-down” method (that is, press a key when a behavior starts, keep the key pressed for as long as the behavior occurs, and release the key when the behavior stops). However, Noldus can provide a separate, programmable event logging keyboard so you can manually score behaviors in EthoVision XT using the “press-and-hold-down” method.

Defining behaviors

- To open the Manual scoring settings, do one of the following:

- From the **Setup** menu, select **Manual Scoring Settings**.



- In the Experiment Explorer, click **Manual Scoring Settings**.

The **Manual Scoring Settings** screen appears.

A screenshot of the 'Manual Scoring Settings' window. At the top, there's a toolbar with a 'Add Behavior...' button. Below it is a table with the following columns: 'Behaviors' (which is a header), 'Keys' (another header), 'Behavior Name', 'Type', 'Description', 'Initially Active', and 'Subject 1'. The table is currently empty.

Behaviors		Keys		
Behavior Name	Type	Description	Initially Active	Subject 1

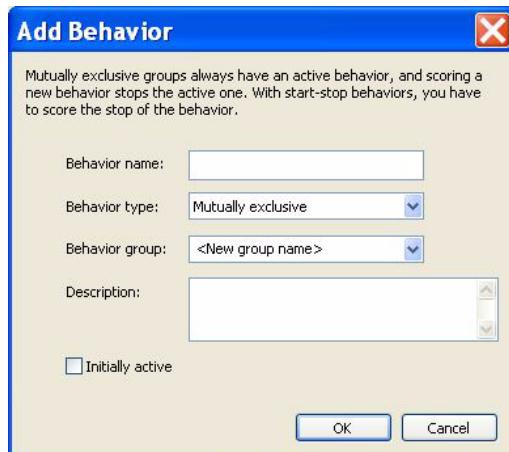
The **Manual Scoring Setting** screen shows the headers of the table that will list the behaviors of your coding scheme.



Think in advance how the different categories of behavior relate to each other. You may for example want to score different types of grooming (*Unilateral stroke*, *Bilateral stroke* etc.). Each type will be a behavior, however these are best organized in a **group**. If you plan to score behaviors that do not relate to each other (for example *Rearing* and *Drinking*), they do not need to be defined in a group.

- Click the **Add Behavior** button at the top of the table. The **Add Behavior** window appears.

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- 3 In the **Behavior name** field, type in the name to the behavioral category you want to score.
- 4 From the **Behavior type** list, choose the type: **Mutually-exclusive** or **Start-Stop**.
 - **Mutually-exclusive** means that the behavior will be part of a group, where one item, when scored, automatically switches off the item currently active. For example, you define a group of two behaviors, *Grooming Head* and *Grooming Body*. When the subject grooms its body, score this behavior. *Grooming Body* is now **active**. As the subject grooms its head, score *Grooming Head*. This state is now active, while *Grooming Body* is deactivated (stopped) automatically.
 - **Start-Stop** means that scoring that behavior is fully independent of other behaviors. To stop that behavior you must press the Stop key (see below).
- 5 If in the previous step you have chosen **Mutually-exclusive**, then the **Behavior group** list becomes available. Type in the name of the group the behavior belongs to, or, if you have already defined it, select it from the list.
If you have chosen **Start-stop**, the behavior cannot be part of any group, and therefore the **Behavior group** list is not available. Go to step 6.
- 6 Enter a **Description** (optional) for the behavior.
- 7 If you think that the behavior is active at the start of the trial, select the **Initially active** option.

Setting up an experiment



For mutually-exclusive behaviors, It is always a good idea to define a default behavior that can be scored when the subject does not show any significant behaviors. Choose this behavior as **Initially active**.

- 8 Click **OK**. The behavior is listed in the table, under the group it belongs to.

Behaviors					Keys
Behavior Name	Type	Description	Initially Active	Subject 1	
Grooming					
Unilateral stroke	Mutually exclusive		<input type="checkbox"/>	u	
Bilateral stroke	Mutually exclusive		<input type="checkbox"/>	b	
No grooming	Mutually exclusive		<input checked="" type="checkbox"/>	0	

Note that:

- Names of groups and start-stop behaviors are displayed in a grey row.
- Names of mutually-exclusive behaviors are displayed in a white row under the corresponding group name.

- 9 In the cell under **Keys**, select the keyboard key that you want to use for scoring that behavior.



It is not required to use key codes. Without key codes defined, you can manually score events using the mouse.

- Select keys that you can press without taking your eyes off the keyboard (for example A, S, D) rather than keys which are easy to remember. If your scoring settings contain so many behaviors that you cannot remember the keys, it is probably too complex.
- For each cell you can enter one key, among those available **a...z**, and **0...9**.
- If your setup includes multiple subjects and arenas, choose a key for each arena-subject-behavior combination.
- For **Start-Stop** behaviors, choose a key to Start the behavior and a key to stop it.
- This list shows all keys available, excluding keys already in use. For **Start-Stop** behaviors, you can select the same key for both starting and stopping a behavior.

Notes



- Names** – Behavior names and group names must be no more than 64 characters long. The characters you use for names must be ASCII characters in the range between 32d and 127d.

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If your computer works with English language settings, you can also use characters in the range between 128d and 255d. To enter a specific character, open the character map – from the Windows **Start** menu choose **All Programs**, then **Accessories**, subsequently **System Tools** and finally **Character Map**. When you click on a character, its ASCII code appears in the lower right corner of the **Character Map**. In EthoVision XT, press and hold **Alt** and type the ASCII code you find on the Windows Character map.

- **Initially active** – Within a mutually-exclusive group, only one behavior can be defined as initially active. If one of the behaviors of a Mutually-exclusive group is already selected as **Initially active** and you check the same option in the **Add Behavior** window for a newly-created behavior, the program asks you whether you want to remove that option for the existing behavior. Click **Yes** to do so. If you click **No**, you return to the dialog, so you can clear the **Initially active** option.
- **How many behaviors?** – You can define as many behaviors as you need. However, since the number of available keys is 36, we advise you to limit the number of arena-subject-behavior combinations to 36, so you can assign a keyboard key to a behavior of a specific subject in a specific arena. If you need to define more combinations, do so without assigning a key code. You can score those behaviors by clicking the corresponding button on the acquisition screen.

Because you will be scoring live, keep the scoring settings as simple as possible, with few behaviors. If you try to score many complex behaviors at once then you may make mistakes.

 - For scoring behaviors, see page 299.
 - For analyzing behaviors, see page 555.
 - For exporting behaviors to The Observer XT, see page 495.

Editing the manual scoring settings



After acquiring at least one trial, the defined behaviors become read-only. See at the end of this section for more information.

Renaming a behavior or behavior group

Click the name of the behavior or behavior group, and make the necessary changes and press **Enter**.

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Modifying the key code

Click the cell under **Key** for a behavior, and type in the new key code, or select another key.



If the new key you want to use is assigned to another behavior, it is not available in the **Keys** list. Pressing that key on your keyboard makes no change in the selected cell.

To delete a key code, click the corresponding cell and press **Backspace**.

Editing the behavior properties

Double-click the behavior row or right-click and select **Edit Behavior**. Make the necessary changes and click **OK**.

Creating a new behavior group

To create a new behavior group, create first a behavior that belongs to that group, then from the Behavior type list select **Mutually-exclusive**, and in the **Behavior group** list enter the name of the new group.

Adding a behavior to a specific group

Right-click the behavior group row and select **Add Behavior**, type in a new name and click **OK**.

Moving a behavior from one group to another

- 1 Double-click a behavior to edit it.
- 2 In the **Edit Behavior** window, select the **Behavior group** you want to move the behavior to and click **OK**.

Deleting behaviors and behavior groups

You can delete a behavior or behavior group only after you have deleted all your trials.

Right-click anywhere on the behavior (or behavior group) row, and select **Delete Behavior** (or **Delete Behavior Group**).



If you delete a behavior group, all behaviors of that groups are removed from the table!

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Editing settings after acquiring data



After acquiring data, the **Manually Scoring Settings** screen is largely read-only to prevent your data from being invalidated by accidental editing.

However, you can:

- Add and rename a new behavior or behavior group.
- Edit the behavior's description.
- Set a behavior as Initially active. This won't modify your existing data, it will only set that behavior as initially active for the next trial.

You cannot move or delete behaviors, unless they have just been added after data acquisition. If you acquire more trials, those new behaviors cannot be deleted.

For **GLP** experiments – After acquisition, no behavior properties can be changed (including the behavior's name).

Checking the manual scoring settings

To have the program check that the coding scheme is consistent, click the **Validate** button at the top of the screen.

Manual scoring settings are valid if:

- For **Start-Stop** behaviors – A key has been selected for both start and stop, or for neither of the two. If you define a start key but not a stop key or vice versa, when leaving the manual scoring settings an error message is displayed.
- For **Mutually-exclusive** behaviors – All behavior groups contain at least two behaviors, and one is selected as "Initially active".
- All behaviors and behavior groups have unique names.



The program checks that the manual scoring settings are valid every time you leave the Manual Scoring Setting screen.

5.6 Setting preferences

EthoVision XT allows you to set preferences for:

- **Default Folders** – The default location of experiment files. See below.

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- **Warnings** – Select in what situations you want to get a warning, for instance, when you are about to delete a variable in the **Trial List**. See page 128.
- **Auto Save** – Enable or disable auto recovery and set a time interval at which you like to back-up your data. See page 129.
- **Default units** - Specify the default units for distance, time and rotation for future experiments.

To set preferences, from the **File** menu, choose **Preferences**. The **Preferences** window opens. You can set preferences by clicking the appropriate option on the left pane.

Defaults

Click the **Defaults** button to return to the default settings. For the **Default Folders** this is the default location for experiments that you specified during installation. When you click the **Defaults** button all the warnings are selected and auto save is enabled. The auto save interval is reset to 5 min.



Please note that when you click the **Defaults** button with the **Default Folders** tab open, the preferences for **Warnings**, **Auto Save** and **Default units** are also reset (and vice versa).

Setting preferences for default folders

From the **File** menu, select **Preferences** and click the **Default Folder** tab to specify a default file location for new experiments. The initially suggested location is the location that you specified during installation. For information on default file locations see page 586. You can change the default location of your new experiments by clicking the appropriate **Browse** button. Choose the location that you prefer. Then click **OK**.

If your video file is not located in the Media folder of your experiment, you can select its location under **Alternative media location**:. Click **Browse** and select the correct location.

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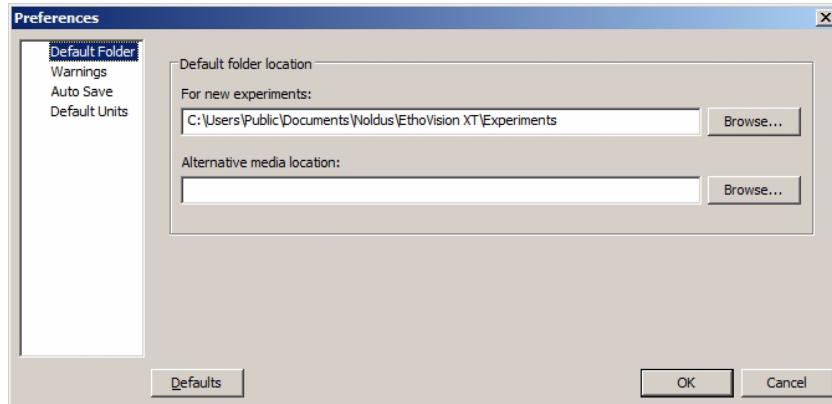


Figure 5.15. The **Preferences** window for Default Folders.

Setting preferences for warnings

From the **File** menu, select **Preferences** and click **Warnings** to select which warnings you want to view.

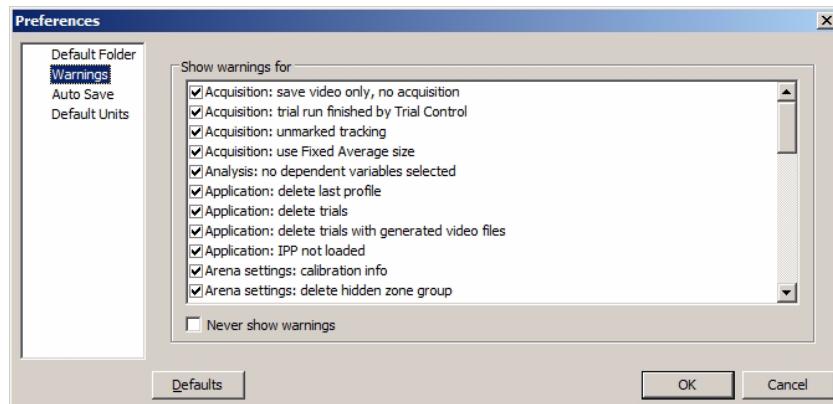


Figure 5.16. The **Preferences** window for warnings.



Please see the EthoVision XT Service Manual for an overview of all warning messages.

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Setting preferences for auto save

From the **File** menu, select **Preferences** and click **Auto Save** to enable or disable auto save and to set a time interval at which you like to back-up your data.

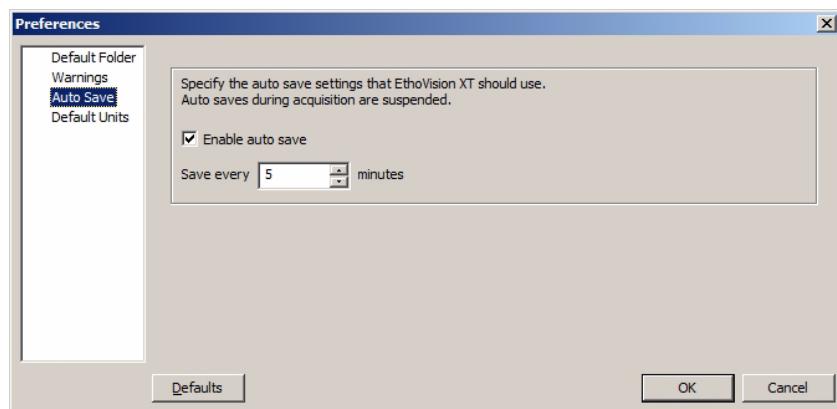


Figure 5.17. The **Preferences** window for Auto Save.

Auto save saves all the data (tracks, independent variables, detection settings, data and analysis profiles, etc.) to a temporary file. You can find this file in the folder with the same name as your experiment. By default, this is: C:\Documents and Settings\All Users\Shared Documents\Noldus\EthoVision XT\Experiments. The temporary file name starts with a "~". Your data are only saved in the experiment file when you manually save them (from the **File** menu, select **Save Experiment**).



Auto saves during acquisition are suspended to prevent auto save disturbing acquisition.

Setting preferences for default units

Specify the default units for distance, time and rotation for future experiments. Changing the default units in the Preferences does not affect the present experiment. If you want to change the units in your present experiment, change the units in the **Experiment Settings** (see page 102). Changing the units does not affect the raw data, only their presentation.

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- **Unit of distance** – EthoVision measures the distance between two points in pixels. To convert these to real values you must calibrate your arena. Here, you can choose what unit of distance you prefer: millimeters (mm), centimeters (cm), meters (m) or inches (inch).
- **Unit of time** – Milliseconds (ms), seconds (s), minutes (min) or hours (hr).
- **Unit of rotation** – Degrees ($^{\circ}$), radians (rad), gradians (grad) or rotations (rot).

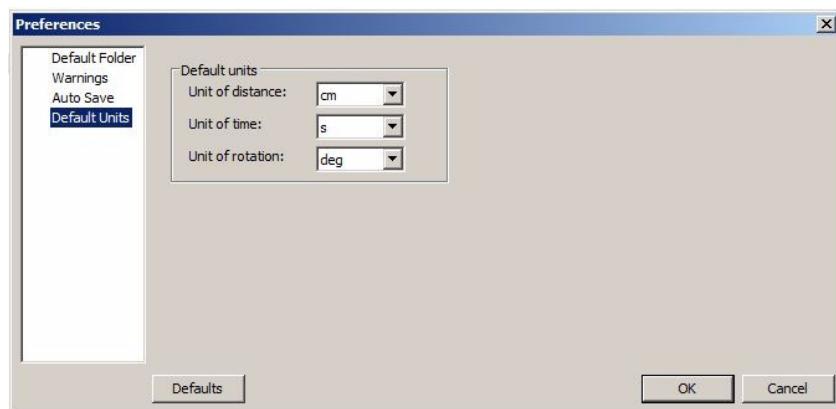


Figure 5.18. The **Preferences** window for Default Units.

6

Arena Settings

This chapter is about:

- **Arena Settings** – Contains information about the size and shape of Arenas and Zones, Points of interest, calibration and the background image.
→ See page 132
- **Arenas** – An Arena is the region on the screen in which EthoVision XT detects one or more Subjects. With the Multiple Arenas add-on, you can define up to 100 Arenas in one screen.
→ See page 133
- **Zones** - Areas of interest defined within an Arena. You can define a Zone to automatically start/stop a trial when, for example, a subject is in a particular Zone. You can also use zones to analyze, for instance, movement between particular zones. Cumulative Zones can be defined to treat separate zones as one. Hidden Zones can be used to define areas in the Arena in which the animal can disappear from view.
→ See page 146
- **Point** - A point of interest can be used to analyze the **Distance** from the animal, the **Heading** to that point or Head direction to a circle around that point (**Head direction to zone**).
→ See page 156
- **Calibration** – To the distance between two points on the screen in pixels to a 'real-world' distance. You can also specify the origin and directions of the X- and Y-axis.
→ See page 160

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- **Drawing Objects** – EthoVision provides you with a number of Drawing Objects to define Arenas and Zones.

→ See page 162

See also [Managing Settings and Profiles \(page 581\)](#).

6.1 Arena Settings

An experiment has at least one **Arena Settings**, defined in a profile called Arena Settings profile. All **Arena Settings** profiles you created are listed under the **Arena Settings** item in the **Experiment Explorer**. You can create as many **Arena Settings** profiles as you want. The definition of Arenas and Zones in the **Arena settings** are used for **Trial Control** (see Chapter 7), **Acquisition** (see Chapter 9), and **Data Selection** (see Chapter 11).

Current Arena Settings

All **Arena Settings** are shown under the **Arena Settings** item in the **Experiment Explorer**. The current Arena Settings are shown in blue. Acquisition is done using the current Arena Settings.

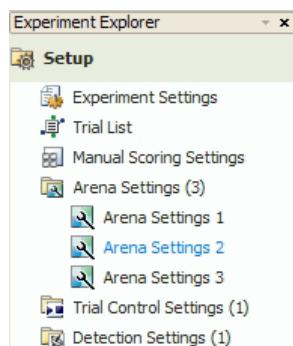


Figure 6.1. The **Experiment Explorer** with the **Arena Settings** item with three **Arena Settings**. Arena Settings 2 is the current one.

Arena Settings

Read-only Arena Settings

Arena Settings that have been used in acquisition are called 'read-only **Arena Settings**' and can no longer be edited. This is indicated by a small lock icon in the **Experiment Explorer** in front of the **Arena Settings** icon.



'Read-only Arena Settings' **cannot** be deleted, but they can be used for acquisition. Alternatively, you can make an editable duplicate of 'read-only' Arena Settings.

For more information on managing Arena Settings, see page 581.

6.2 Arenas

An **Arena** is the region in the video image on the screen in which a moving Subject is tracked by EthoVision XT. You can have one or more (up to 100) separate Arenas in one screen. If you have multiple arenas, they must not overlap.

Why use Arenas?

There are three main reasons to define Arenas:

- **To reduce information to be processed** – EthoVision XT ignores all activity outside Arenas, so only movement inside the region of interest is tracked. You should make sure that the whole region of interest is defined as an Arena, otherwise EthoVision will not be able to detect all of the subject's movement.
- **To reduce noise** – By defining Arenas you exclude parts of the screen containing unwanted movement, such as the hands of an experimenter, or system-generated movement, such as a clock in the corner of the screen.
- **To carry out multiple trials simultaneously** – If you have multiple, independent regions of interest (e.g., cages, petri-dishes) within one screen, each with its own Subject, you can define each region as an Arena, and track all animals simultaneously and separately. The tracking data from each Arena

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are stored in a separate track file and in the Analysis are treated as an independent replicate.

Defining an Arena

Defining an Arena involves the following basic steps:

- 1 Opening Arena Settings and grabbing a background image – see below.
- 2 Drawing one or more Arenas – see page 135.
- 3 Optionally, you can add Zones and Points of interest to an Arena – see page 146.
- 4 Calibrating your Arenas – see page 160.

Grabbing a background image

- 1 To open **Arena Settings**, in the Experiment Explorer, under Arena Settings, click one of the **Arena Settings**.
- 2 If you have selected “**From video file**” as **Video Source** in the **Experiment Settings**, and you only have one **Arena Settings**, the **Grab Background Image** window opens.
- 3 Click the **Browse** button, locate the video file you want to use and click **Open**.
- 4 When the **Video** window shows an unobscured, representative background, click the **Grab** button. You can move the video file to another position by using the slider in the **Video** window.

If the **Grab background Image** window does not open, click the **Grab background Image** button in the **Arena Settings** window.



When it is not possible to grab a reference image without the animal being visible, you can use the **Learn** button to create an empty arena (see page 237 of Chapter 8 Configuring Detection Settings).

If necessary, adjust the aspect ratio of the video file (see “Checking the aspect ratio” on page 136).

Arena Settings

Defining an Arena

- 1 Depending on the number of Arenas you have set in the **Experiment Settings** (see page 96), and the EthoVision XT add-on module you have, the **Arena Settings** window shows one or more Arenas (see Figure 6.2.). By default, the **Arena** covers the whole Background image.
- 2 Click one of the Arena rows; this activates the **Arena** in the **Video** window.
- 3 Select a **Drawing Object** on the **Arena Settings** component tool bar at the top of the window by clicking it or by pressing the corresponding shortcut key (see section 6.5 Drawing Objects).

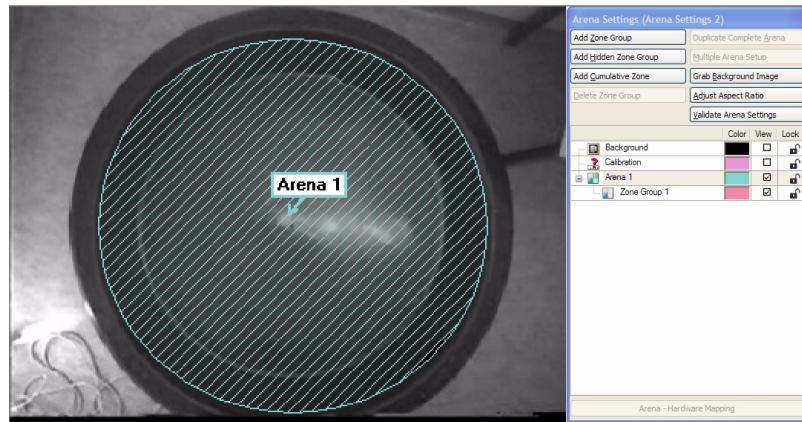


Figure 6.2. Part of the **Arena Settings** screen. In this example, a circular arena has been defined. In the Arena Settings window, the **Arena** contains one default **Zone group**.

- 4 Create a shape that covers the region on the screen in which you want EthoVision XT to track the Subject. Next, make sure the arrow point of the **Arena Label** is inside the **Arena** you just created (see also Figure 6.2.).



The shape is filled with the color visible next to the zone name in the Arena Settings window. If you want to choose a different color, click the color and pick another color. You can also change the fill color by right-clicking the label of Arena, select **Change Fill Color** and select a new color.

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Important notes



- The size of an Arena should be so that the animal always stays inside the Arena but that unwanted bright spots at the brim of the Arena are excluded.
- See page 162 for a description of how to create shapes with the **Drawing Objects**.
- If you have multiple Arenas with the same size and shape, you do not have to draw each Arena separately. You just draw one Arena and duplicate this one. See “Duplicating an Arena” on page 142 for more information.
- **Arenas** must not overlap. EthoVision XT checks for this automatically.
- You cannot delete an Arena that has been used for Acquisition; it has become ‘read-only’.



Checking the aspect ratio

The aspect ratio is the relation of the horizontal to vertical size of an image. MPEG encoders almost never record video with exactly the same aspect ratio as that of the source camera: rather, they change it, resulting in a distorted image. This could, for example, mean that a perfectly circular water maze may look ellipse-like in EthoVision XT after you grabbed a background image from the video file. This might affect the calibration and the calculation of certain variables, like the distance moved.

In some cases (often MPEG-2 files), the change in aspect ratio caused by digitizing is stored in the video file and EthoVision XT reads this aspect ratio from the file. If the video appears distorted, you can manually adjust the aspect ratio in the Arena Settings in EthoVision XT.

How do I know that the video is distorted?

- If the shape of your arena as displayed in the video image in the Arena Settings in EthoVision XT deviates from the shape that you know it should have (for example, a circular arena looks like an ellipse or a square arena looks like a rectangle), it is likely that EthoVision XT could not read the aspect ratio. See below for a description of the procedure how to manually adjust the aspect ratio.
- MPEG-4 files created with the Noldus MPEG Recorder 1.1/2.0. or the Media Recorder do not contain information about the aspect ratio. See page 137 for a description of how to manually adjust the aspect ratio for MPEG-4 files created with the Noldus MPEG Recorder 1.1/2.0.

Arena Settings

- For H.264 files created with the Media Recorder the aspect ratio also needs to be adjusted manually. See below for a description on how to adjust the aspect ratio for H.264 files created with the Media Recorder.

When you create a video file from within EthoVision XT, you do not need to adjust the aspect ratio. This is automatically done by EthoVision XT.

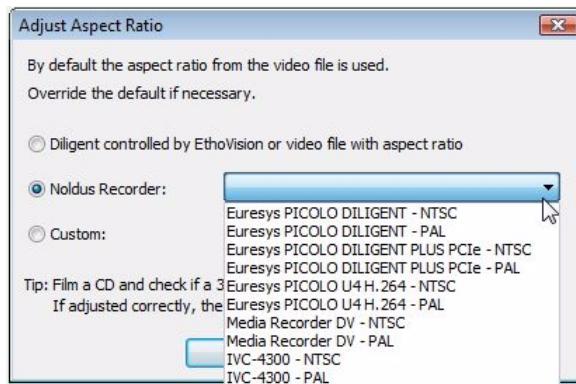
Adjusting the aspect ratio

The procedure depends on how you created the video file:

- With the Noldus MPEG Recorder or Media Recorder - See below.
- With EthoVision, but in another experiment - See page 138.
- With another media recorder - See page 139.

Adjusting the aspect ratio for MPEG-4 or H.264 files from the Noldus MPEG Recorder 1.1/2.0 or the Media Recorder

- 1 Open the video file (see page 134 for a description of how to do this).
- 2 In the **Arena Settings** window, click the **Adjust Aspect Ratio** button.
- 3 In the **Adjust Aspect Ratio** window, select **Noldus Recorder** and select one of the options from the list:



- Select **Euresys Picolo Diligent** when you used the Noldus MPEG-4 Recorder 1.1 to create the video file.
- Select **Euresys Picolo U4 H.264** when you used the Media Recorder with the Picolo U4 H.264 board.

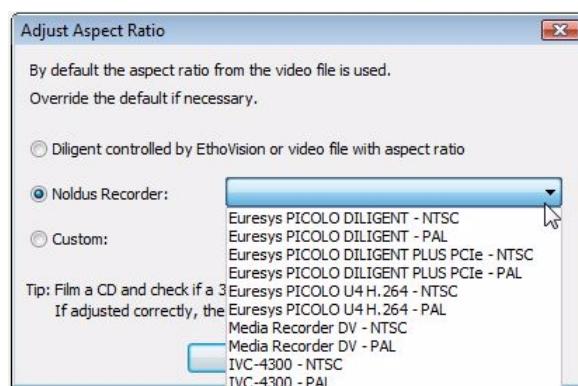
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- Select **Media Recorder DV** when you used the Media Recorder with the Picolo Diligent board.
- Select the **IVC-4300** video capture card when you used the Noldus MPEG Recorder 2.0 to create the video file.

Adjust the aspect ratio for files recorded in a different EthoVision experiment

If you record video with EthoVision (track live and record video, or record video only), and use the video in the same experiment you do not have to adjust the aspect ratio. However, if you copy the video files to another experiment it may be necessary to adjust the aspect ratio.

- 1 Open the Arena Settings by clicking one of the Arena Settings in the Experiment Explorer.
- 1 Open the video file (see page 134 for a description of how to do this).
- 2 In the **Arena Settings** window, click the **Adjust Aspect Ratio** button.
- 3 Keep the default option **Diligent controlled by Ethovision or video file with aspect ratio** selected if you use the video file in the same experiment in which it was created. If not, select **Noldus Recorder** and select one of the options from the list:



- Select **Euresys Picolo Diligent** if the file was created with the **Picolo Diligent** board.

Arena Settings

- The option **Euresys Picolo Diligent Plus PCIe** is only for users that have upgraded EthoVision and have created video files with a **Euresys Picolo Diligent Plus PCIe** board.
- Select **Euresys Picolo U4 H.264** when the file was created with the Picolo U4 H.264 board.

Adjust the aspect ratio for files recorder with another media recorder

First check the possible distortion of your video image (step 1 – 3), then adjust the aspect ratio (step 4).

- 1 Grab a frame from the video file as your Background Image (see page 134 how to do this).
- 2 Open the file in an image editing program (such as Paint.NET, which you can download for free from <http://www.getpaint.net/>). The bitmap file of your Background Image can be located in the folder **Bitmap Files** of your experiment folder:
 - **Windows XP** – C:\Documents and Settings\All Users\Documents\Noldus\EthoVision XT\Experiments\.
 - **Windows 7** – C:\Users\Public\Public Documents\Noldus\EthoVision XT\Experiments\.

Alternatively, you can use the fact that the 3-point circle Drawing Object in EthoVision is a perfect circle. Draw a 3-point circle on top of your Arena and see in what dimension it is distorted. Delete the circle, adjust the aspect ratio (see step 4 below) and redraw the 3-point circle over the adjusted Arena.

- 3 Draw a shape exactly around the arena: note the dimensions of the shape you have just drawn.

If the ratio (for instance, width/height) of the dimensions of the drawn shape deviates from the ratio of the real-world dimensions of the arena, this means that the image is distorted.

- 4 In EthoVision XT, in the **Arena Settings**, click the **Adjust Aspect Ratio** button. In the **Adjust Aspect Ratio** window, select **Custom** and enter the adjusted aspect ratio.

Example 1 – You have a perfectly circular water maze with a diameter of 100 cm. When you open the Background Image of this water maze in an image editing program and draw an ellipse around the maze, the ratio of width/height does not equal '1' but is 1.019. This means that you need to adjust the aspect ratio of the Background Image using this ratio: in the **Adjust Aspect**

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Ratio window, in the **Custom** field, divide the width (box on the left) of the image by 1.019. Do not change the height.

Example 2 – You have a square open field measuring 40 x 40 cm. When you open the Background Image of the open field in an image editing program and draw a rectangle around the open field, the ratio of width/height does not equal '1', but is 0.8. This means that you need to adjust the aspect ratio of the Background Image using this ratio: in the **Adjust Aspect Ratio** window, in the **Custom** field, divide the width of the image (box on the right) by 0.8. Do not change the height.

View Settings

You can display **Arenas**, **Zones**, **Calibrations** and **Background** in the **Video** window by doing one of the following:

- Select an **Arena**, **Zone Group**, **Calibration** or **Background** in the **Arena Settings** window by clicking its row in the **Arena Settings** window. This way you can also view multiple layers (that is, an Arena, Zone Group, Calibration or Background).
- To hide a layer, de-select the corresponding check box in the **View** column.
- If you want to view more than one layer at the same time in the video image, select the appropriate check boxes in the **View** column (see Figure 6.3.below).
- If you have multiple arenas, you can view or hide layers and labels of all arenas for better viewing. In the **Arena Settings** window, right-click an Arena and select one of the options:
 - **View all arena layers.**
 - **Hide all arena layers.**
 - **View all arena labels.**
 - **Hide all arena labels.**

Arena Settings



Figure 6.3. Example of **View Settings** in the **Arena Settings** screen. In the Arena Settings window, the **Arena** is selected. **Background** is also selected in the **View** column and therefore visible in the Background image.

Editing an Arena

Moving an Arena

- 1 In the **Arena Settings** window, click the **Arena** row to display the corresponding **Arena** in the video image.
- 2 Right-click the **Arena** row and select **Select Complete Arena** to display the edit box in the **Background** image.
- 3 To **move** a complete **Arena**, including its **Zone group**:
 - i In the **Arena Settings** window, right-click the **Arena** and select **Select complete Arena**.
 - ii In the **Video** window, move the mouse over the **Arena** to make it a move pointer and move the complete **Arena**, including **Zones**. Alternatively, you can use the arrow keys on your keyboard to move the complete **Arena**.



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Resetting an Arena

To **reset** an **Arena**, right-click the border of the **Arena** and select **Delete**.

Renaming an Arena

To **rename** an **Arena**, do one of the following:

- In the **Video** window, double-click the **Arena Label** and type in the new name in the **Edit Name** window.
- In the **Arena Settings** window, right-click the **Arena** and type in the new name in the **Edit Arena Name** window.

For a description how to duplicate an Arena, see below.



For a description of how to **move**, **rotate**, **scale** or **change the shape** of an **Arena**, please refer to section 6.5 **Drawing Objects** (page 162), under the corresponding **Drawing Object**.

Duplicating an Arena

Duplicating an Arena is especially useful if you have multiple Arenas with similar shape and size.



You can only duplicate a complete **Arena** when you have defined more than one **Arena** in the **Experiment Settings**.



Before duplicating an Arena, make sure you have created all the Zones for that Arena.

See also “Creating a new experiment based on a pre-defined template” on page 92 how to use the guided setup to create a new experiment.



For instructions how to make Arena Settings for a multi-well microtiter plate to track zebra fish larvae, refer to the DanioVision Manual which can be found on the installation DVD.

Duplicating an Arena involves the following steps:

- 1 Duplicate an Arena to a specific other Arena or to all other Arenas (see below).
- 2 Adjust the position, shape and size of individual Arenas (see “Editing an Arena” on page 141).

Arena Settings

- 3 Use the Multiple Arena Setup if you have arenas that are evenly distributed in the video image. In the Multiple Arena Setup you can move, scale, rotate and space all arenas (see below).

Duplicating an Arena

- 1 In the **Arena Settings** window, click the **Arena** you want to duplicate.
- 2 Click the **Duplicate Complete Arena** button or right-click the Arena and select **Duplicate Complete Arena**.
- 3 In the **Duplicate Arena** window, select one of the options from the list:
 - **All other arenas** – Select this option to duplicate the selected Arena to all other Arenas
 - **Arena [number]** – Select this option to duplicate the selected Arena to a specific other Arena.

An Arena to which another Arena is duplicated, loses its previous definitions and contents.



If you select the option **Duplicate content to existing arenas**, the position of existing arenas is maintained. If you do **not** select this option, the position of each arena is adjusted based on the number of arenas and their layout (see "Using the Multiple Arena Setup" below).

Using the Multiple Arena Setup

You can use the Multiple Arena Setup to configure multiple arenas which are regularly distributed (for example, a 96-well microtiter plate or four adjacent open fields). Taking into account the number of arenas and their layout (that is, the number of arenas on rows and on columns), EthoVision creates a virtual grid in which the arenas are initially evenly distributed. You can then configure the multiple arenas with the Multiple Arena Setup.

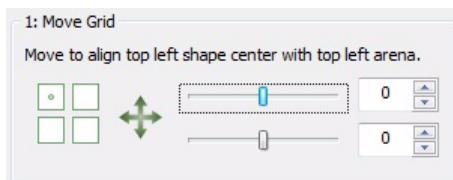
There are two ways to open the Multiple Arena Setup:

- The Multiple Arena Setup window opens automatically, when you duplicate an Arena to all other Arenas.
- In the Arena Settings window, click the **Multiple Arena Setup** button.

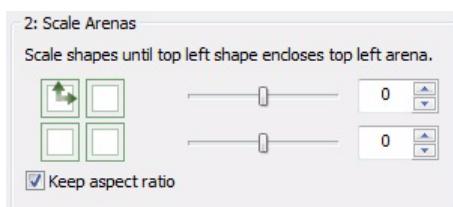
In the Multiple Arena Setup window, you can configure the following:

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- 1 Move Grid** - You can move all shapes (including zone shapes) both horizontally and vertically, by using the slider, by pressing the up/down arrows of the corresponding box or by entering a number in a box.

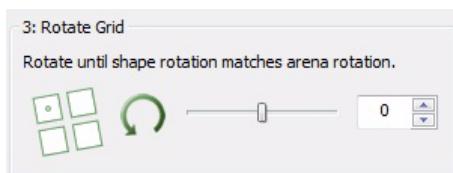


- 2 Scale Arenas** - You can resize all shapes by using the sliders, by pressing the up/down arrows of the corresponding box or by entering a number in a box.

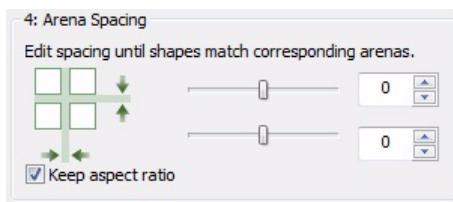


By default the option **Keep aspect ratio** is selected. As a result, both the height and width of all shapes are changed while the original shape and position is kept. If you do not select the option **Keep aspect ratio**, you can resize the height and width of all shapes separately.

- 3 Rotate Grid** - You can rotate all shapes. The shapes rotate around the center of gravity of Arena 1.



- 4 Arena Spacing** - You can change the space between the shapes.



Arena Settings

BY default, the option **Keep aspect ratio** is selected. As a result, spacing is done both in horizontal and vertical direction relative to Arena 1. If you do not select **Keep aspect ratio**, you can change the space between the shapes separately in the vertical and horizontal direction (relative to the first row and first column, respectively).



To reset a configuration, change both values to '0' (make sure to de-select the option **Keep aspect ratio**). Alternatively, you can press the **Cancel** button; this will reset all configurations!

Adding and deleting an Arena

You cannot add or delete an arena in the **Arena Settings**. You should do this in the **Experiment Settings**: Open the **Experiment Settings** and increase or reduce the number of Arenas in the drop-down list next to the **Number of Arenas** group.



You cannot add/delete an Arena in the Experiment Settings once you have carried out Acquisition in the Experiment.

Exporting an image of the Arena

You can export a picture of the Arena Settings.



The image contains all layers that are currently visible. See **View Settings** on page 140 how to display layers.

- 1 In the **Arena Settings** components tool bar, click the camera icon .
- 2 Type in a name or accept the default file name, select a picture format (*.png, *.emf, *.jpg, *.bmp, *.gif) and click **Save**.

Mapping hardware devices in your arenas

If your experiment is set to **Use of trial control hardware** (see page 96), the **Arena-hardware mapping** button at the bottom of the **Arena Settings** window is available. Click this button to assign hardware devices to each of your arenas. If

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your experiment consists of only one arena, the hardware is automatically assigned to this arena, so you do not have to use the **Arena - hardware mapping** button. If you have more than one arena, you need to assign the hardware to the arenas manually.

Arena - hardware mapping



For more information, see **Assigning devices to arenas** in the EthoVision XT Trial and Hardware Control Manual, which you can find on your installation DVD.

6.3 Zones

Within an Arena you can optionally define specific areas of interest, called **Zones**. For example, in a Morris water maze with a hidden platform (see Figure 6.4.) you define this platform as **Zone 'Platform'**.

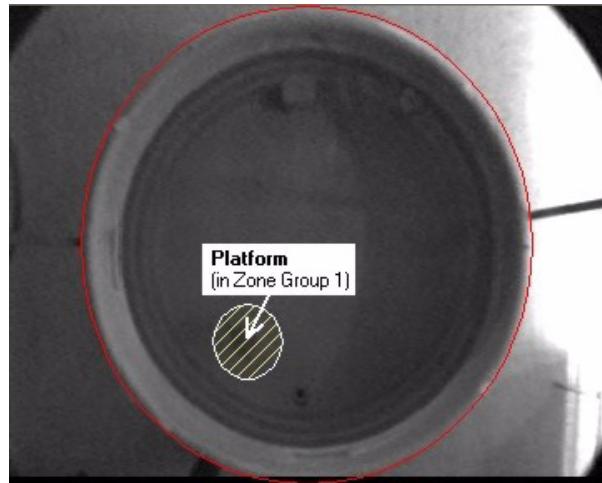


Figure 6.4. Video window showing a Morris water maze with a Zone Platform

A **Zone** can be used:

- for **Analysis** - A **Zone** is used in the analysis to calculate, for example, the time a **Subject** spent in a **Zone** or the number of visits to that **Zone**.

Arena Settings

- for **Acquisition** - A **Zone** is used in the Trial Control (see page 191) to, for example, stop a trial when the **Subject** is in a particular **Zone**.
- as **Cumulative Zone** (see page 157)- This type of **Zone** combines two or more different **Zones** and treats them as one **Zone**.
- as **Hidden Zone** (see page 151) - This type of **Zone** is used to deal with subjects that are out of view of the camera, for example, when an animal moves into a shelter. When this shelter is defined as a normal **Zone**, EthoVision XT produces missing samples when the animal is inside the shelter. However, when this shelter is defined as a **Hidden Zone** the animal is registered as being in the shelter.

How Zones are organized

Zones are organized in Zone Groups. You can add an unlimited number of Zone Groups to each Arena. Each Zone Group contains a number of non-overlapping Zones. If you want to define overlapping Zones in an Arena, you should define them in separate Zone Groups or use Cumulative Zones. A Zone Group can also contain one or more Points of interest (see page 156).

Example - In an Arena, a circular open-field, you want two circular Zones (Center and Border) and four quadrant Zones (NW, NE, SW, SE) and you want to analyze these Zones separately. To do this you need to create two Zone groups: one with the two circular Zone, one with the four quadrant Zones (see Figure 6.5. below).

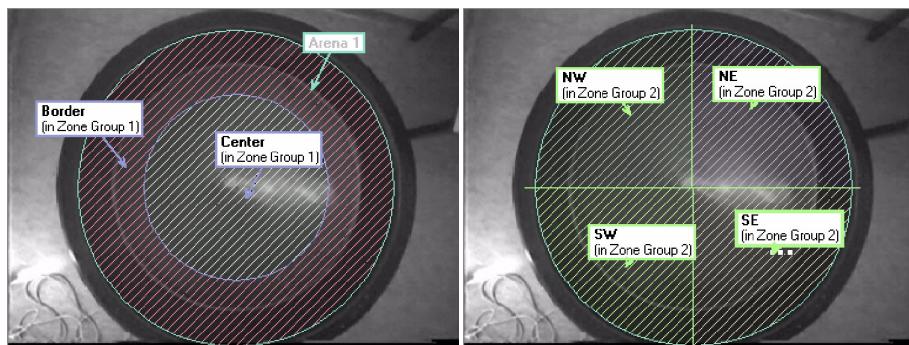


Figure 6.5. Arena with Zone Group 1 (containing Zones Center and Outer, picture on the left) and Zone Group 2 (with Zones NW, NE, SW, SE, picture on the right).

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Hidden Zones are organized in Hidden Zone Groups. You can add an unlimited number of Hidden Zones to a Hidden Zone Group. Each Arena can only contain one Hidden Zone Group. A Hidden Zone Group contains one Entry Zone Group. You can add an unlimited number of Entry Zones to an Entry Zone Group.

Defining a Zone

After you have defined one or more Arenas (see page 134), you can define Zones for each Arena. By default, one Zone Group is added to each Arena. This Zone Group is displayed in the Arena Settings window as part of an Arena (see the figure below).



The color of the box in the Color column of the Zone Group row indicates the color of the border of the Zones in that Zone Group.

	Color	View	Lock
Arena 1	Green	<input type="checkbox"/>	
Zone Group 1	Light Green	<input type="checkbox"/>	
Calibration	Light Purple	<input type="checkbox"/>	
Background	Black	<input checked="" type="checkbox"/>	

If you have only one Arena

To define one or more Zones in a Zone Group:

- 1 In the **Arena Settings** window, click a **Zone Group** to make it active in the Video window.
- 2 Select a **Drawing Object** from the **Arena Settings** component tool bar (see also section 6.5).
- 3 Create a shape that covers the area of interest within the Arena.
- 4 Click the **Add Zone** button on the **Arena Settings** component tool bar (or press 'Z') and click in the shape you have just created.



The Zone is filled with the color visible in the **Set Next Fill Color** drop-down menu. If you want to choose a different color, press the drop-down button and pick a color. You should do this before you assign a label to a Zone.

Arena Settings



You can also change a color of a Zone or Arena afterwards: right-click the label of the Zone or Arena, select **Change Fill Color** and select a new color.

5 Next, you can:

- Add a new Zone Group with Zones to this Arena (see page 158).
- Create additional Zones in this Zone Group by repeating steps 2-4 above.

Subdividing an Arena or a zone

You can automatically divide an Arena into a number of Zones or a zone into a number of sub-zones.

Example - You have a rectangular open field and want to define the four quadrants as Zones.



This only works for Arenas/zones with four sides, so created with the rectangle or closed polyline tool. For example, if you create four quadrants as zones, each side of the Arena is divided in half by a subdivision line.

To subdivide an Arena/zone:

- 1 Create an Arena/zone, using the rectangle or closed polyline tool (see page 135).
- 2 Right-click the Arena/zone border and select **Subdivide**.



If you want to subdivide an Arena to create zones, in the **Arena Settings** window, select the **Arena View** check box and click a **Zone Group** to make it active in the Video window.

- 3 In the **Quadrangle Subdivision** window, enter the number of vertical (left box) and horizontal subdivisions (right box) you want to create.

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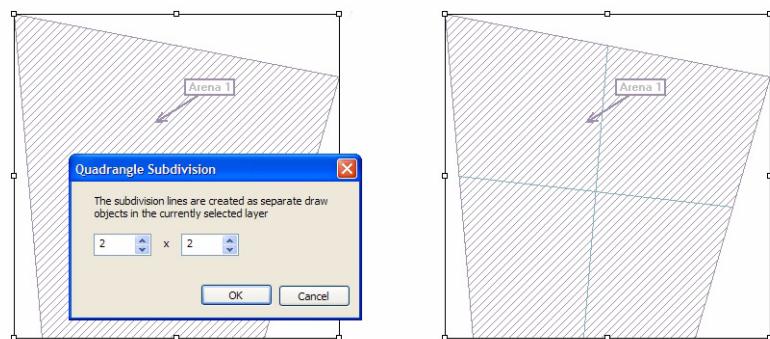


Figure 6.6. Example of subdividing a four-sided Arena into 4 zones. The picture on the left show the **Quadrangle Subdivision** window. The picture on the right shows the result of the subdivision.

- 4 Next, proceed with assigning a label to each zone/sub-zone (see step 4 on page 148).



To remove the subdivisions, in the Zone Group, delete the subdivision lines.

If you have multiple Arenas

If you have multiple Arenas with the same size and shape (for instance, 4 similar cages in one camera-view or 96 wells on a microtiter plate), you do not need to define each Arena and its Zones separately. You can define one Arena with its Zone Groups, Zones and Points of interest and duplicate this to another or to all other Arenas.

To define Zones for multiple Arenas:

- 1 Follow steps 1-4 described above for the first Arena.
- 2 Make sure this first **Arena** is selected in the **Arena Settings** window and click the **Duplicate Complete Arena** button.
- 3 In the **Duplicate Arena** window, select an **Arena** from the drop-down menu and press **OK**.

Arena Settings



- 4 Move the duplicated **Arena** to the appropriate location.
- 5 Repeat steps 2-4 for all consecutive **Arenas**.

You can resize the Arena and its Zones: see page 167 how to do that.



When you duplicate an Arena, all Zone Groups and Zones in this Arena are also duplicated. Calibration is also duplicated if it is 'unshared' (see page 162).



Notes

- You **cannot** edit **Zone groups** that contain **Zones** that have been used in Acquisition (for example, in a Trial Control Profile) or have been used as **Hidden** or **Entry Zone**.
- You **can** add new **Zone groups** with new **Zones** to **Arena Settings** that have been used for Acquisition. These new **Zones** then can be used, for example, for Analysis.

Working with Hidden Zones

Optionally, you can define one or more Hidden Zone in each Arena. When you create a Hidden Zone, you also need to define at least one Entry Zone for that Hidden Zone. The Entry Zone is used to determine whether the animal has entered or left the Hidden Zone.

Entering a Hidden Zone

Suppose you have a square shelter defined as Hidden Zone and an Entry Zone on the side of the shelter where the animal can enter it (see Figure 6.7.). When the animal enters the Entry Zone, one or more body points are tracked in that zone. As soon as a body point is not detected anymore, that body point is assumed to have entered the Hidden Zone and this body point is positioned in the center of the Hidden Zone during the time the animal is inside the shelter.

Leaving a Hidden Zone

By default, the animal is considered to have left the shelter when it is next detected anywhere outside of the Hidden Zone, the Entry Zone does not play any role in this process. However, every time an animal pokes its nose out, this may be recorded as an exit. Subsequently, retracting back into the shelter - without ever really leaving the shelter - would count as entering again. This results in an

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overestimation of how often the animal enters/exits the hidden zone. Therefore, EthoVision XT versions 5.1 and up have the possibility to **also use the entry zone as leaving zone**. The animal is then considered to have left the hidden zone when it was first considered to be in a hidden zone, and subsequently detected outside of the entry zone (see Figure 6.7.). In other words: the exit is counted only when the body point crosses the border between the entry zone and the rest of the arena. We recommend you use this function if you want to prevent an overestimation of entrances into, and exits from the hidden zone.

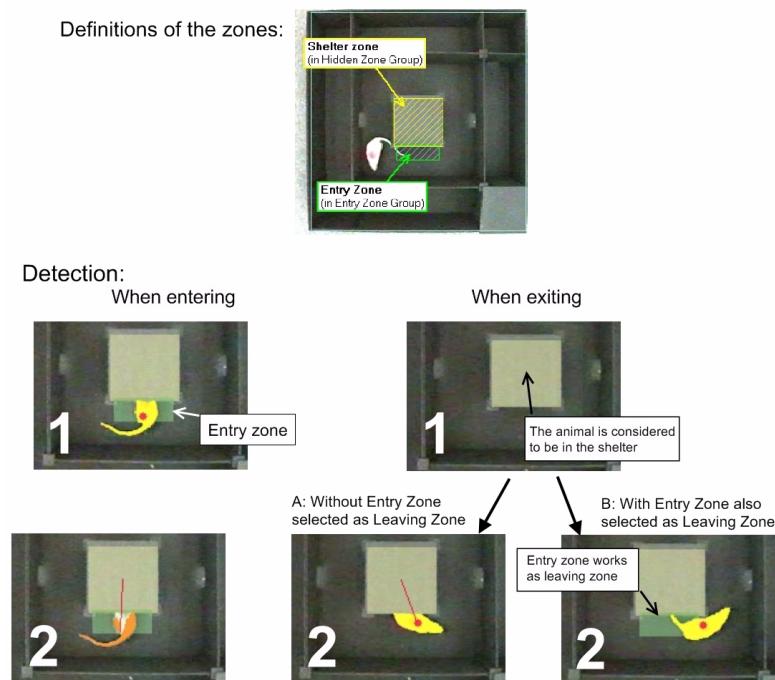


Figure 6.7. Left: If the animal's center point is first detected in the Entry Zone, and then disappears (because the apparent size of the animal becomes smaller than the minimum size set, see Subject size in Chapter 8), then the animal is considered to be in the shelter. Right: The detection for exiting the hidden zone differs for not using or using the entry zone as a leaving zone also, respectively; A: The subject is detected as having left the hidden zone as soon as the minimum subject size is detected anywhere within the Arena. Depending on the minimum size set, only poking out the nose could count as "leaving". B: The subject is detected outside the Hidden zone, but will only be recognized as "outside of the hidden zone" as soon as it crosses the border of the entry zone into the rest of the arena.

Defining a Hidden Zone

If you have only one Arena

You define a Hidden Zone in a similar way as a 'normal' Zone (see page 148).

To define one or more Hidden Zones (each with at least one Entry Zone):

- 1 In the **Arena Settings** window, click the **Add Hidden Zone Group** button.
- 2 Create a Zone covering the area you want to define as a Hidden Zone and label it.
- 3 In the **Arena Settings** window, select the **Entry Zone Group** row.
- 4 Make a Zone that covers the area, overlapping the **Hidden Zone**, to define as an **Entry Zone**.

The size of the entry zone is critical, it must be wide enough, but not too wide so that:

- **When entering**, the animal never goes straight away from the area outside the entry zone to the hidden zone; There should be at least one instance of the animal's body point within the entry zone, before disappearing from view. If necessary, increase your sample rate to make sure that this happens.
- **When exiting**, the animal's body point is far, but not too far from the hidden zone when it crosses the entry zone border to the rest of the arena; just enough for it to be considered as having left the hidden zone. This only applies if you select **Use this entry zone also as leaving zone** (see next step). You can use the **Time Event Plot** function to help you set the right size (see Chapter 13).

- 5 In the **Add Entry Zone** window, link this **Entry Zone** to the appropriate **Hidden Zone** by selecting the **Link** box in the **Linked hidden zone** field. You can link more than one Entry Zone to a Hidden Zone, but you cannot link more than one Hidden Zone to an Entry Zone.
- 6 If you want to define the entry zone as leaving zone also, select the check box for **Use this entry zone also as leaving zone**. This will be applied on **all hidden zones** in your Arena Settings. Each entry zone will be also used as leaving zone for the hidden zone associated to it.
- 7 Press **OK** to assign the label to the **Entry Zone**.



While adjusting the detection settings and during data acquisition, the video window does not display the data points corresponding to the animals being in the hidden zone. It looks like the animal is missing (this

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can also be checked in the **Analysis Results** pane, which shows the statistics of **Subject not found** constantly increasing). However, the program considers the animal to be in the hidden zone. After ending the trial, inspect the actual tracks (from the **Visualize** menu) to view the samples that have been recorded within the hidden zone. Also check the proportion of samples in which no subject was found in the Trial list (see page 103).



To display the Hidden Zone while drawing the Entry Zone, in the Arena Settings window, select the check box in the **View** column of the Hidden Zone Group row, next to the **Color** column (see also Figure 6.3.).

What next?

- Repeat steps 2-5 to define additional **Hidden Zones** and **Entry Zones** for this **Arena**.
- Create one or more **Hidden Zones** and **Entry Zones** in other **Arenas**, in case you have multiple Arenas (see below).

Linking a Hidden Zone and Entry Zone

If you did not link an Entry Zone to its Hidden Zone when you defined the Hidden Zone (see above), you can do the following:

- 1 In the **Arena Settings** window, either select the Hidden Zone Group row or Entry Zone Group row.
- 2 In the video image, right-click one of the following:
 - the Hidden Zone label. Select **Link Entry Zones** and select the **Link** check box of the appropriate Entry Zone in the **Links to Entry Zones** window.
 - the Entry Zone label. Select **Link Hidden Zones** and select the **Link** check box of the appropriate Hidden Zone in the **Link to Hidden Zone** window.
- 3 Click **OK**.

Changing the Hidden Zone subject coordinates

In previous versions of EthoVision XT, when a subject entered a Hidden Zone, its body points were assumed to be in the center of the Hidden Zone. However, if you have a very large Hidden Zone, the distance the subject moves from the Entry Zone to the center of the Hidden Zone every time it enters the Hidden Zone,

Arena Settings

contributes rather significantly to **Distance moved**. In these cases, you can now use the arrow tip of the Hidden Zone label to set the coordinates.

To set the Hidden Zone subject coordinates:

- 1 First, define a Hidden Zone (see page 153).
- 2 Right-click the Hidden Zone label and select **Hidden Subject Coordinates**.
- 3 Now, select one of two options to set the Hidden Subject Coordinates:
 - **Center of the zone, unless the center is outside the zone (then it is the arrow tip location)** - Select this option when you have a small Hidden Zone (for an example, see Figure 6.8.A).
 - **Arrow tip location** - Select this option when you have a large Hidden Zone (for an example, see Figure 6.8.B).

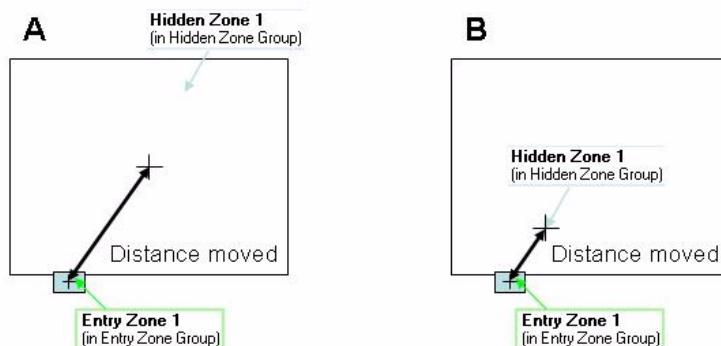


Figure 6.8. Example of setting the Hidden Subject Coordinates. **A** - The Hidden Subject Coordinates are set to the center of the Hidden Zone, thereby making a large contribution to the Distance moved. **B** - The Hidden Subject Coordinates are set to the Arrow tip location, thereby minimizing the contribution to Distance moved.

If you have multiple Arenas

If you have multiple **Arenas** with the same size and shape (for instance, 4 similar cages in one camera-view), you do not need to define each **Arena** and its **Hidden Zone Group** and **Entry Zone Group** separately. You can define one **Arena** with a **Hidden Zone Group** and **Entry Zone Group** and duplicate this for all other Arenas.

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See **Duplicating an Arena** on page 142 to see a description of how to create multiple copies of an **Arena** and its (**Hidden**) **Zone Groups**, **Entry Zone Groups** and **Calibration**.

If you have multiple **Arenas** that do not have the same size or shape, you manually create **Hidden Zones** and **Entry Zones** by doing the following:

- 1 First define the **Arenas** (see page 134).
- 2 In the **Arena Settings** window, click in the row of the **Arena** for which you want to define one or more **Hidden Zones**.
- 3 Continue to follow the instructions in the section **Define a Hidden Zone** on page 153 to define one or more **Hidden Zones** and **Entry Zones** for an **Arena**.
- 4 Repeat steps 2 and 3 above to define **Hidden Zones** and **Entry Zones** for the other **Arenas**.

Defining a Point

You can define one or more **Points** in a **Zone Group**.

You can use a **Point** to:

- calculate the **Distance to point** (see page 507).
- calculate **Heading to point** (see page 510).
- calculate **Head direction to zone** (see page 539).

To define a **Point**:

- 1 In the **Arena Settings** window, select the **Zone Group** in which you want to add one or more Points by selecting the **Zone Group** row.
- 2 Click the **Add point** icon on the **Arena Settings** component tool bar.
- 3 Click in the **Background image** to position the **Point**.
- 4 In the **Add Point** window, you can type in the name of the **Point**.
- 5 Click **OK** to define the **Point**.



You can position a **Point** outside an **Arena**.

Copying a Zone from one Arena to another

If you have multiple Arenas with the same size and shape you can duplicate a complete Arena including all Zone Groups and Zones (see page 142).

You cannot copy individual zones from one Arena to another. You can redraw the Zone in the destination Arena and use a label with the same Zone name.

- 1 In the **Arena Settings** window, select the **Zone Group** into which you want to add a **Zone**.
- 2 Make a zone that covers the area of interest within the **Arena**.
- 3 Click the **Label Zone** button on the **Arena Settings** component tool bar and click in the shape you have just created.
- 4 In the **Add Zone** window, select **Use existing zone** and select one of the **Zones** from the list.
- 5 Click **OK**.

Defining a Cumulative Zone

You can add several **Zones** to make a **Cumulative Zone**, so that when the different zones are analyzed they are treated as a single zone.

Example - In a novel object test, you define three non-overlapping zones. To analyze when the animal enters (or gets close to) *any zone*, define a cumulative zone from the three existing ones.

To make a **Cumulative Zone**:

- 1 In the **Arena Settings** window, click on a **Zone Group** in which you want to create a **Cumulative Zone**.
- 2 Click the **Add Cumulative Zone** button.
- 3 In the **Add Cumulative Zone** window you have two options:
 - **Create new cumulative zone** - Type in the name of the New Cumulative Zone.
 - **Use existing cumulative zone** - Choose the name of an existing **Cumulative Zone** from the drop-down list.



The latter option only works if the current Arena contains Zones with the same Zone Labels as the Zones in the existing Cumulative Zone.

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Important notes

- A zone cannot extend outside the border of the arena because the **Draw Objects** used to define the arena are also used to define the zone. The result of a zone that extends outside the arena can be seen in Figure 6.9.

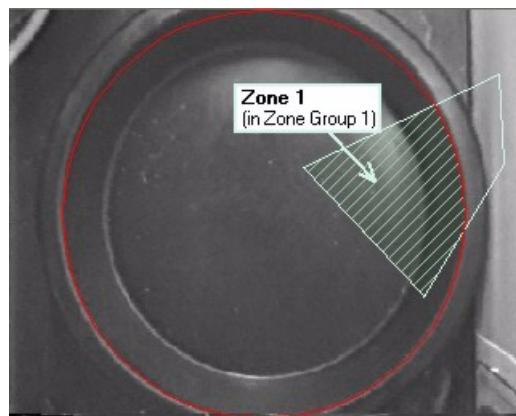


Figure 6.9. Example of a Zone that extends outside the (circular) Arena. The (non-hatched) area outside the Arena is ignored during tracking.

- You cannot create overlapping zones in the same zone group. If you want to create overlapping zones you have to define more zone groups and add each zone in a separate zone group. The overlapping area falls within each of the overlapping zones. You can also create cumulative zones.



See page 157 for more information on cumulative zones.

How to...

Add a Zone Group to an Arena

To add a Zone Group to an Arena:

- 1 In the **Arena Settings** window, select the Arena to which you want to add a **Zone Group** by clicking the **Arena** row or one of its **Zone Groups**.
- 2 Click the **Add Zone Group** button.

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You can now add Zones to the Zone Group (see page 148).

Delete a Zone Group

To delete a Zone group and all its Zones: In the **Arena Settings** window, click the row of the Zone Group you want to delete, and click the **Delete Zone Group** button.

Add a Hidden Zone Group

To add a **Hidden Zone Group** to an Arena:

- 1 In the **Arena Settings** window, select the Arena to which you want to add a **Hidden Zone Group** by clicking the **Arena** row.
- 2 Click the **Add Hidden Zone Group** button.

You can now add one or more **Hidden Zones** and **Entry Zones** to the **Hidden Zone Group** (see page 153).

Delete a Hidden Zone Group

To delete a Hidden Zone Group and all the Hidden Zones and Entry Zones it contains: In the **Arena Settings** window, click the row of the Hidden Zone Group you want to delete, and click the **Delete Zone Group** button.

Change the color of an Arena or Zone Group

You can change the color of arenas and zones:

- 1 In the **Arena Settings** window, select the Arena or (Hidden) Zone group you want to edit by clicking in the Arena row or **(Hidden) Zone Group** row, respectively.
- 2 Click the box in the **Color** column, select a new (custom) color and click **OK**.

Edit a (Hidden) Zone label

To edit a Zone:

- 1 In the **Arena Settings** window, select the (Hidden) Zone group to which that Zone belongs by clicking the **(Hidden) Zone Group** row.
- 2 To edit the name of the **(Hidden) Zone Label** do one of the following:

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- Double-click the label and in the **Edit name** window, type in a new name.
- Right-click the label, select **Rename** and type in a new name in the **Edit name** window.



For a description of how to **move**, **rotate**, **scale** or **change the shape** of a **Zone**, please refer to section 6.5 **Drawing Objects** (page 162), under the corresponding **Drawing Object**.

Delete a (Hidden) Zone

To delete a (Hidden) Zone you can just delete the (Hidden) Zone label: Click on the label so the arrow gets selected and press **Delete**.

6.4 Calibration

Calibration enables you to convert pixel coordinates to real-world coordinates. Two restrictions apply to your experimental setup: a) the camera should be far enough from the Arena(s) to prevent spherical distortion and b) the axis of the camera should be perpendicular to the Arena surface to prevent perspective distortion.

Use the following procedure to calibrate your **Arena**:

- 1 Measure the real-world size of your Arena or the real-world distance between features in your Arena.



Measure this distance at the height of your animal, not, for example, on top of the arena.

- 2 In the **Arena Settings** window, select **Calibration**.

- 3 Click the **Create Calibration Scale** button on the **Arena Settings** component tool bar.

- 4 In your image, click the mouse pointer (which has changed into a '+' sign) on a feature of your Arena, keep the mouse button pressed, drag the mouse pointer to another feature of the Arena and release the mouse button.

- 5 In the **Calibration Distance** window, type in the real-world distance between the two pixel coordinates in your screen.

You should use more than one Calibration line, to minimize the drawing error. These lines should be both horizontal and vertical to compensate



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for the fact that pixels in many cameras are not perfectly square. EthoVision XT then uses the average of the lines.

You can change the distance unit in the **Units** group of the Experiment Settings.

You can optionally use the **Calibration Axes** drawing object to specify the orientation of the real-world coordinate system and for one pixel point specifies the real-world coordinates.

- 1 Click the **Create Calibration Axes** drawing object on the **Arena Settings** component tool bar.
- 2 In the **Calibration Coordinate System** window, type in the real-world origin of the X-axis and Y-axis relative to the position of the origin of the coordinate system on your screen.



By default, the origin of the **Calibration Axes** drawing object is put in the middle of the image, the positive x-axis points to the right, and the positive y-axis points upward. Use the **Normal Mode** arrow to move the **Calibration Axes** drawing object. To flip the Y-axis of the Calibration Axes drawing object, double-click the **Calibration Axes** drawing object and select the **Flipped Y-axis** check box. Use the **Rotation Mode** arrow to rotate the Calibration Axes drawing object.

When do I use the Calibration Axes drawing object?

You normally do not need to use the **Calibration Axes** drawing object, therefore its use is optional. You can use this tool when you have a good reason to have the coordinates system different from the default one. For example:

- You want to export the real-world coordinates and you want these coordinates expressed relative to a point of reference in the real world that is **not** in the middle of your image.
- You want the dependent variables that use angle (that is, Head direction, heading, Meander, Turn angle), to be expressed relative to a reference line that is different from the default one (horizontal and pointing to the right). For example, you have an external cue to the north-east of your arena and you want to express the animal's orientation relative to this cue. In this case, you rotate the Calibration Axes so the x axis points towards the cue.

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Shared/Unshared Calibration

By default, **Calibration** is shared. This means that, in case of multiple **Arenas**, the same **Calibration** is used for all **Arenas**.

Making Calibration unshared

To make the default shared **Calibration** unshared: In the **Arena Settings** window, right-click the **Calibration** and select **Unshare This Calibration**.



Using unshared Calibration is useful when you have multiple arenas but the distance between individual arenas and the camera is different.

Making Calibration shared

In case of multiple **Arenas** and an unshared **Calibration**: In the **Arena Settings** window, right-click the **Calibration** you want to share and select **Share Copy Of This Calibration**.

World point / Pixel point

The Status Bar at the bottom of the screen in the Arena Settings shows the pixel coordinates as **Pixel point: (X), (Y)** relative to the top-left corner of the background image:

- The Status Bar indicates that you have an **uncalibrated** Arena by displaying '**No calibration**'.
- The Status Bar indicates that you have a **calibrated** Arena by displaying the real-world coordinates as **World point (X), (Y)**. The origin of the real-world coordinates is in the center of the Background image, unless you specify it differently with the Calibration Axes drawing object (see page 161).

6.5 Drawing Objects

The Drawing Objects are used to create and edit geometrical shapes which are used to define Arenas and Zones. The following drawing objects are available:

- **Line** - A Line can be used to, for example, divide an Arena in two Zones.
- **Rectangle** - A Rectangle can be used to create a square or a rectangular shape.

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- **Open Polyline** - An Open Polyline can be used to create any shape with straight sides.
- **Closed Polyline** - A Closed Polyline can be used to create any shape with straight sides.
- **Ellipse** - An Ellipse can be used to create a circle or an ellipse.
- **3-Point Circle** - A 3-Point Circle can be used to easily create a circle.



You need to create closed shapes for the definition of Arenas and Zones. You can do this by using one or more drawing objects to define closed shapes.

Working with Drawing Objects

You can draw objects by clicking the appropriate **Drawing Object** button on the **Arena Settings** component tool bar (or by pressing the corresponding shortcut key; see below) and subsequently draw the object in the Background image (see below).

Selecting Drawing Objects

You can select a single drawing object by left-clicking on the edge of the object. As a result, the outline of the object becomes bold and a rectangle with resize handles appears around the object (see Figure 6.10. below).

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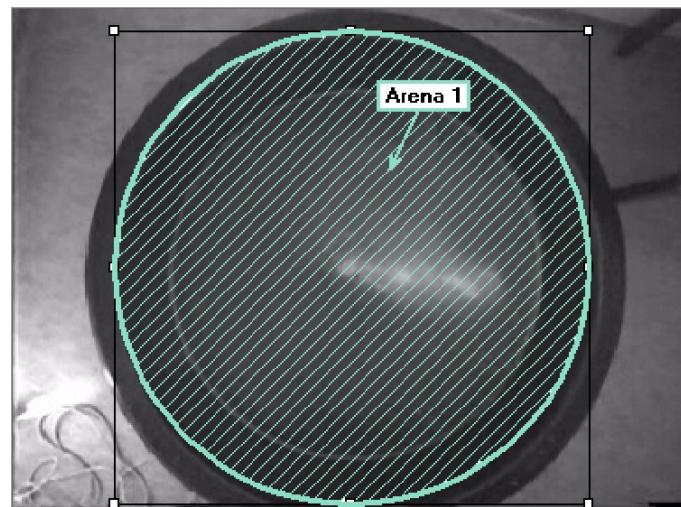
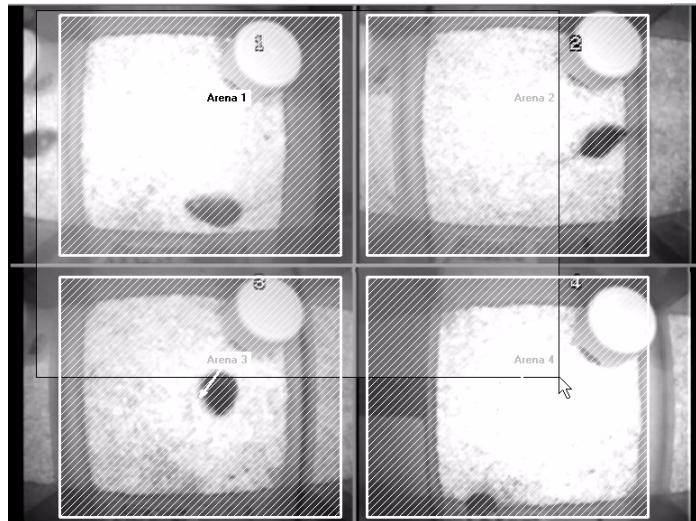


Figure 6.10. An example of a selected, circular, Arena.

To select multiple drawing objects, do one of the following:

- Press <Shift> and left-click on the edge of the objects.
- Left-click anywhere on the background image, keep the left mouse button pressed and draw a rectangle around the objects you want to select (see figure below).

Arena Settings



To de-select objects:

- Press **<Shift>** and left-click on the edge of an object to de-select a single object.
- Left-click anywhere on the background to de-select all objects at once.



With **Normal mode** selected on the **Arena Settings** component tool bar, you can **move** objects by selecting them and dragging the objects to a new position. In this mode you can also **scale** objects by selecting the object in the Background image and dragging one of the resize points on the border of the object to a new position. You can also select **Normal Mode** by pressing 'V' on your keyboard with Caps Lock on.



With **Rotation Mode** selected on the **Arena Settings** component tool bar, you can **rotate** objects by applying it to the selected object. You can also select **Rotation Mode** by pressing 'R' on your keyboard with Caps Lock on.



With **Point Edit Mode** selected on the **Arena Settings** component tool bar, you can **change the shape** of an object (**Line**, **Rectangle** and **Polyline** only) by moving, removing or adding one or more resize points. You can also select **Point Edit Mode** by pressing 'P' on your keyboard.



Whenever you move, scale, rotate or change the shape of an Arena or a Zone, make sure the arrow point of the corresponding label stays within the shape.

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Creating Drawing Objects

Line, Rectangle, Ellipse

To create a **Line**, a **Rectangle** or an **Ellipse**, do the following:

- 1 Click the appropriate **Drawing Object** button on the **Arena Settings** component tool bar or press 'L' for a **Line**, 'S' for a **Rectangle** and 'E' for an **Ellipse**.
- 2 Click on the Background image with the left mouse button to define the first point of the **Drawing Object**, keep the mouse button pressed, drag the pointer to the end point to create the desired shape and release the mouse button.

Polyline

To create an **Open** or **Closed Polyline**, do the following:

- 1 Click the appropriate **Drawing Object** on the **Arena Settings** component tool bar or press 'O' for an Open Polyline and 'Y' for a Closed Polyline.
- 2 Click in the Background image to position the first point, move the mouse pointer to the position of the second point and click in the Background image, move the mouse pointer to a new position for the third point, etc.
- 3 Double-click to finish drawing the **Polyline**.

3-Point Circle

To create a **3-Point Circle**, do the following:

- 1 Click on the **3-Point Circle** button on the **Arena Settings** component tool bar or press 'T'.
- 2 Click in the background image to position the first point, move the mouse and click to position the second point.
- 3 Move the mouse to position the **3-Point Circle** and click to finish.

When you resize a 3-Point circle, it becomes an Ellipse (see page 167). If you don't want that, press the **CTRL**-button while you resize the circle.



You can show or hide the center of the bounding rectangle of a Drawing Object by, respectively, doing the following:

- Right-click the Drawing Object and select **Show center**.
- Right-click the Drawing Object and select **Hide center**.

Moving a Drawing Object

To move a **Drawing Object**:



- 1 Click the **Normal Mode** button on the **Arena Settings** component tool bar or press '**V**'.
- 2 Move the mouse pointer over the **Drawing Object**, so the pointer becomes a move pointer.
- 3 To move the **Drawing Object** do one of the following:
 - Click with the left mouse button on the **Drawing Object**, keep the mouse button pressed and drag the **Drawing Object** to a new position.
 - Click with the left mouse button on the **Drawing Object** to select it. Use the arrow keys on your keyboard to move the **Drawing Object**.

Rotating a Drawing Object

To rotate a **Drawing Object**:



- 1 Click the **Rotation Mode** button on the **Arena Settings** component tool bar or press '**R**'.
- 2 Move the mouse pointer over the **Drawing Object**, so the pointer becomes a rotation pointer.
- 3 Click with the left mouse button on the **Drawing Object**, keep it pressed and move the pointer to rotate the **Drawing Object**.
- 4 Select **Normal mode** when you are done rotating.

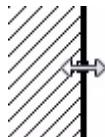
Resizing a Drawing Object

To resize a **Drawing Object**:



- 1 Click the **Normal Mode** button on the **Arena Settings** component tool bar or press '**V**'.
- 2 Select the drawing object (see "Selecting Drawing Objects" on page 163).
- 3 Position the mouse pointer on one of the middle resize handles, so the pointer becomes a resize pointer (see the figure below), click the left mouse button, keep it pressed and drag the resize handle to a new position.

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When you resize a Drawing Object as described above, the Drawing Object is resized relative to the opposite side of the bounding rectangle; this means that the opposite side or opposite corner does not move. When you press the **CTRL**-button while you move a resize handle, the Drawing Object is resized relative to the center of the bounding rectangle. This means that the other side of the Drawing Object is moved in the opposite direction.



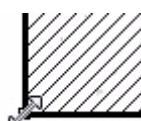
Resizing Drawing Objects as described above works the same for a selection of Drawing Objects. You get a selection of Drawing Objects when you, for instance, duplicate a complete Arena, including Zones.

Rescaling a Drawing Object

You can rescale a drawing object; in this case the size is changed but the aspect ratio is maintained. To rescale a drawing object:



- 1 Click the **Normal Mode** button on the **Arena Settings** component tool bar or press '**V**'.
- 2 Select the drawing object (see "Selecting Drawing Objects" on page 163).
- 3 Position the mouse pointer on one of the corner resize handles and do one of the following:



- Press the **CTRL**-button and move the resize handle: the object is rescaled with the centre-point of the object as the reference point.
- Press the **Shift**-button and move the resize handle: the object is rescaled with the opposite corner resize handle as the reference point.

Reshaping a Drawing Object

You can reshape a **Line**, **Rectangle** and **Polyline** by doing the following:



- 1 Click the **Point Edit Mode** button on the **Arena Settings** component tool bar or press 'P'.
- 2 Move the mouse pointer over the **Drawing Object**, so the pointer becomes a move pointer and click the left mouse button to select the **Drawing Object**.
As a result, resize handles appear at the corners of the **Drawing Object**.
- 3 Position your mouse pointer on one of the resize handles, so the pointer becomes a reshape pointer, click the left mouse button, keep it pressed and drag the resize handle to a new position (see Figure 6.11.).

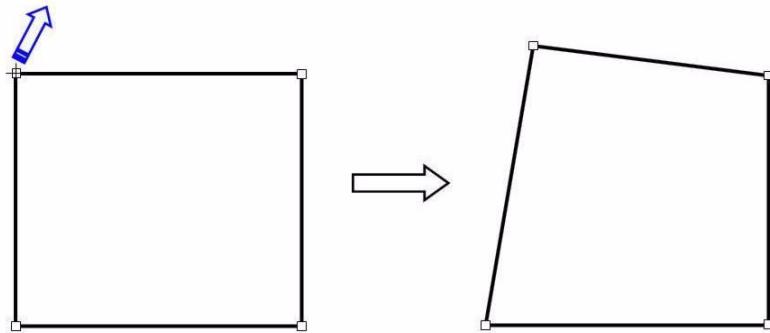


Figure 6.11. Example of resizing a Rectangle.

- 4 Select **Normal mode** when you are done reshaping.

Adding/removing resize handles



- You can add an extra resize handle to a selected Drawing Object by clicking anywhere on the Drawing Object when in **Point Edit Mode**.
- You can remove a resize handle from a selected Drawing Object by right-clicking one of the resize handles and selecting **Remove Point**, when in Point Edit Mode.

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Subdividing a Drawing Object

You can automatically divide an Arena into a number of Zones.

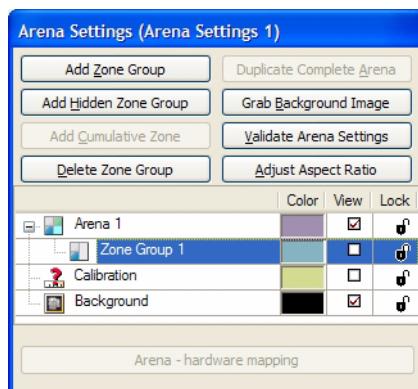
Example - You have a rectangular open field and want to define the four quadrants as Zones.



This only works for Arenas with four sides, so created with the rectangle or closed polyline tool. For example, if you create four quadrants as zones, each side of the Arena is divided in half by a subdivision line.

To subdivide an Arena:

- 1 Create an Arena, using the rectangle or close polyline tool (see page 135).
- 2 In the **Arena Settings** window, select the **Arena View** check box and click a **Zone Group** to make it active in the Video window (see figure below).



- 3 Right-click the Arena border and select **Subdivide**.
- 4 In the **Quadrangle Subdivision** window, enter the number of vertical (left box) and horizontal subdivisions (right box) you want to create.
- 5 Next, proceed with assigning a label to each zone (see step 4 on page 148).



To remove the subdivisions, in the Zone Group, delete the subdivision lines.

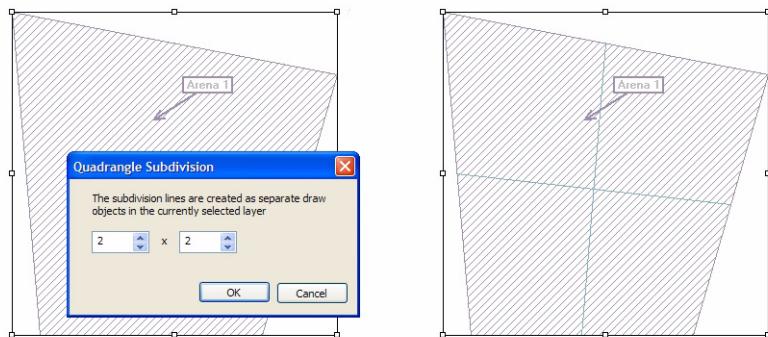


Figure 6.12. Example of subdividing a four-sided Arena into 4 zones. The picture on the left show the **Quadrangle Subdivision** window. The picture on the right shows the result of the subdivision.

Copying and enlarging a Drawing Object

You can create a copy of a Drawing Object in the same Group by doing the following:

- 1 Right-click the Drawing Object and select **Duplicate**.
 - 2 In the **Duplicate** window, you can enter an **Enlargement factor** that results in a larger or smaller copy of the original Drawing Object:
 - An **Enlargement factor > 1** results in a larger copy.
 - An **Enlargement factor < 1** results in a smaller copy.
- When you want to create a copy with the same size, enter an **Enlargement factor** of '1'.

- 3 Click **OK** to create the copy.

7

Trial Control

This chapter is about:

- **Introduction to Trial Control and the Trial Control screen.**
→ See the next page and page 180
- **Programming Trial Control** – Basics of programming the automatic control of an experiment using conditions and actions.
→ See page 189 and page 202
- **Analysis of Trial Control events** – To analyze for example the time from the start of tracking to the time the animal enters a particular zone.
→ See page 208



For a detailed overview of conditions, creating *sub-rules* and controlling hardware devices, see the **EthoVision XT Trial and Hardware Control Manual** which you can find on your installation DVD.

7.1 Introduction to Trial Control

Why use Trial Control?

Trial Control allows you to automate your experiment. For example:

- You want to **set a maximum duration for your trials**.

→ See page 199

- You want to **automate the start and/or stop of data acquisition**.

A few examples:

- Start recording when the rat is first detected in the open field.
- Stop recording when the rat has reached the platform in the Morris water maze.
- Start recording at exactly 12:30:00.
- Stop recording after the animal has been in the closed arms of the plus maze for 5 minutes.

→ See page 202

To use Trial Control:

- 1 Open the Trial Control screen (see page 180).
- 2 Define the conditions that, when met during your trial, trigger specific actions. Organize conditions and actions in a sequence (see page 189).
- 3 Before starting data acquisition, make sure that those **Trial Control Settings** are active.

See also page 581 for instructions how to manage Trial Control Settings.



With the **Trial and Hardware Control** add-on module, you can also automate conditioning schedules using *sub-rules*, or operate *hardware devices* like a pellet dispenser. If you have this module, see the **EthoVision XT Trial and Hardware Control Manual** on the installation DVD under **Documentation**.

The EthoVision XT Trial and Hardware Control Manual also includes the information you find in this chapter.

Conditions and actions

A **Condition** is a statement that EthoVision evaluates. An **Action** is a command executed on a variable or a hardware device. You can therefore control your experiment by linking conditions with actions.

- Example – In a Morris water maze test, stop tracking when the rat is detected on the platform (provided that the platform has been defined as a **zone**).

The action is *Stop tracking* and the condition is *Rat detected on the platform*.

You define and link conditions with actions in a graphical form. The example above can be represented by the following:

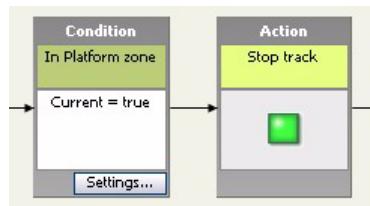


Figure 7.1. A condition is followed by an action. The condition checks that the animal is in the zone named "Platform". The action "Stop track" is taken when the condition is met.

- For more information on conditions, see page 190.
- For more information on actions, see page 192.

The Start-Stop trial rule

Conditions and actions are organized in a logical sequence named called the *Start-Stop trial rule*. This can be viewed as a set of instructions executed for starting and stopping data recording.

For more information on the Start-Stop trial rule, see page 202.



The Trial Control function also allows you to analyze events that occurred during the trial, or the time between two specific events. For example, the time from the condition A being activated to action X being taken. For the detailed procedure, see page 208.

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With the **Trial and Hardware Control** add-on, you can also define subroutines called *Sub-rules*. The sub-rules are meant to carry out specific actions. They can start at specific times and be repeated according to user-specified conditions. For more information, see the **EthoVision XT Trial and Hardware Control Manual** on the EthoVision XT installation DVD.

How Trial Control instructions are executed

The instructions contained in the Trial Control Settings are carried out from the moment you start a trial, to the moment the trial is stopped. Only the instructions in the Trial Control Settings currently active (that is, highlighted in blue in the Experiment Explorer) are carried out.

The program evaluates the Trial Control sequence at each *sample time*. The rate at which this happens depends on your chosen *sample rate*, not on the video frame rate.

The program remembers which Trial Control box was evaluated (*active*) in the previous sample. Depending on the type of this box:

- For a **Condition** box – EthoVision XT checks whether the condition is met. If it is not, the condition *becomes false*. The program waits until the condition is met. When this happens (condition *becomes true* - see 3 in Figure 7.2.), the program passes control to the next box in the sequence. The condition becomes then *inactive* (see 4 in Figure 7.2.).
- For an **Action** box – EthoVision XT carries out the action (see 4 in Figure 7.2.), and passes control to the next box, which becomes active. Then, the Action box *becomes inactive* (see 5 in Figure 7.2.).
- For Sub-rules and their References, see the EthoVision XT Trial and Hardware Control Manual.

When a box *becomes active*, the previous *becomes inactive*.



- Boxes combined in parallel using operators (see page 196) are evaluated at the same time, in unspecified order. This means that one cannot establish which condition is evaluated/which action is taken first.
- If two or more boxes are evaluated at the same time, action is taken only after all active boxes that must be evaluated in that sample have been evaluated.
- If the next box to be evaluated contains a condition that is fulfilled immediately, the program passes control to the next box. Therefore, within

Trial Control

one sample time the program can pass control to two or more boxes to the right.

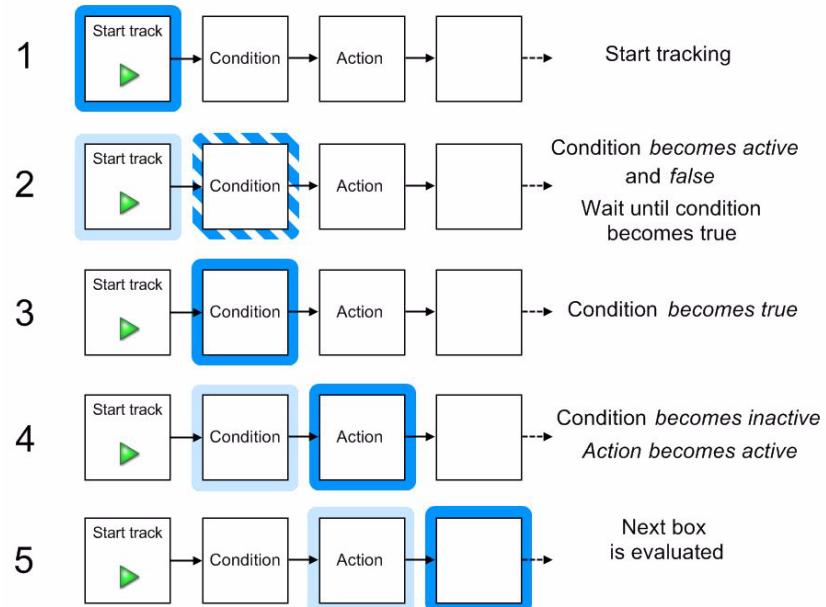


Figure 7.2. Schematic representation of how Trial Control Instructions are executed. The scheme shows an example of a Start-Stop trial rule (see page 202). **1**-Tracking starts, either manually or because a previous condition has been met. **2**- Control passes to a Condition box (for example, "Is mouse on top of Shelter?" which *becomes active*. The condition is evaluated. Since the condition is not met immediately, it *becomes false*. **3**- The Condition is met. **4**- Control passes to the next box. In this case, it is an Action. Actions are taken immediately. **5**- The Action box *becomes inactive*, and the next box *becomes active*.
For clarity, step 3 and 4 have been placed separately. In reality, when a condition is met it becomes inactive at the same time, and control passes to the next box.
Hatched outlines - Condition Box *becomes active*. Dark outlines - Condition becomes true or Action is taken. Pale outlines - Box *becomes inactive*

- When you stop the trial or the Maximum trial duration has been reached, all Trial Control boxes are deactivated.
- When the **Rule End** box of the Start/Stop trial rule is evaluated, data recording stops. From that moment, Trial Control is deactivated, even in those sub-rules that were ongoing in the meantime.

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Trial Control in multiple arenas

If your experimental setup includes two or more arenas, Trial Control is applied to each arena separately. This means that, if a condition is met in one arena, EthoVision XT takes the corresponding action in that arena, not the others.

In the following example, a setup includes four cages, each defined as an arena. A Trial Control **In zone** condition (see page 191) has been defined so that tracking starts when the animal is first detected in the arena. When you first put an animal in Arena 2, the condition is met in this arena and tracking starts for that arena. When you release the second animal in Arena 4, 2 seconds later, tracking in that arena starts 2 seconds later than in Arena 2 (see Figure 7.3.).



Figure 7.3. **Trial Control** in multiple arenas. The time values displayed on the monitor are the times elapsed since the start of tracking in a particular arena. Tracking started earlier in Arena 2 than in Arena 4 (see text), therefore at any time the **Elapsed** time (duration of tracking) is longer in Arena 2 than in Arena 4.

The advantage of Trial Control in multiple arenas is that you can put one animal at a time into the arenas, and EthoVision XT will start tracking in each arena at the appropriate moment.

If your setup includes multiple arenas, you cannot define a condition/action specific to one arena. This means that the zone which a condition is based on must be present in all arenas, and have the same name.

Trial Control

- If a zone is not present in an arena, and a condition is based on that zone, Trial Control cannot progress for that arena. Therefore, tracking does not stop unless you set a Maximum trial duration or tracking reaches the end of the video.
- At any sample time, Trial Control carries out the instructions for each arena. However, you cannot establish which arena is evaluated first at a specific sample time.

Your EthoVision license and Trial Control

Your EthoVision XT license determines which type of Trial Control you can set up.

- EthoVision XT **Base** license – You can define a rule to start and stop data recording (*Start-Stop trial rule*; see page 202). You cannot control hardware devices. Note: Trial Control in a Base license is equivalent to Trial Control in EthoVision XT 4 and 5.
- EthoVision XT Base + **Trial and Hardware Control** add-on – You can define a *Start/Stop trial rule* (see page 202), and in addition *sub-rules*. Moreover, you can control hardware devices.



The **EthoVision Trial and Hardware Control Manual**, which you can find on your installation DVD, includes the following:

- Programming Trial Control (extended)
- The Start-Stop trial rule
- Sub-rules
- Overview of conditions
- Controlling hardware devices
- Analysis of Trial Control data



To acquire data in an experiment made with the Trial and Hardware Control add-on, you must have a hardware key enabled for Trial and Hardware Control plugged in your computer.

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7.2 The Trial Control screen

To access the Trial Control screen, click **Trial Control Settings 1** in the Experiment Explorer, or from the **Setup** menu, select **Trial Control Settings**, then click **Open** and select **Trial Control Settings 1**. Next, click **OK**. The Trial Control screen appears, showing the default Trial Control settings.

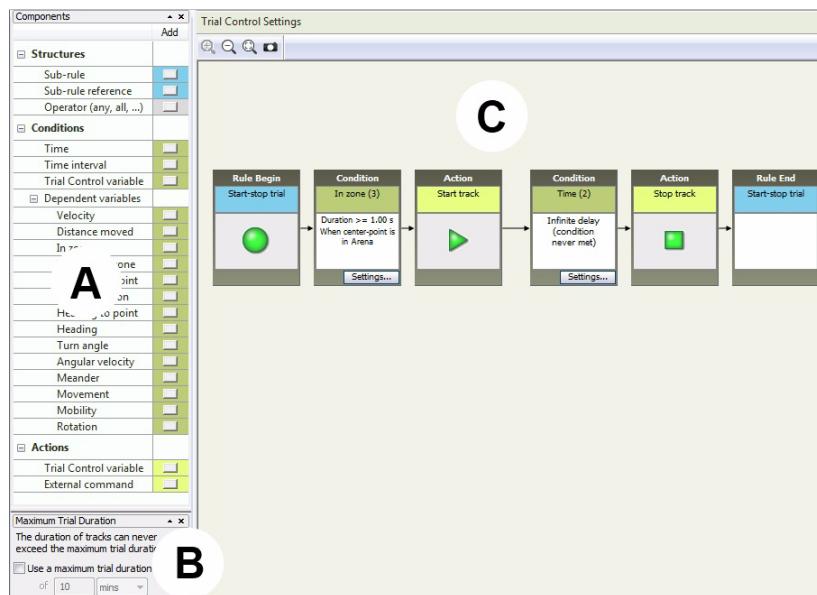


Figure 7.4. The Trial Control Settings screen. A - **Components** pane. B - **Maximum Trial duration** pane. C - Trial Control window.

To access the Trial Control screen, you can also create a new Trial Control Settings, or open one other than **Trial Control Settings 1** (see page 581).

- The **Components** pane, listing the conditions on which you can base your actions and the operators which you can use to combine conditions. See the next page.
- The **Trial Control** window, showing the **Trial Control Settings** that are active. It contains a sequence of boxes connected by arrows. See page 183.
- The **Maximum trial duration** pane that enables you to define a maximum duration of the trial. See page 189.

Trial Control



You can show/hide the **Components** pane and the **Maximum trial duration** pane by clicking the **Show/Hide** button on the component tool bar and selecting/deselecting the corresponding option in the menu.

The Components pane



If you do not see the Components pane, click the **Show/Hide** button on the components tool bar and select **Components**.

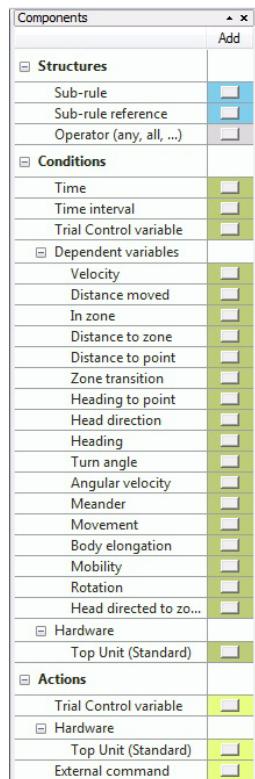


Figure 7.5. The **Components** pane for Trial Control.

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With the **Components** pane (see Figure 7.5.) you choose the blocks that build up your trial control rules. Not all the components listed below may be available on your screen, depending on what EthoVision XT license you have on your computer (see page 179).

- **Structures**

- **Sub-rule** – To define a subroutine that can be called from a specific point of the Trial Control sequence.

- **Reference** – To insert a call to a sub-rule within a sequence of instructions.

- **Operator** – To combine two or more conditions in such a way that an action is taken when All, Any or "N of All" conditions are met. See page 196.

- **Conditions** (see page 190):

- **Time** – To define a condition based on time.

- **Time interval** – To define a condition based on a time interval.

- **Trial Control variable** – To define a condition based on a Trial control variable.

- **Dependent variables** – To define a condition based on a variable that describe the animal's behavior, for example velocity, presence in a zone, movement etc.

Under **Dependent variables**, you can view the list of variables available.

- **Hardware** – To define a condition based on the state of a hardware device (only with the **Trial and Hardware Control add-on**).

- **Actions**

- **Trial Control variable** – To define an action on a Trial control variable. See page 193.

- **Hardware** – To define an action on a hardware device (only with the **Trial and Hardware Control add-on**).

- **External command** – To control external applications. With an External command action you can, for example, start an external application or run a batch file.



For more information on Sub-rules, References to sub-rules and hardware devices, see the **EthoVision XT Trial and Hardware Control Manual** that you can find on your installation DVD.

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How to use the Components pane

To define a sub-rule, condition, action or operator:

- Double-click its name.
- Click the button next to it.
- Drag the name from the **Components** pane to the Trial Control window.

A new Trial Control box appears in the top-left corner of the **Trial Control** window. Insert the new box in the sequence of boxes (see page 186).

For the complete procedure for Programming Trial Control, see page 189.

The Trial Control window

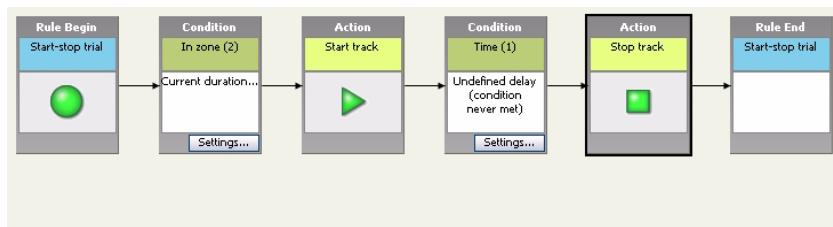


Figure 7.6. The Trial Control window, with the default *Start-Stop trial* rule.

The Trial Control window contains the sequences of instructions (*rules*) currently present in the Trial Control Settings. When you create a new **Trial Control Settings** profile, the **Trial Control** window contains the default *Start-Stop trial rule*, consisting of six boxes connected by arrows (see page 202).

You can then define your own conditions in the *Start-Stop trial rule* that determine the start and stop of data recording.

For more information:

- About programming Trial Control – See page 189.
- About the Start-Stop trial rule – See page 202.

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Grid

The trial control boxes automatically snap to a grid. You can change this by clicking the **Show/Hide** button on the component tool bar and selecting/deselecting the two Grid options (**Snap to Grid** and **Show Grid**).

Zoom

The component tool bar of the Trial Control Settings shows three zoom icons:

- **Zoom in**  – You can keep zooming in until all trial control boxes fit in the window.
- **Zoom out** .
- **Zoom to fit**  – Clicking this button fits all trial control boxes into the window.



The Trial Control window is ‘dynamic’: this means that it expands when you move trial control boxes to the right. In this case, you can navigate ‘from left to right’ in the Trial Control window by using the scroll bar at the bottom. Use the **Zoom to fit** button in the component tool bar to make all trial control boxes visible.

Working with Trial Control boxes

A Trial control box has the following information:

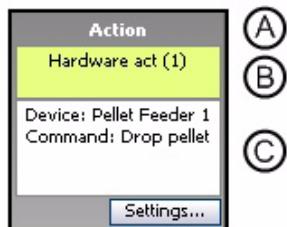


Figure 7.7. An example of a Trial Control box.

- **A** - Type of control (**Rule Begin/End**, **Action**, **Condition**, **Operator**, **Reference**). You cannot change this text.

Trial Control

- **B - Name** – Text describing the control. To change this text, click the **Settings...** button and enter the text under **Name**, for example *Drop one food item*. You can also add a longer description under **Comment** (this is not shown).
Names of Trial Control boxes must be unique, unless you make a copy of an existing box (see page 198).
- **C - Properties** – Depending on the type of control, it contains the option chosen, the formula or the command to be given, or the sub-rule that reference refers to.

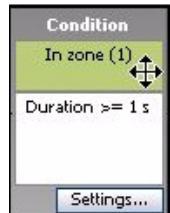
Colors

Trial control boxes have different colors:

- Blue - for the Start-Stop trial rule, sub-rules and sub-rule references.
- Olive green – for conditions.
- Light green – for actions.
- Grey – for operators.

Moving a box

- 1 Hover the mouse on the margin or the colored area of the box. The mouse cursor changes to a four-headed arrow.

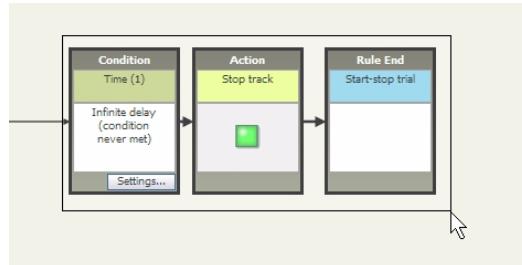


- 2 Drag the box to the position you require.

Moving a group of boxes

- 1 Draw a box around the boxes you want to move (see figure below) or click on the boxes you want to select while holding the **Ctrl** key.

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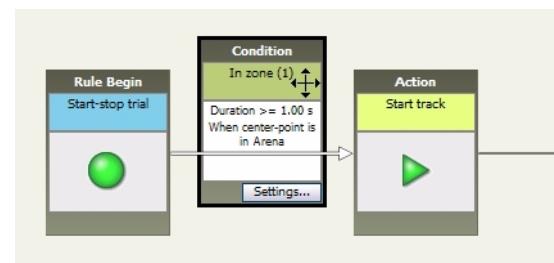


As a result, the selected boxes get a gray, dark border.

- 2 Hover the mouse on the margin or the colored area of one of the selected boxes. The mouse cursor changes to a four-headed arrow.
- 3 Drag the group of boxes to the position you require.

Inserting a box in a sequence

- 1 Drag the **Trial Control** box between two boxes until the connecting arrow turns white.

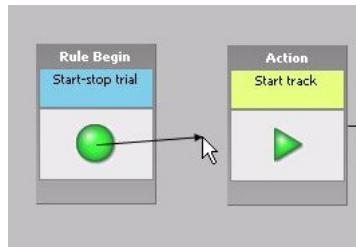


- 2 Release the mouse button. The new box is inserted.

Connecting two boxes

- 1 Point the mouse to the center of the first box, press and hold the left mouse button and drag toward the center of the other box.

Trial Control



- 2 Release the mouse button when the pointer has reached the center of the other box. The two boxes are connected.
 - You cannot create connections from the **Rule End** box to any other box, nor from any box to the **Rule Begin** box.
 - **Operator** boxes can have one, two or more input arrows; all other boxes have no more than one input arrow.
 - All boxes can have 1 or more output arrows, pointing to different boxes.
 - You cannot create a circular sequence of Trial Control boxes.

Modifying the settings in a box

Follow the instructions below when you have inserted a **Trial Control** box, and you want to modify the properties of that box.

- 1 Locate the **Trial Control** box that specifies the condition or operator you want to change. You can find the name of the condition/operator in the upper green/grey area of the box.
- 2 Click the **Settings** button in the lower part of the box.
- 3 Make the appropriate settings in the window that appears (see the corresponding section above for defining conditions and operators).

Deleting a box

- 1 Click the title of the box. The box border is highlighted.
- 2 Press **Delete**.

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Deleting a group of boxes

- 1 Draw a box around the boxes you want to delete or click on the boxes you want to select while holding the **Ctrl** key.
- 2 Press **Delete**.



You cannot delete the **Rule Begin**, the **Rule End** box, the **Start track** box and the **Stop track** box.

Deleting a connecting arrow

- 1 Click the arrow you want to delete. The arrow turns bold to show it is selected.
- 2 Press **Delete**.



You cannot delete the arrow connecting the **Stop track** box and the **Rule End** box.

Exporting Trial Control Settings

You can export an image of the Trial Control Settings:

- 1 Click the **Export image** button in the component tool bar.
- 2 Select a location to save the image to, type in the **File name** or accept the default one and select an image type from the **Save as type** list.
- 3 Click **Save**.



The complete Trial Control window is exported, irrespective of the zoom factor.

Maximum trial duration pane

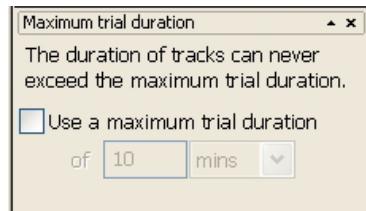


Figure 7.8. The **Maximum Trial duration** pane.

In the Maximum trial duration pane you define the maximum duration of the trials. For further information, see page 199.



If you do not see this pane, click the Show/Hide button on the component tool bar and select the **Maximum Trial Duration**.

If the text in this pane is greyed out, the Trial Control Settings are read-only.

7.3 Programming Trial Control



If you just want record data for a specific time, you can do so by setting the *Maximum trial duration* (page 199).

Procedure

- 1 Before defining Trial Control in the program, it is helpful to draw your experimental procedure as a flow diagram, where each block represents an action or a condition which, when met, triggers other actions or conditions.
- 2 From the **Setup** menu, select **Trial Control Settings**, select **New**, enter a name of the new Trial Control Settings or accept the suggested one, and click **OK**. The default *Start/Stop trial rule* appears on the screen.
- 3 Build the Trial Control sequence outlined in step 1, using the components available.

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- To define a *Condition*, click one of the buttons under **Conditions**.
→ See page 190
 - To define an *Action*, click the button under **Actions**.
→ See page 192
- Insert the box in the appropriate place in the sequence.
- 4** Test the Trial Control sequence.
→ See page 200
- 5** Apply Trial Control to your trials.
→ See page 200



- When you create a new action or condition, and another of the same type has already been defined in this or other Trial Control Settings, a message appears asking you whether you want to create a new element or make a copy of the existing element. For more information, see page 198.
- You can also combine multiple conditions. To combine multiple boxes, see page 196.

Using Conditions

A Condition is a statement that EthoVision checks during the trial. When the Condition is met (*True*), the program evaluates the next Trial Control element (another condition, an action or a reference to a sub-rule).

Examples of conditions (in *italics*):

- *When the rat reaches the platform*, stop tracking.
- *When the mouse is detected in the open field*, start tracking.
- *When the animal enter zones A*, increment the variable **Count** by 1.

How to define a condition

- 1** In the **Components** pane under **Conditions**, locate the type of condition you want to define.
-  **2** Double-click the condition name or click the button next to it.

- 3 If the **Add a condition** window appears, it means that there is at least one condition of the same type in your experiment. You are asked to choose between creating a new condition, or re-use an existing one (see page 198). Choose the option you require and click **OK**. If this window does not appear, skip this step.
- 4 Next to **Condition name**, type in the name you want to give to the condition, or accept the default name.
- 5 Specify the condition properties.
- 6 Enter a **Comment** (optional), then click **OK**.
- 7 Insert the condition box in the sequence.



- If the condition is complex (for example, "stop the trial either if the rat has reached the platform or it has been swimming for 60 seconds"), then you must define separate conditions and combine them (see page 196).
- See also the examples on page 204.
- For a detailed overview of conditions, see the **EthoVision XT Trial and Hardware Control Manual** on your installation DVD.

Types of condition

- **Time** – Helps you defining a time interval that must elapse before an action be taken.
Example – Start tracking *after a delay of 2 seconds*, or start tracking *at 12h00*.
- **Time interval** – This condition makes sense when it is combined with another condition.
Example: Stop tracking when the animal is found in Zone A (In zone condition) *between 5 and 10 minutes* (time interval condition).
- **Trial Control variable** – Helps you make a comparison between a Trial Control variable and a value, another variable or a formula at the time the condition *becomes active* (for the meaning of *becomes active*, see page 176).
Example – Stop tracking *when the variable Counter has reached 10*.
- **Dependent variables** – To define a condition based on the behavior of the subject. Choose one of the dependent variables to create the condition.
Example 1 – Stop tracking *when the subject has visited 10 times in the Target zone* (**In zone** condition).
Example 2 – Stop tracking when the subject has been walking for more than 5 minutes (**Movement** condition).

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- **Hardware** – To define a condition based on the signal given by a hardware device. To use hardware devices with EthoVision, you must have the **Trial and Hardware Control** add-on. See the **EthoVision XT Trial and Hardware Control Manual** on your installation DVD.



For a detailed overview of conditions, see **Overview of conditions** in the EthoVision XT Trial and Hardware Control Manual, which you can find on your installation DVD.

Using Actions

An Action is a command that EthoVision carries out during acquisition and that influences the trial.

Examples of actions (in *italics*):

- When the animal is detected in the arena, *start tracking*.
This is an example of system actions (start tracking and stop tracking).
- When the animal enters the maze's left arm, *do C= C+1*.
This is an example of an action taken on a Trial Control variable. See page 193.
- When the animal comes out of the shelter, *start a recording with the Noldus Media Recorder*.

The actions *Start tracking* and *Stop tracking* are already defined in the *Start-Stop trial rule*. Beside these, you can define actions on Trial control variables.



- You cannot create additional actions of the "Start track" and "Stop track" type, nor can you delete the existing ones.
- If your EthoVision license includes the **Trial and Hardware Control** add-on module, you can also define actions on hardware devices. See the **EthoVision XT Trial and Hardware Control Manual** on your installation DVD.

How to define a Trial Control variable

- 1 In the **Components** pane, click the button next to **Trial Control variable** under **Conditions** or **Actions**. Next, click the **Variables** button.
- 2 The **Trial Control Variables** window lists the variables currently in the experiment (also those defined in other Trial Control Settings). To add a new variable, click **Add variable**.

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- 3 A new row is appended to the table. Under **Name**, type in the name you want to give to the variable. Under **Initial Value**, enter the value of this variable at the start of the trial (default: **0**).
- 4 Click **OK**. In the **TC-variable action/condition** window, define the action or condition you require. Click **Cancel** if you do not want to create a condition or action based on this variable at this point.



- To delete a variable, click the variable name in the **Trial Control Variables** window and click the **Delete variable** button.
- To rename a variable, click the variable name in the **Trial Control Variables** window and edit this name.
- The default name of a new trial control variable is **VarN**, where N is a progressive number.
- The variable name cannot contain blank spaces.

How to define an Action on a Trial Control variable



- 1 In the **Components** pane, under **Actions** click the button next to **Trial Control variable**.
- 2 If the **Add an action** window appears, it means that there is at least one action of the same type in your experiment. You are asked to choose between creating a new action, or re-use an existing one (see page 198).
- 3 Next to **Action Name**, enter the name of the action (for example, *Increment Counter*) or accept the default name.
- 4 Under **Action to perform**, select the variable from the list. You can also create the variable by clicking **Variables** if you have not yet done so.
- 5 Next to the "=" symbol, do one of the following:
 - To assign the same value of another variable (for example **A = B**), select the other variable (**B**) from the second list.
 - To enter a formula, click the double-arrow button, select the operator from the list and specify the formula in the second and third lists. For example, **A= A + 1**.



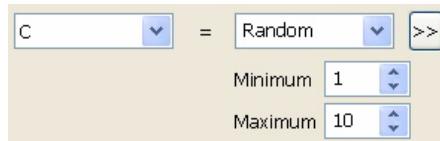
Action to perform

Variables...

A = A + 1 <<

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- To assign a random value, select **Random** from the second list, and select the **Minimum** and **Maximum** limits (only integer values, 0 up to 999) in which the random value must lay.



- Enter a **Comment** (optional), then click **OK**.
- Insert the resulting **Action** box in the Trial Control rule.

Notes



- If your setup includes multiple arenas, each arena receives an instance of the variable. Thus, a variable can have different values in different arenas.
- You cannot combine **Random** with a formula (for example, to compute $A = Random + 1$). The equivalent solution is the following: define first an action **B = Random**, and then one more action **A = B + 1**. Place the two resulting Action boxes in sequence.
- To generate a random value, the maximum limit must be greater than the minimum.

How to define an External command



- In the **Components** pane, under **Actions** click the button next to **External command**.
- Next to **Action Name**, enter the name of the action (for example, *start recording*) or accept the default name.
 A green circular icon with a question mark symbol, representing help or additional information.
- Under **Actions to perform**, select which file you want to run by clicking the ellipsis button.
 A blue square icon with three dots, representing an ellipsis button.
- Next, select one of the file types from the list:
 - Executables (*.exe)**.
 - Batch Files (*.bat)**.

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- All Files (*.*).
- 5 Locate the file and click **Open**.
- 6 Optionally, enter a **Command line option**.

Example - You carry out live tracking during a 24-hour period and you want to make a recording in the Media Recorder but only when the animal leaves the shelter (defined as a Hidden Zone, where it spends most of its time). First, start up the Media Recorder using an External command box: select **MRCmd.exe** as the Executable to run and enter **/E** as a Command line option to start the Media Recorder. Next, insert a Condition *Out of shelter* and combine this with a Time condition to make sure that the Media Recorder is started before recording starts (see Figure 7.9. for an example). Then, insert an External command box: select **MRCmd.exe** as the Executable to run and enter **/R** as a Command line option to start recording with the Media Recorder. Similarly, you can stop recording (Command line option: **/S**) when the animal enters the shelter again.



There may be a delay between the command Start Recording and the moment the Media Recorder actually starts recording. Run a test recording to test how long this delay is.

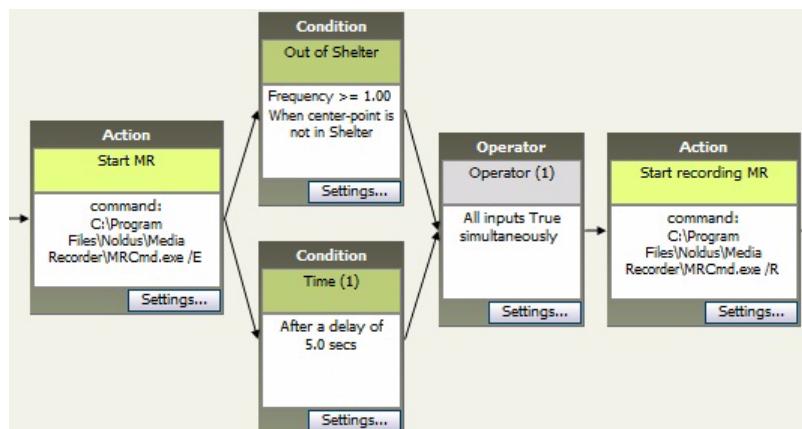


Figure 7.9. Example of the External command action to start a recording with the Media Recorder when the animal leaves a shelter. The left *Start MR* action box starts up the Media Recorder. The *Start recording MR* action box on the right starts the recording when both the *Out of Shelter* and *Time(1)* conditions are true, that is, the center-point of the animal has left the shelter at least 5 seconds after the Media Recorder was started.

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Using Operators

The Operators help you combine actions, conditions and sub-rules in various ways. For example:

- *When at least one of the two conditions A and B is met, then do ...*

This is an example of conditions combined by an operator of the "Any" type (*OR logic*).

- *When two conditions are met at the same time, then do ...*

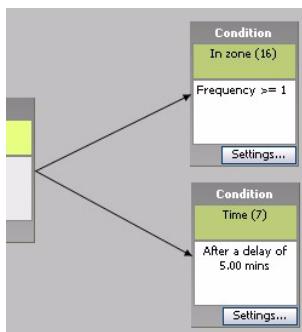
This is an example of conditions combined by an operator of the "All" type (*AND logic*).

- *When at least/at most/exactly 4 of 8 conditions are met, then do ...*

This is an example of conditions combined by an operator of the "N of All" type.

To combine conditions/actions/rules:

- 1 Define the conditions/actions/rules that you want to combine. Place them in your Trial Control sequence as parallel branches. The connecting arrows must originate from the condition/action that precedes the combination of elements you want to define.

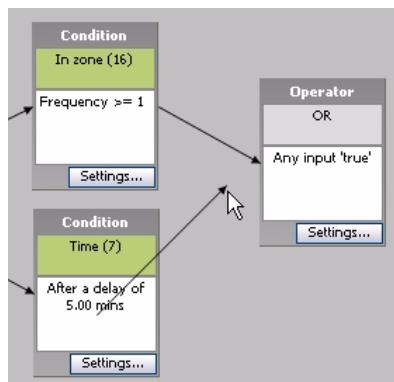


- 2 In the **Components** pane under **Structures**, double-click **Operator** or click the button next to it.
- 3 If the **Add an operator** window appears, it means that there is at least one operator of the same type in your experiment. You are asked to choose between creating a new operator, or re-use an existing one. If this window does not appear, skip this step.
 - **Create a new operator** – A new operator is created.
 - **Reuse an existing operator** – Select the name of the operator already present in your experiment. See page 198 for more information.

Trial Control

Click **OK**. The **Operator** window appears.

- 4 Under **Name**, enter the **Operator name** or accept the default name **Operator (n)**, where **n** is a progressive number.
- 5 Under **Operator triggers when**, select the option that applies:
 - Any (at least one) of the inputs is 'true'.
 - All inputs are simultaneously 'true'.
 - N of All inputs are simultaneously 'true'.Where 'true' means a condition met, an action carried out, or a sub-rule finished (depending on the elements you want to combine).
 - If you choose the third option, specify how many inputs must be 'true': = (exactly equal to), **not=** (not equal to), \geq (at least), \leq (a maximum of), etc. Specify the number in the box.
- 6 Enter a **Comment** (optional) to describe this operator, and click **OK**.
- 7 A new **Operator** box appears in the Trial Control. Place the box right of the elements defined in step 1, and connect each element (or ending element, in the case of a sequence) to the operator.



- 8 Connect the operator to the next element that should be activated.



- Names of operators must be unique in your experiment. You cannot define two operators with the same **Operator name**, even if these are defined in two different Trial Control Settings.
- An Operator can also have just one input box. In that case the operator is of no use, because control passes immediately to the next box as soon as the

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input condition becomes true or the input action is carried out. EthoVision informs you about this.

Re-using Trial Control elements

All elements of Trial Control (conditions, actions, operators, sub-rules and sub-rule references) can be duplicated that you have defined in other Trial Control Settings can be duplicated and re-used in your current Trial Control Settings to reduce your time spent editing.

To re-use all the elements defined in your current Trial Control Settings profile, make a copy of it: right-click the profile in the Experiment Explorer and select **Duplicate**.

How to re-use a Trial Control element



- 1 Click the button next to the category of element that you want to re-use.
- 2 The **Add** window appears. Select **Reuse an existing condition/ action**.
This window does not appear when the experiment contains only one Trial Control Settings profile, or the experiment contains more Trial Control Settings profiles but none of them contains an element of the same type as that you have chosen.
- 3 Select the name of the existing element from the list next to the option.
The second list shows the Trial Control Settings profile that contains that element. If the element is present in multiple Trial Control Settings, choose the appropriate one from the list.
- 4 Click **OK**.
- 5 A window appears for the type of element chosen. The **Name** and settings specified here are the same as in the element chosen in step 3.
 - To create an identical copy of the element, click **OK** and go to step 7.
 - In all other cases, edit the settings and click **OK**, then go to step 6.
- 6 If you have changed any property of the new element (including name and comment), a window appears showing two options:
 - **Apply the new settings only in the current trial control profile.**
 - **Apply the new settings in all writable Trial Control profiles.**

The program asks you whether you want to apply the properties only to the new copy, or to extend those changes to the original elements in all Trial

Trial Control

Control Settings that are writable (that means, not locked after acquisition). Choose the option you require and click **OK**.

- 7 Insert the resulting box in the Trial Control sequence.

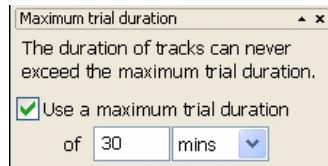


- If you choose the option **Apply the new settings in all writable trial control profiles**, changes are not made in those profiles made read-only after data acquisition.
- You cannot re-use a Trial Control element from the same Trial Control Settings. This is because the Trial Control elements must be unique in order for correct analysis to be done.

Defining a maximum Trial duration

If the conditions to stop the trial (see page 202) are never met, EthoVision XT waits indefinitely and the trial never ends. To prevent this from happening, you can define a maximum trial duration. For example in a novel object test, if you define a condition 'stop the track when the mouse enters the zone with the familiar object' it may happen that the mouse completely ignores the familiar object and only pays attention to the novel object.

- **Use a maximum trial duration** – Select this check box to define a maximum trial duration and enter the maximum duration of the trial (in hours, minutes or seconds).



When you set a Maximum trial duration, the trial stops when that time has been reached, regardless of whether one or more rules are being evaluated.



Instead of using a **Maximum trial duration**, you can also define a condition based on time and place it immediately before the **Stop track** box (see page 202). However, there are two important differences:

- If you use **Maximum trial duration**, the program counts the time from the start of the trial (this is indicated by the **Start-Stop trial** box). Instead, a condition placed immediately before the **Stop track** box considers the time from the start of data recording (this is indicated by the **Start track** box). The two starting points may not be the same if you have a condition between

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Start-Stop trial and **Start track** that makes data recording start some time later than the trial.

- With a multi-area setup, a **Maximum trial duration** stops the trial (and thus data recording) in all the arenas simultaneously, even when data recording had started at different times. Instead, a time condition placed between the **Start track** and the **Stop track** box stops data recording in one arena when the condition is met in that arena. This means that you can have data recording stop at different times in different arenas.

For example, you set to start data recording when the animal is detected for the first time (*In zone* condition). Next, you define a delay condition of 5 minutes immediately before the **Stop track** box. If the animals are detected for the first time at different times in different arenas, data recording stops at different times too because of the constant delay for all arenas. The trial ends when the recording stops in the last arena.

Testing the Trial Control sequence



It is not easy to make a complex Trial Control sequence work right the first time. To check that Trial Control works as expected, see **Testing the trial control sequence** in the **EthoVision XT Trial and Hardware Control Manual** on your installation DVD.

Applying Trial Control to your trials

To apply Trial Control to your trials, make sure that the appropriate Trial Control Settings profile is highlighted in blue in the Experiment Explorer.



Test your setup thoroughly before carrying out the actual trials (see above).

- For setups with multiple arenas** – Trial Control is applied to each arena independently.
- Locked Trial Control Settings** – When a Trial Control Settings profile is used for acquiring at least one trial, it becomes locked. Locked settings are indicated by a lock symbol in the Experiment Explorer, and cannot be edited. To edit a locked Trial Control Settings profile, make a copy of it and edit this copy. See page 581.

Trial Control

- **Tracking from video files** – When you track from video files, Trial Control checks conditions using video time instead of the real time.
 - **Conditions based on Delays** – If you select the **Detection Determines Speed** option, Trial Control is carried out at the speed set by EthoVision in order not to skip video images (see page 273). This results in the video playing faster or slower than normal (1x), depending on the processor load necessary to detect subjects. For example, if detection requires little processor work, the program tracks the subject faster than normal. A Delay condition (for example, Delay 60 s) is therefore met earlier than at real time.
 - **Using Clock time** – If you define a condition based on clock time, or schedule a sub-rule with Clock time, this is translated into the **video start time**, that is, the date and time the video file used for tracking was created.

Example 1 – You set a Time condition to start tracking **After clock time 11:30**. The video file was created on March 6, 2008 at 11:00. once you start the trial, the condition is met half an hour later in the video.

If you had set to start tracking **After clock time 10:30**, tracking would start immediately after starting the trial.

Example 2 – You set a sub-rule to start at **10:00 (1st day)**. The video file was created on March 6, 2008 at 11:00. Once you start the trial, the sub-rule never starts, because the planned start occurs before the initial time of the video. To make a sub-rule start when tracking from that video, set the start time between 11:00 and the video end time.
- **Recording video, then tracking** – If you choose to record video first and then acquire data from the resulting video file (see page 290):
 - When recording video only, Trial control is turned off. You get an appropriate message when selecting the **Save video file only** option in the **Acquisition** window.
 - When you track from that video, Trial Control for Start-Stop is activated, but you cannot control hardware devices.
- **Re-doing a trial** – For video files recorded with EthoVision, you can re-do the corresponding trial (see **Redo trials** in Chapter 9). However, if you re-do a trial the Trial Control log files recorded with the previous instance of the trial are deleted.
- **Stopping a trial** – When you stop the trial, all rules active in the Trial Control Settings are ended immediately, and hardware devices are reset.

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7.4 The Start-Stop trial rule

The Start-Stop trial rule is displayed on your screen when you create or open Trial Control Settings. It can be recognized by the first box with a green circle: With this rule, you control the start and stop of data acquisition (tracking). You can only modify the initial Start-Stop trial rule.

You cannot:

- Delete the Start-Stop trial rule, including the Rule Begin, Rule End, Start track and Stop track boxes.
- Define an additional Start-Stop trial rule.



Always keep a Condition box that determines when the track is stopped right before the Stop track Action box, otherwise the trial stops immediately after you start the trial.

The default Start-Stop trial rule

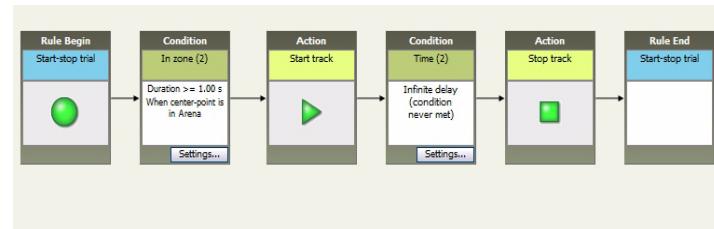


Figure 7.10. The default Start-Stop trial rule. See explanation in the text.

The default Start-Stop trial rule is a sequence of six boxes:

- **Rule Begin - Start-Stop trial** – Activated when you start the trial (from the Acquisition menu, select **Start Trial**, or click the **Start Trial** button, or press **Ctrl+F5**). Once you start the trial, control passes to the next box.
- **Condition - In Zone - Duration >= 1.00 s When Center-Point is in Arena** – This is the default Start track condition. It is fulfilled when center point of the subject (or of any subjects, in the case of an arena with multiple subjects) has been detected in the arena for 1 second after you started the trial.

If you start the trial and the animal is not detected yet, the program waits until

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it detects the animal for 1 second, then it starts tracking.

The condition is applied separately for each arena. This means that tracking can start at different times in different arenas in the same trial.

- **Action - Start track** – Activated when the condition on its left side is met. Once this box is activated, data recording (tracking) starts. If the condition placed between the Start-Stop trial box and this box is not met immediately, tracking starts later than the time you start the trial.
- **Condition - Time - Infinite delay (condition never met)** – This is the default Stop track condition. This condition is never met. The trial stops when you give the Stop command or the time exceeds the Maximum trial duration (when this has been set).
- **Action - Stop track** – Marks the end of all tracks (and trial).
- **Rule End - Start-Stop trial** – This box is just the delimiter of the rule, it does not take any action.

An important distinction: Trial vs. track

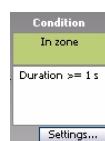
- **Trial** – A **Trial** can be viewed as a container for the data collected in one recording session. It starts when you give the Start command in acquisition and stops when the tracks for all arenas and subjects have stopped.
- **Track** – A **Track** corresponds to the actual recording of a subject's position and behavior. The start of a track may or may not coincide with the start of the trial. This depends on your **Trial Control Settings**. If you use the default **Trial Control Settings**, the track starts 1 second after the animal has been detected in the arena and stops when you stop the trial.
- A Trial may contain one or more tracks. For example, if you track two subjects simultaneously, each trial includes two tracks, one per subject. Similarly, if your setup contains four arenas with two subjects each, each trial includes 4 arenas x 2 subjects = 8 tracks.
- In a multiple-area setup, the end of a track does not necessarily mean the end of the trial. The trial ends when all tracks come to an end.

Customizing the Start-Stop trial rule

Modifying the Start track condition

The default Start track condition is an **In zone** condition.

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- To modify that condition, click the **Settings** button. In the window that appears, click **Settings** and specify the zone in which the animal should be in order for the program to start tracking.
- To use another condition (for example: start recording exactly 1 minute after starting the trial), delete first the current condition (click that box and press **Delete**) and insert the new one. For an overview of conditions, see page 191.
- To start recording as soon as you start the trial, delete the Start track condition: Click the box immediately before the **Start track** box and press **Delete**.

Modifying the Stop track condition



The default Stop track condition is a **Time** condition.

- To modify that condition, click the **Settings** button, and choose the option you require.
- To use another condition, delete first the current condition (click that box and press **Delete**) and insert the new one (see page 190).



If you want to stop tracking when a specific time has elapsed, see page 199.



Keep at least one condition between **Start track** and **Stop track**. If you do not do this, tracking stops immediately after tracking starts, resulting in no data.



For more information on conditions, see **Overview of conditions** in the **EthoVision XT Trial and Hardware Control Manual**.

Examples of Start-Stop trial rules

General

● Starting data recording at a specific time

You want to start recording at a time you are not in the lab, for example at 23:00 h.

Delete the default Start track condition (see page 203). Define a **Time** condition (see page 190). Select **After clock time** and enter 23:00:00. Click **OK** and place the resulting box before the **Start track** box.

Before leaving the lab, click the green button to start the trial. The program waits till 23:00 to start data recording.

Trial Control

- **Stopping data recording after the maximum time has elapsed**

Click **Settings** in the **Condition** box immediately before the **Stop track** box. Select **After a delay of** and enter the maximum time. Instead of using a Time condition, you can also use the Maximum trial duration option (see page 199).

Open field with multiple arenas

Starting data recording when the animal has been detected in the open field. The start command is given to each arena independently.

In this setup, four open fields are treated as separate arenas. You want to start acquisition when the animal is detected in the open field independent of what happens in other arenas. This can be achieved by using the default Start-Stop trial rule. As soon as an the subject is detected in an arena, tracking starts for that arena, not the others. This way you do not have to release all the animals at the same time.

Water maze

- **Stopping the trial when the animal has found the platform.**

In the Arena Settings, make sure that the platform has been defined as zone. In the Trial Control Settings, delete the default Stop track condition (see page 203). Next, define a **In Zone** condition (see page 190).

- If you want the program to stop recording as soon as the animal is over the platform, select **Frequency** as **Statistic** and choose ≥ 1 . Click **Settings** and select the platform zone.
- Sometimes the animal swims over the platform, but it does not stop there. In such cases the program would stop recording while the animal has not 'found' the platform. Instead of selecting **Frequency**, choose **Current duration** and the minimum time the animal must stay on the platform (for example, 3 s). Click **Settings** and select the platform zone.

Click **OK** and place the resulting box before the **Stop track** box.

- **Stopping the trial either when the rat has found the platform, or when it has been swimming in the water maze for 60 seconds.**

The Arena Settings and the condition "rat has found the platform" are similar to those in the example above. The condition "rat swimming for 60 s" can be translated to "delay from tracking ≥ 60 s".

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The track stops when either condition is met. The two conditions are combined with **OR** logic (see Figure 7.11.).

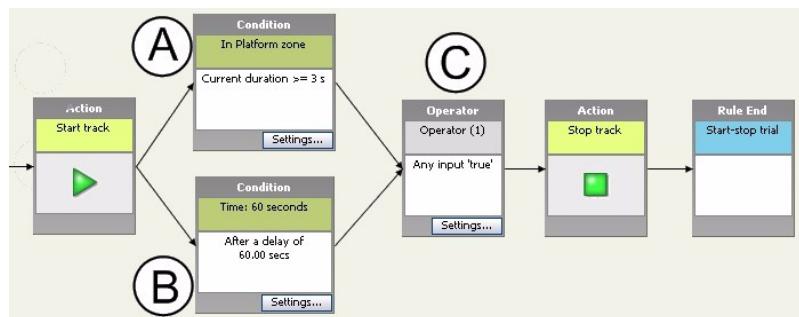


Figure 7.11. Example of a Start-Stop trial rule for a water maze. The trial stops when the animal has been in the platform zone for at least 3 s without break, or the time since the start of tracking is 60 s.

A - **In zone** condition that specifies that the animal must be for at least 3 seconds over the *Platform* zone. Select **Current duration** \geq 3s.

B - **Time** condition that specifies a delay of 60 s since the track started.

C - 'Any' operator box.

This solution results in tracks of different duration: less than 60 s for the animals that found the platform, and 60 s for the others.



Instead of two **Condition** boxes in the example above, you can also define the **In zone** condition box and set a **Maximum Trial duration** (see page 199).

Eight-arm radial maze

Stopping the trial when the animal has been in four arms within 10 minutes.

This can be done by combining eight conditions, that is, that the animal must be in the arm specifying that at least four must be met, no matter which arm the animal visits.

- 1 Create a **In zone** condition (see page 191) and specify that the Frequency for Arm 1 must be ≥ 1 . That is, the animal must have visited Arm 1 at least once. Do the same for each of the other arms.
- 2 Connect the resulting eight condition boxes in parallel using the **N of All** operator (see Figure 7.12.).

Trial Control

- 3 Set the **Maximum trial duration** (see page 199) to 10 minutes to stop tracking in the case the animal fails to visit four arms in the meantime.

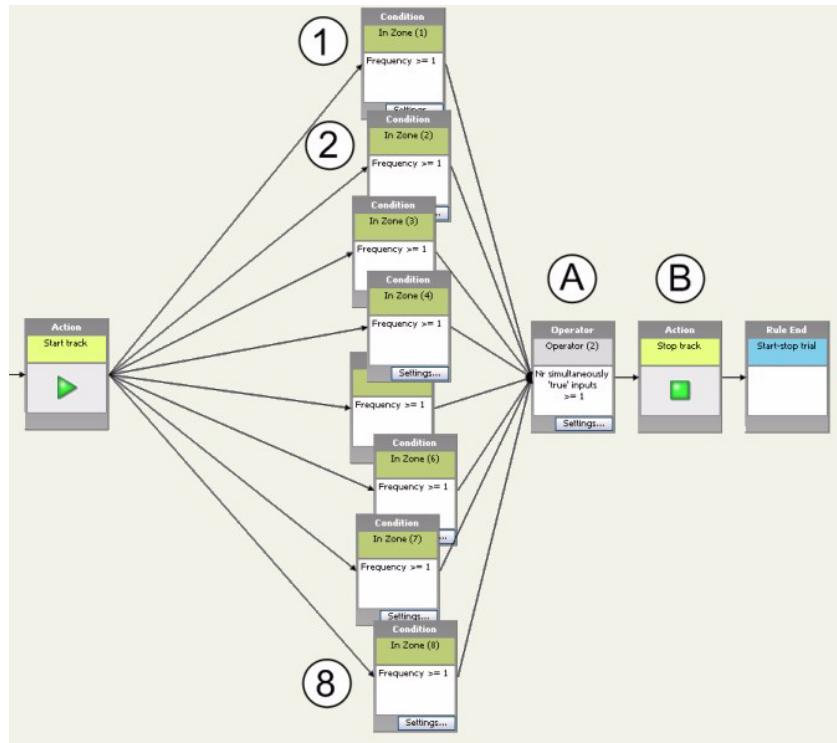


Figure 7.12. A Trial Control sequence for an eight-arm radial maze. The trial must stop when the animal has visited four of the arms at least once.

1, 2,... 8 - In zone condition boxes for Arm 1,2,... 8 respectively. A condition is met when the **Frequency** of In zone for that arm is greater than or equal to 1.

A - Operator that checks that at least four of the eight conditions are met.

B - **Stop track** box. When four conditions are met, the trial is stopped.

For more information on "**N of All**" operators, see page 196.

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7.5 Analysis of Trial Control data

With the EthoVision analysis function you can analyze the events that occur during a trial by means of statistics or time plots.

- Trial Control events – For example, when exactly does a condition become *true*?
- Trial Control states – To analyze the time between two Trial Control events. For example, how much time elapsed from the moment a condition became *active* to when the condition became *true*?

Analysis of Trial Control data is generally carried out for testing purposes, or to analyze the subject's response to presentation of stimuli (for instance, in conditioning tests).

To analyze Trial Control data, in the Analysis Profile choose **Trial Control event** to analyze simple events, or **Trial Control state** to analyze time intervals between specific events. Next, calculate statistics (from the **Analyze** menu select **Calculate Statistics**) or visualize the data (from the **Visualize** menu select **Plot Integrated Data**).



For more information on analysis of trial control data, see **Analysis of Trial Control data** in the EthoVision XT Trial and Hardware Control Manual, which you can find on your installation DVD.



- If you want to analyze the behavior of your subjects, see Chapter 14.
- If you want to calculate statistics/visualize data within intervals defined by Trial Control events, then you must first define those intervals as **Nesting intervals** in the Data Profile. See page 424.

Exporting Trial Control data

You can export Trial Control events (for example, *Action becomes active*, or *Condition becomes true*) and Trial Control states (for example, **From Action becomes active To Condition becomes true**). For more information, see page 571.

8

Configuring Detection Settings

This chapter is about:

- **Why configure Detection settings** – Short introduction to the Detection settings.
→ See below
- **Configuring Detection settings** – To specify how EthoVision XT must detect the subject(s) and/or body points.
→ See page 212
- **Working with nose-tail base detection** – To optimize detection of nose-point and tail-base of rodents.
→ See page 258
- **Customizing the Detection settings screen** –
→ See page 264

See also *Managing Settings and Profiles* (page 581).

8.1 Why configure detection settings

EthoVision XT needs a few criteria to track moving subjects.

For example, you need to specify how different the subject is from the background in terms of gray scale or color values, you need to select a method to distinguish the subject from the background, how many images per second you want EthoVision XT to analyze and to set the average subject size. Such criteria make up your **Detection Settings**.

You can define different Detection settings in the same experiment. For example, you can have one set for detecting white animals, and another to detect dark ones. For more information, see page 581.

Which settings are available in the Detection Settings screen first of all depends on the version of EthoVision XT:

- **EthoVision XT Base version** – In this version, you can track the center-point of the body of a single animal. For the detection of the animal's body, four detection methods are available. The base version also allows tracking of a color marker on a single animal; in this case the color marker is treated as the center-point of the animal.
- **EthoVision XT Multiple Body Points Module** – In this version, you can track the center-point, the nose-point and the tail-base of a single animal. For the detection of multiple body points, three detection methods are available.
- **EthoVision XT Social Interaction Module** – This version allows you to track two or more animals in one arena. You can use Color marker tracking or Marker assisted tracking. You can use this version in combination with the Multiple Body Points Module to study social interactions in detail.



Tracking multiple subjects requires that you carefully adjust the Detection Settings. Make sure you follow the General procedure of configuring Detection Settings in the order described below (see General procedure on page 212).

We recommend to only use Tracking from video files if you use the Multiple Body Points module in combination with the Social Interaction Module.

Opening the Detection Settings



Before opening the Detection Settings, make sure that you have valid Arena Settings.

To open the Detection Settings, do one of the following:

- In the Experiment Explorer, click the folder **Detection Settings** to expand it and click on one of the **Detection Settings** to open the Detection Settings screen.
- From the **Setup** menu, select **Detection Settings**. Select **Open**, select one of the Detection Settings from the list and click **OK**.

Result – The Detection Settings screen opens. By default, the **Detection Settings** window, the **Video Source** and **Playback Control** window are displayed. You can use the **Show/Hide** button on the component tool bar to change the view settings.

The Detection Settings window



Depending on the Number of Subjects per Arena and the Detected Body Points you selected in the Experiment Settings (see page 96), the layout of the Detection Settings window differs.

The Detection Setting window contains the following sections (see also Figure 8.1.):

- **Methods (A)** – This section contains the methods for Subject Detection, Nose-Tail detection (if applicable) and options to Use scan window and to apply Marker assisted tracking.
- **Detection (B)** – In this section you configure the Subject Detection settings.
- **Subject Identification (C)** – This section is only available when you have multiple animals.
- **Video (D)** – In this section you can select your video if you track from video, adjust video settings if you track live, set the Sample rate and Pixel smoothing.
- **Subject Size (E)** – In this section you set the Subject Size for one or more animals.
- **Subject Contour (F)** – In this section you can erode and dilate the detected body to optimize detection.

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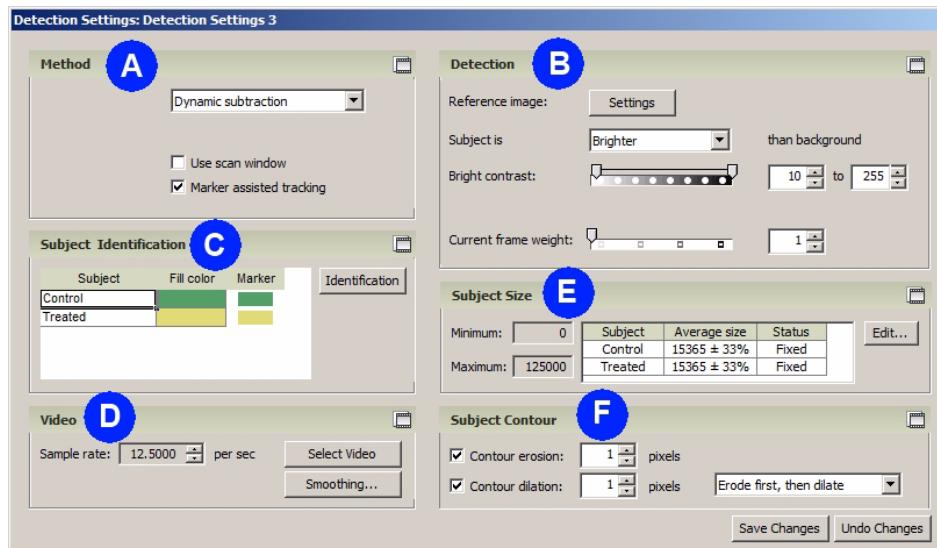


Figure 8.1. The **Detection Settings** window. See the text above for an explanation of the letters.

8.2 Configuring Detection Settings



You can use a pre-defined template to automatically configure detection settings for commonly used experimental setups (see "Creating a new experiment based on a pre-defined template" on page 92). After you have done this, you must still adjust the detection settings (as described in this chapter) before you can track any animal correctly.

General procedure

Subject detection works well if there is good contrast between the subject and the background in the video image, and for the whole duration of the trials. Increasing the contrast (for example, by changing the background so it differs as

Configuring Detection Settings

much as possible in color from the subject) is far more effective than any detection setting.

Make sure you carefully follow the order of steps as described below. If a particular step does not apply to your setup, proceed to the next step.

Experiment Settings

In the **Experiment Settings** window (see also page 96):

- 1 Select the **Number of Subjects per Arena**.
- 2 Select one of the options from **Detected features**.

Method section - 1



Which methods and options are available in the Method section, depends on the Experiment Settings.

- 3 Make the following selection:
 - **Use scan window** – Make sure this option is NOT selected while you are configuring Detection Settings.
 - **Marker assisted tracking** – Select this option when you want to track more than one animal.

→ see page 257

Subject Identification section

- 4 You can use **Subject Identification**, if you have multiple subjects and you have either selected **Color marker tracking (treat marker as center-point)** in the Experiment Settings or **Marker assisted tracking** in the Detection Settings.

→ see page 216

Video section

- 5 In the **Video** section, you can have the following options:
 - **Select video** (only if you track from video) - Click this button and browse to your video if it is not automatically selected.
 - **Video settings** (only if you track live). Click this button to adjust the settings of your camera. The options depend on your camera or frame grabber board.
 - **Sample rate** – The sample rate is the number of video images per second you want EthoVision XT to analyze among those available.

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- **Smoothing** – Select the option you require.

→ see page 223

Method section - 2



Which methods and options are available in the Method section, depends on the Experiment Settings.

- 6 Select one of the following:

- **Method** – These subject detection methods (Gray scaling: page 233, Static subtraction: page 235, Dynamic subtraction: page 240, Differencing: page 245) must always be selected.
- **Nose-Tail detection** – These nose-tail detection methods (Shape-based (XT4), Model-based (XT5), Advanced Model-based (XT6)) are only available when you have selected **Center-point**, **nose-point** and **tail-base detection** for a single animal in the Experiment Settings.



If you select **Center-point**, **nose-point** and **tail-base detection** with **2 or more Subjects per Arena** in the Experiment Settings, the Nose-Tail detection in the Detection Settings is automatically set to Advanced Model-based (XT6) and therefore the Nose-tail detection methods are not displayed.

→ see page 232 for Detection methods and page 258 for Nose-tail detection methods

Detection section

- 7 In the **Detection** section, you can configure the subject detection method (**Gray scaling**: page 233, **Static subtraction**: page 235, **Dynamic subtraction**: page 240 and **Differencing**: page 245) you selected in the previous step.

→ see page 233

Subject Contour

- 8 In the Subject Contour section, set the level of **Erosion** and **Dilation**.

→ see page 249

Subject Size

- 9 In the Subject Size section, click the **Edit** button to set:

- **Detected subject size** – Here you can set the Minimum and Maximum subject size.

Configuring Detection Settings

- **Modeled subject size** – Here you model the subject size when you have multiple subjects or when you use the Nose-tail detection method Advanced Model-based (XT6) for one or more subjects.
- **Advanced Subject Size settings** – Here you can set **Maximum noise size**, **Shape stability** and **Modelling effort** in case you have multiple subjects or when you use the Nose-tail detection method Advanced Model-based (XT6) for one or more subjects.

10 Once the subject is detected well, in the Method section, select **Use scan window** (see page 257) and click **OK**.

→ see page 252

You are now ready to acquire data (see Chapter 9).

Notes



- Every time you apply changes in the **Detection Settings** window, you can see the consequences in the **Video Source** window.
- To save the detection settings, click the **Save Changes** button at the bottom of the window. If you have made more changes and you want to return to the last saved settings, click the **Undo Changes** button.
- EthoVision XT offers a number real-time statistics on the quality of detection that you can check while you adjust detection settings.
- **Keep in mind that detection in the Detection Settings is real-time, whereas with Detection determines speed (page 273) during acquisition the quality of detection can be better!**

Marker assisted tracking

When do I use Marker assisted tracking?

You use marker assisted tracking when you have more than one subject per arena and when you have NOT selected Color marker tracking in the Experiment Settings (see page 96). Marker assisted tracking is optimized for use with rodents.

How to use Marker assisted tracking?

In the **Method** section of the **Detection Settings** window, select the **Marker assisted tracking** check box. The **Identification** button in the **Subject Identification** section now becomes enabled.

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Follow the steps in the **Subject Identification** section below to set up **Marker assisted tracking**.



See also "Tips for marker tracking" on page 222.



When you do NOT select the Marker assisted tracking check box, you will carry out unmarked tracking. You can carry out unmarked tracking when you analyze the variables on a group level (so the identity of the animals is not important) or when the animals cannot touch.

What is the difference between Marker assisted tracking and Color marker tracking?

With Marker assisted tracking, EthoVision tracks the animal's body and uses the marker to determine the animal's identity. When you use Color marker tracking, EthoVision tracks just the marker.

Color marker tracking

When do I use Color marker tracking?

Color marker tracking can be used for tracking any species (that can be marked) whereas Marker assisted tracking is optimized for rodents only.

How to use Color marker tracking?

Select **Color marker tracking (treat marker as center-point)** in the **Experiment Settings** (see page 102).

When you open the **Detection Settings** (see page 211), the Detection Settings window shows the **Subject Identification** and **Video** section.

Follow the steps in the **Subject Identification** section below to set up **Color marker tracking**.



See also "Tips for marker tracking" on page 222.

Subject Identification

You carry out the procedure described below for either **Marker assisted tracking** or **Color marker tracking**.

Configuring Detection Settings

- 1 Put the marked animals in the arena or play the video. Optimize the camera setup (see page 58), lighting conditions (see page 60) and marker characteristics (page 222).



Make sure you select a point in the video where the animals do not touch each other!

If you use multiple body point detection, it is normal that the nose is not correctly identified at this point.

- 2 In the **Subject Identification** section, select one of the subjects and click the **Identification** button.

Result – The **Identification of Subject #** window and the **Marker detection** window open.



You should enlarge the **Marker detection** window by dragging its bottom-right corner.

- 3 Move the mouse pointer to the **Marker detection** window so the pointer becomes an eyedropper.
- 4 Move the eyedropper on top of the color marker of the subject you want to identify (see figure below) and click the left mouse button.



The **Identification** window now displays the color you just picked and the pixels with the initial color are highlighted in the Color marker effect window. In the **Identification** window, you can change the following (see also Figure 8.2.):

- **Hue** – Hue is the predominant wavelength of the marker color and represents what is usually referred to as color in everyday life (red, green, blue, etc.). The range of values for Hue of the picked color are shown and this range is represented by the box on the vertical color bar on the right.

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- **Saturation** – Saturation represents the purity of a color. Saturation decreases when a pure color is mixed with white; "red" is saturated, "pink" is less saturated. The range of values for Saturation are shown and this range is represented by the width of the box on the Color map.
- **Brightness** – Brightness (or Intensity) represents the amount of light reflected by the colored surface. The range of values for Brightness are shown and this range is represented by the height of the box on the Color map. If you set this range too broad, you will not be able to separate the colors well.



If the marker is not detected completely or not detected in all areas of the arena, expand the range of Hue, Saturation and Brightness slightly.

➔ see **Fine-tuning color settings** on page 219

The detected marker can be eroded or dilated in order to compensate for specific scenarios. For example, you can dilate the marker if the marker is partly masked by cage bars or you can use erosion to round the marker which will prevent the center-point from jittering.

➔ see **Fine-tuning color settings** on page 219

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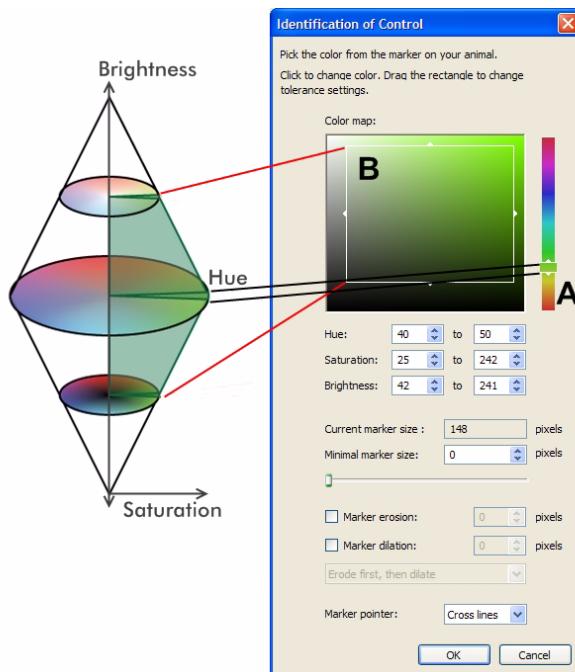


Figure 8.2. The **Identification** window and its relation with the HSI color model. **A** = Color bar: the box represents Hue which corresponds to an angle on the circle in the HSI color model (for example, 0 degrees means red, 240 degrees means blue). **B** = Color map: the height of the box represents the Brightness (or Intensity) range which corresponds to the vertical position of the color circle. The width of the box represents the Saturation range which corresponds to the horizontal position on the circle between the center and the edge.

Fine-tuning color settings

When you first pick a marker color in the Color marker effect window, EthoVision selects all pixels in the video image with the same initial color. Groups of pixels with this initial color are highlighted by an outline with the opposite color. Because a marker in the video image can consist of different shades of the same color, it is possible that initially not the complete marker is selected (see Figure 8.3.).

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The figure below shows part of the Color marker effect window and part of the Identification window.

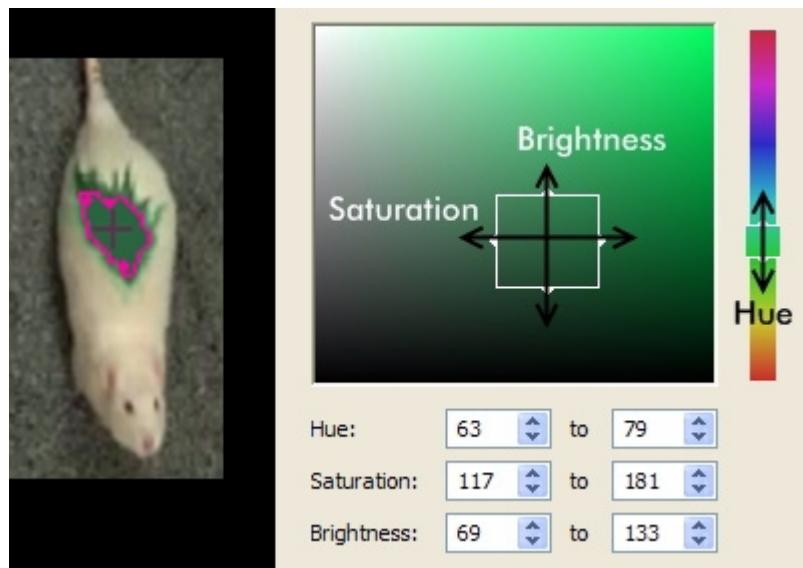


Figure 8.3. The initial color that is picked in the Color marker effect window (left picture) and the corresponding range for color settings **Hue**, **Saturation** and **Brightness** in the **Identification** window. The arrows indicate how changing the boxes changes the corresponding color setting.

You can fine-tune the color settings by adjusting the Hue, Saturation and Brightness in the **Identification** window.

- 5 Change the range of color settings by changing the numbers or by resizing the Hue box on the vertical color bar, or resizing/moving the box in the color map (horizontally to adjust Saturation, vertically to adjust Brightness).

As a result, the outline covers (almost) the complete marker (see Figure 8.4. below).

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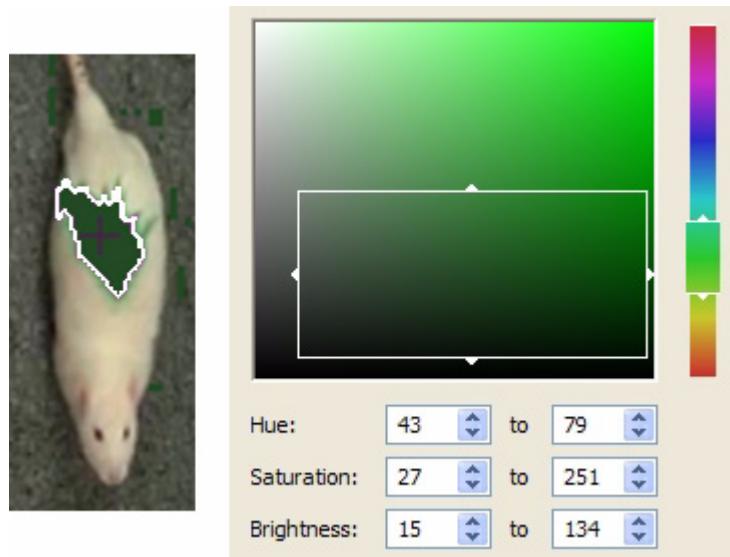


Figure 8.4. The color of the marker after fine-tuning the color settings. Most of the marker is now selected as indicated by the white outline (see also Figure 8.3.)

- 6 Next, play the video to see in the Color marker effect window whether the marker is detected correctly in different parts of the arena.
If the marker 'dances' then your color settings are too sensitive. Go back to step 5 and make the box larger.
- 7 Continue with setting the following:
 - **Marker erosion** – Set the number of pixels to erode. By selecting Erode first, then dilate, you can make the marker more round to prevent the center-point of the marker to start jittering.
 - **Marker dilation** – Set the number of pixels to dilate. By selecting Dilate first, then dilate, you can prevent the marker from being masked or divided in two separate markers by, for instance, a grid on top of the arena.
 - **Minimal marker size** – Set the **Minimal marker size** to prevent noise to be detected as the marker. First, increase the Minimal marker size until noise is not detected anymore. Next, enter a value for the **Minimal marker**

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size that is somewhere in between this lower threshold and the value of the **Current marker size**.

- **Marker pointer** – Select a Marker pointer from the list. With relatively small markers it is useful to select **Cross lines**.

8 Click **OK** when you are done.

Repeat steps 2-8 for all subjects you want to identify.

Tips for marker tracking

Color characteristics

- Use a color scale (for example from a paint company) to find out which colors are most easily recognized by EthoVision in your setup and lighting conditions. Do this before applying color markers to your animals.
- Use colors that have different hue values. For example, use red and green and not red and orange.
- It may be wise to avoid using red for marking, since it looks like blood.
- Note that marking your animals may stress them, and therefore affect their behavior. If necessary, ensure that you select a marking method that lasts for a longer period of time.

Marker characteristics

- Make sure that the marker is as round as possible, this will ensure that the relative movement of the center of gravity of the marker is the same in all directions when the edges of the marker change due to posture changes or otherwise. For color marker tracking it will help to prevent the jitter of the marker.
- When you use marker assisted tracking, make sure the marker is not too big; the marker can interfere with proper detection of the body contour. For example, make sure that a dark marker on a white animal does not cover the complete width of the animal because it can cause the body to be split in two.

Lighting conditions

- Use a sensitive camera if possible. A low light intensity makes it difficult to separate different colors. When it is not possible to use a sensitive camera or strong illumination in your setup, try using fluorescent marker colors with UV lighting.

Configuring Detection Settings

- For optimal color separation, illuminate your setup with lamps that approximate to day-light in color temperature, that is, have a wide spectrum range.

Subject roles

The names under **Subjects** in the **Subject Identification** section are the **Subject roles** entered in the Experiment Settings (see page 96). You can use Subject roles "Control" and "Treated", for instance, to plan to give the control animals a blue marker in some trials and treated animals the blue marker in other trials. To do this, define multiple sets of Detection Settings, one for each combination of marker color*treatment level. Before acquiring the data, make sure that you use the Detection Settings that correspond to the current animals.

Video adjustments - Sample rate

The Sample rate is the rate at which EthoVision analyzes the images to find the subject. It is expressed in samples per second.



Selecting a certain sample rate does not mean that the program can always analyze data at that rate. If the computer processor load is too high, EthoVision XT may skip a sample and analyze the next one. Skipped samples result in **missing samples** (see below).

The maximum sample rate is the frame rate set by the TV standard of your video. For PAL video, frame rate is 25 frames/s, therefore the maximum sample rate is 25 samples per seconds. For NTSC video, the maximum sample rate is 29.97 samples per seconds.

The sample rate you set in EthoVision XT can only be the frame rate divided by an integer. For example, for PAL video it is 25, 12.5, 8.33, etc.



Some digital cameras support different frame rates than the sample rates supported by EthoVision XT. If your camera supports different frame rates, we recommend to adjust the frame rate in the camera settings so EthoVision does not have to discard too many samples.

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What is the optimal sample rate?

Setting the correct sample rate is very important. If the rate is too high, the noise caused by small movements of your animal will be picked up and give an overestimate of dependent variables such as the distance moved. If the sample rate is too low, you will lose data and get an underestimate of the distance moved.



When you have selected both nose-tail tracking and Marker assisted tracking, we recommend a sample rate of 12.5 samples per second.

The table below gives some general recommendations taken from the published literature. These sample rates have successfully been used to track animals with previous EthoVision versions. However, we strongly recommend that you determine the optimum sample rate for your specific setup and animals (see below). Note that if, for instance, your treatment causes hyperactivity, you will need a higher sample rate for hyperactive animals than somnolent animals.

Animal	Samples/second
Damselfish	5
Goldfish	0.5
Zebrafish larvae (analog camera)	25
Zebrafish larvae (FireWire camera)	30*
Mites	1
Mouse	12
Parasitic wasps	2
Rat	5
Rodent's nose	25 (PAL) 30 (NTSC)
Tick	3
Tree-shrew (Tupaia)	6-12

* For rapid movements you may want to track with a higher sample rate. It depends on the number of tracked subjects, the video resolution, and the processor speed of your computer whether that is possible.

The optimal sample rate is the minimum sample rate that provides an accurate estimation of the dependent variables (distance, velocity, etc.) without including the redundant information due to phenomena other than the 'real' locomotion. For example, for an animal walking in a straight line the data points will never be in a straight line because the center-point of the subject shifts laterally with each step. In order to distinguish between 'real' movement and effects like the one described above, you can calculate dependent variables like distance moved using the maximum or a lower sample rate.

- 1 Create new **Detection Settings** (see page 212) and specify the maximum sample rate (25 or 29.97, depending on your TV standard). With a FireWire

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camera this sample rate may be higher. However, whether this is possible depends on the performance of your computer, the number of animals you track, and the video resolution.

- 2 Start **Acquisition** and acquire data with those Detection Settings (see Chapter 9).
- 3 Start the **Analysis** module and calculate the dependent variable you are interested in (see Chapter 13). Export the data for example to Excel (see page 496) and plot the dependent variable values against the sample rate. In the example below, distance moved is considered.
- 4 Repeat steps 1 to 3 by selecting smaller sample rates.

Once the data are plotted as in Figure 8.5., there should be a range of sample rates for which the dependent variable value does not change much (plateau). This means that slight changes in the sample rate do no result in loss of information, or addition of redundant information (noise and movements like body wobble).

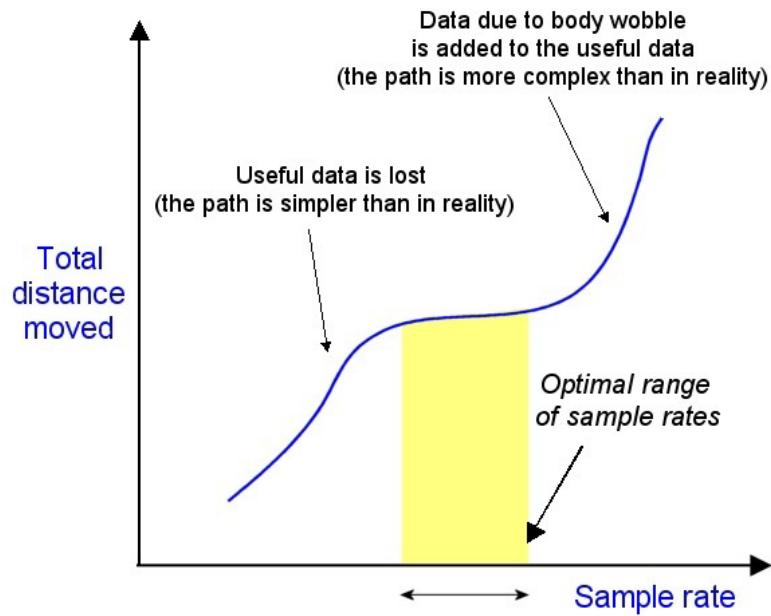
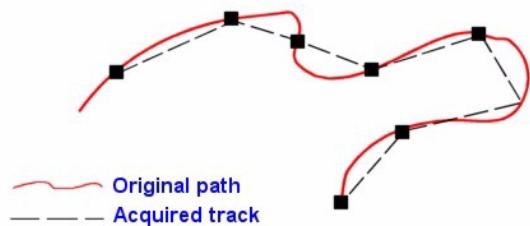


Figure 8.5. Detecting optimal sample rate from a collection of distance moved recorded with different sample rates.

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Low sample rates result in loss of useful information, because the sinuosity of the original path is removed. Therefore, the total distance moved is usually decreased (see figure below).



High sample rates result in acquisition of redundant information. In the case of body wobbling, and assuming that the animal is moving along a straight line, the lateral shift of the body center causes the total distance moved to be longer than the 'real' one.



With Track Smoothing (see page 378) and Minimal distance moved (see page 380) you can filter out 'noise' as a result of body wobble.

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Missing samples

The actual sample rate may be lower than the maximum you set, because an image cannot be captured until the previous one is processed. If the sample rate you define is too high, EthoVision will miss samples (up to 1% is acceptable) and the processor load will be high. The percentage of missed samples is shown in the Analysis Results pane (see page 266) and in the Trial List as a System Variable (page 103). You can calculate the number of missing samples in acquired tracks with the Number statistic of continuous variables (e.g., velocity). If your processor load is larger than 100, and there are large amounts of missed values, you will have to lower the sample rate. The following factors may cause the processor load to be too high:

- **Track from video files** (see next page)!
- **Computer memory, processor speed and video card capacity** – See the system requirements on page 36. In general, using a computer with a dual-core CPU helps you to work with higher sample rates than normal computers do.
- **Other programs installed** – Do not install other video software (for example, video editing programs, DVD burning software), because this can interfere with EthoVision's video processing and cause a reduction in performance.
- **Other programs are running** – Make sure you shut down all other programs, including those running in the background such as e-mail programs and virus scanners. These are usually shown in the System Tray in the bottom-right corner of your screen.
- **Windows Classic** – The performance will considerably increase if you set the Windows Theme to Windows Classic when using Windows 7. See the note on page 36 of Chapter 2 Installation).
- **Image resolution** – For live video tracking, In the Experiment Settings you can choose between Medium and High resolution for your video image (see page 100). Medium resolution is recommended.
- **Size of arenas** – Make arenas as small as possible (but including the entire area the animal can be in).
- **Number of arenas** – If you track live and use more than four arenas in a trial, check first that no samples are missed. If the number of missing samples is too high, first make a MPEG-4 file (provided that you have the Picolo Diligent board installed on your PC), then track from that. More generally, if you track from video files the number of arenas is never a problem as long as you select **Detection determines speed** (see page 273).

When making detection settings, you could start with making an arena definition with only one area which speeds up the detection process. After

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you have finished configuring detection settings for one arena, you can add the others to the arena definition.

- **Display options** – You can decrease processor load by minimizing the number of Track Features to be displayed (see page 264) and by closing the Analysis Results and Scoring pane (see page 277).
- **Real time analysis** – Hiding the **Analysis Results** pane results in saving processor power.
- **Detection method** – If possible, use the Gray scaling method which requires less processor load than Static subtraction. Static subtraction requires less processor load than Dynamic subtraction and Differencing.
- **Area to search for subjects** – If you cannot achieve the optimum sample rate, make sure that you select **Use scan window** (see page 257), but only after you are finished configuring the detection settings.



After acquisition you can see the proportion of missing samples in the Trial list as one of the System Variables.

Tracking from video files

You can switch the speed at which EthoVision acquires data from real time (1x) to the highest achievable by the computer, by selecting **Detection determines speed** (see page 273). This option allows you to:

- Ensure that you do not lose any frames when the video frame rate is faster than your processor can handle. The video is played slower than real time, without missing samples.
- Acquire data faster than in real time when the video frame rate is slower than the processor can handle.

Video Adjustments - Select Video

If you track from video, you may want to acquire data from a video that differs from the one you used to create the **Arena Settings**. By default, the **Detection Settings** uses the video you grabbed a background image from in the Arena Settings. If you want to track from another video file, click **Select Video** under **Video**. Browse to the location of your video and click **Open**. This option is only available if you chose track from video files in the **Experiment Settings**.

Video Adjustments – Video Settings

If you track live, you can adjust the live video signal before EthoVision XT analyzes it for detection. For example, you can adjust contrast and brightness.

Click the **Video Settings** button above **Smoothing**. In the window that appears, adjust the properties you require (if you have a color camera, there are additional options (hue, saturation etc.). **Contrast** increases the range of available gray scale values, **Brightness** increases the magnitude of the values. The Video Adjustment Settings also affect the image that you can save to a video file (see page 229).

The **Video Settings** button is only available if your experiment is set to **Live tracking**. It might not be available for all types of FireWire camera.

The properties window that appears when you click the **Video Settings** button depends on the type of frame grabber board and camera. If you have a DV camera connected or an 'old' Picolo board installed, the properties window is not available.



Always try adjusting the lighting and camera aperture settings before changing the Video Adjustment Settings.

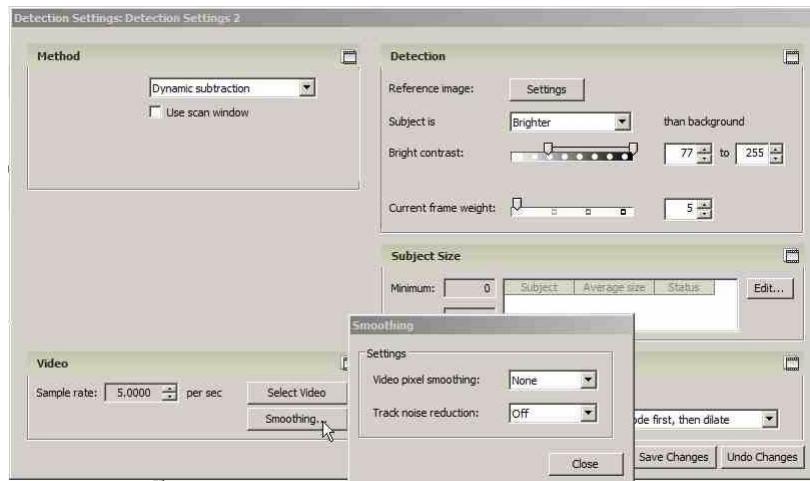


If you change settings, you need to redefine your detection thresholds (see above) and make a new reference image.

Video Adjustments - Smoothing

In some cases you may want to adjust the quality of the video image before acquiring data. If your video contains fine-grained noise, this may be improved by using Video pixel smoothing. If the detected body contour is 'flickering', using Track Noise Reduction may improve the quality of the track. Click the **Smoothing** button and adjust one of the options below.

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Video pixel smoothing

Select a **Video Pixel smoothing** value to reduce the influence of fine-grained noise on detection. Because of fine-grained noise, adjacent pixels that are expected to have the same (or similar) gray scale value may have very different values. In such cases, EthoVision XT may occasionally detect groups of pixels as irrelevant subjects.

The **Video Pixel smoothing** option reduces the difference between adjacent pixels prior to detection, by smudging the image, that is, replacing the gray scale value of each pixel with the median of the surrounding pixels.



Pixel smoothing does not affect Color marker tracking. It does affect detecting the body contour in Marker assisted tracking.

Choose one of the values:

- **None** (default) – No pixel smoothing. The video image is analyzed for subject detection as it is.
- **Low** – Each pixel is blended with the 8 nearest pixels (pixel distance =1).
- **Medium** – Each pixel is blended with the 24 nearest pixels (pixel distance 1 or 2).
- **High** – Each pixel is blended with the 48 nearest pixels (pixel distance 1, 2 or 3).

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Example – A bright pixel (gray value= 240) is surrounded by dark pixels:

150	150	180
180	240	150
140	180	130

If you select **Video Pixel smoothing= Low**, that pixel gets the median value calculated among the 8 nearest pixels plus that pixel itself. In that case the median is 150, so that pixel will look darker. If you specify **Video Pixel smoothing= Medium**, the median is calculated over the 24 nearest pixels plus the pixel itself. If you specify **Video Pixel smoothing= High**, an even bigger group of surrounding pixels is considered.



A high Video Pixel smoothing level requires a significant amount of processor capacity.

Why use the Video Pixel smoothing option?

- Select a moderate **Video Pixel smoothing** value or leave **None** selected If adjacent pixels in the background are relatively constant. Using more surrounding pixels for the smoothing effect does not bring up better results.
- Select a high **Video Pixel smoothing** value if adjacent pixels in the background are on average very different. For example, when the cage's bedding material looks grainy. In such cases you need to smooth each pixel using more surrounding pixels to compensate for this variation.



Using Video Pixel smoothing may result in losing information in the video image important for detection. For example, sharp borders of subjects, etc.

Track Noise Reduction

If the detected centre point of your animal is continuously moving, while in fact your animal is sitting still, the total distance moved will be overestimated. You can use track smoothing to correct for this after you have acquired your data. See page 378 for more information.

In some cases better quality tracking can be obtained by reducing track noise during acquisition. This may especially be the case if you use Trial and Hardware Control. As an example, if the center point of an animal is detected in a zone, you

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want the pellet dispenser to drop a pellet. If the detected center point is moving rapidly because of noise, this may result in a number of consecutive pellets to be dropped, every time the center point crosses the border of the zone. Track Noise Reduction may solve this problem.

With Track Noise Reduction, rapid changes in the distance moved will be compensated for and the path will be smoothed. Using Track Noise Reduction in the Detection Settings influences the acquired track, and therefore it is not possible to change it back after acquisition. This is in contrast to Smoothing and Minimal Distance moved in data selection (see page 378), where you can use profiles to calculate analysis results with and without those filters applied. Also, do not use Track Noise Reduction if you are particularly interested in rapid movements of your animal, for example, if you study the startle response of zebra fish larvae.

Track Noise Reduction makes use of the Gaussian Process Regression method. Track Noise Reduction is applied during acquisition. Hence, it alters the acquired tracks, which cannot be undone afterwards.

With Gaussian Process Regression, the sample points are smoothed, using the x-y coordinates of the previous twelve sample points. This differs from the Smoothing method in data selection (see page 379) that uses samples before and after the sample point to be smoothed. This is not possible during acquisition, because the x-y coordinates of future samples are not yet known.

If you use noise-tail tracking, the paths of the nose point and tail base are smoothed independent of the path of the center point.

Detection methods: which one do I use?

There are four methods available to distinguish the animal from the background:

Use **Gray scaling** when:

- The animal's grayness differs from the background in all places that can be visited.
- The background cannot change during a trial.
- Lighting is even (minimal shadows and reflections) during the trial.

Example – tracking a white rat in a uniform black open field with no bright objects.

Use **Static Subtraction** when:

- The Gray scaling method does not work (because other objects in the arena have a similar color as the animal).

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- The background does not change in time.
- The light is constant during the trial.

Example – Tracking a white rat in an open field with unavoidable reflections or bright objects.

Use **Dynamic Subtraction** when:

During trials light conditions gradually change or the background changes (bedding material is kicked around, food pellets are dropped, droppings appear etc.).

Example – Tracking a mouse in a home cage provided with bedding material. The activity of the mouse causes the bedding to change appearance in the video image.

Use **Differencing** when:

There is a lot of variation in contrast between a subject and the background within an arena. Variation in contrast can be caused, for example, by a gradient in light intensity in the arena or in the fur of the animal, e.g. hooded rats.

Detection method: Gray scaling

How does the Gray scaling method work?

The video image is converted to monochrome. Each pixel in the image has a **gray scale value**, ranging from 0 (black) to 255 (white). With Gray scaling, you define which range of gray scale values should be considered as the subject. The remaining gray scale values are considered as background.

Procedure

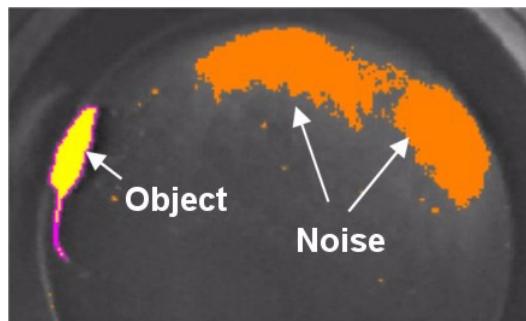
- 1 Select **Gray scaling** in the **Methods** section of the **Detection Settings** window.
- 2 Insert the subject in the arena, or position the media file at a point where the subject is moving.



With the Gray scaling method selected in the Detection settings window, it is not possible to grab a frame or to select another video file because the Gray scaling method does not use a Reference image.

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- 3 In the Detection section, move the two sliders next to **Select range** or type the values in the corresponding fields to define the lower and higher limits of gray scale values (range from 0=black to 255=white) of the animal. The background cannot contain gray scale values outside these limits.
- 4 Check on the **Video** window the quality of detection resulting from the current gray scale range. The detected subject shows the features and colors you have chosen in the Track Features window (see page 264).
 - If the detected area is too small relative to the real subject, you need to increase the range (at least in one direction - brighter or darker).
 - Areas marked as **Noise** (by default, these are shown in orange; see page 264), indicate that the gray scale range is too wide – you need to narrow it in at least one direction.



- 5 Move the sliders until the subject (or the part which is of interest) is detected fully, and the noise is minimized. Check that the subject is properly detected in all parts of the arena by moving the video slider, or by waiting for the live animal to move.



It is important that the complete animal's body is detected for optimal tracking. Proceed with the Contour adjustments (see page 249) to optimize body detection.

Detection method: Static subtraction

How does the Static subtraction method work?

The video image is converted to monochrome. Each pixel in the image has a **gray scale value**, ranging from 0 (black) to 255 (white). With the Static subtraction method, you choose an image of the arena without the subject, named *Reference Image*. When analyzing the images, EthoVision XT subtracts the gray scale value of each pixel in the reference image from the gray scale value of the corresponding pixel in the current image (live or from video). The pixels with non-zero difference are considered the subject.

You can remove small non-zero differences by defining the contrast between current image and background that must be considered as the subject (see the procedure below). The remaining pixels are considered as the background (see Figure 8.6.).



Figure 8.6. An example of how the Static subtraction detection method works. The gray scale value of each pixel of the reference image is subtracted from the gray scale value of each pixel of the live image. The result is '0' for every pixel; if the difference > '0' and within the gray scale range you have set, these pixels are considered to be the subject. So, with this method your task is to specify the contrast that optimizes the detection of the subject.

Procedure

- 1 Select **Static subtraction** in the **Method** section of the **Detection Settings** window.
- 2 Under **Detection**, click the **Settings** button next to **Reference Image**. The image on the left is the Reference Image that is used at the start of the track.

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The options on the right of this window are greyed out.

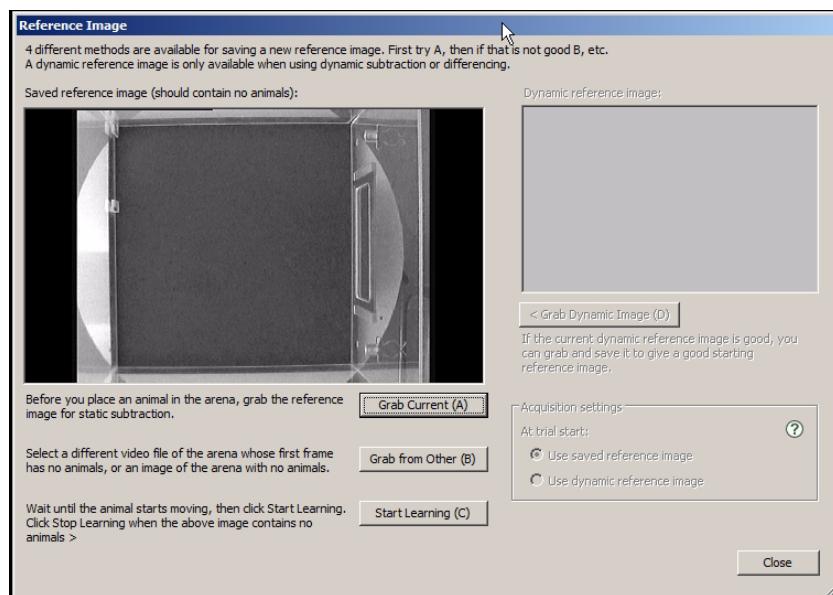


Figure 8.7. The **Reference Image** window of static subtraction and live tracking. If you track from video file, the text in this window is slightly different but the options are the same. Follow the procedure in consecutive order until the left image is without animals.

The aim is to obtain a reference image that does not contain images of the animals you want to track. To do so, follow the instructions below in consecutive order. If A fails, move on to B, if that fails move on to C.

- 1 **Grab Current (A)** - Scroll through the video until you find an image without animals. If you track live, make sure that there are no animals in the arena. Click **Grab Current (A)**. This image will be the initial reference image. Skip Steps 2 and 3 and click **Close**.
If your video does not contain images without animals, continue with option 2. Also continue with option 2 if you track live and you cannot start with an empty arena.
- 2 **Grab from other (B)**- You may have a video with an identical background as the one you use for tracking, but without animals. Or you may have an image of a background without animals. If this is the case, click **Grab from Other**

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and select this video file or image file. If you select a video file, the first frame of this file will be used as an initial reference image. If you select an image file, this has to have the same resolution as the video file you use for tracking. Browse to this file and click **Open**. Skip step 3 and click **Close**. If you do not have such video or image, proceed with option 3.



By default, the reference images are stored in the folder **Bitmap Files** of your experiment. If the background has not changed, you can use these images as reference images in other experiments.

- 3 **Start learning (C)** - With this option an average image of the entire video will be made. If the animals are moving, learning will average out the pixels of the animals, resulting in an initial reference image without animals.

If you track live, you have to click **Start Learning**, and subsequently click **Stop Learning** as soon as you have obtained an initial reference image without animals. Click **Close**.

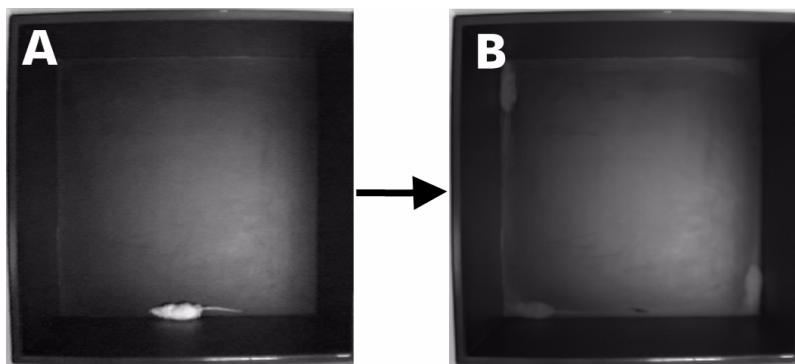


Figure 8.8. The **Learning** process in the Reference Image window. **A**-The video image in which the animal is in the view at all times, **B**-The result of applying **Learn**: the moving animal is removed from the background.

- 4 Click **Close** when you are finished grabbing a Reference Image.
- 5 From the **Subject is ... than background** list, select one of the following, depending on the color of the subject you want to track:
 - **Brighter than background** – For example, to track a Wistar rat in a black open field.
 - **Darker than background** – For example, to track a C57BL6 mouse in an open field with white bedding.

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- **Brighter and darker than background** – For example, to track a DBA2 mouse in a home cage with white background and a black shelter, or a hooded (black and white) rat in a uniform gray open field.

Result – Depending on the selection above, different contrast sliders become available:

- For Brighter than background – **Bright Contrast** slider.
- For Darker than background – **Dark Contrast** slider.
- For Brighter and darker than background – Both sliders.

For each slider, the contrast varies from **0** (no contrast) to **255** (full contrast).



Unlike with Gray scaling, the values selected with the sliders represent the difference between the current and the reference image, not absolute gray scale values.

When the subject is brighter and darker than the background, detection only works well when there is enough contrast between the areas of different brightness and the background. For example, tracking a hooded rat works well when the background is intermediate between black and white.

- 6 Release the subject in the arena, or position the media file at a point where the subject is moving.
- 7 Move the appropriate slider or type the values in the corresponding fields to define the lower and higher limits of the contrast that corresponds to the subject.

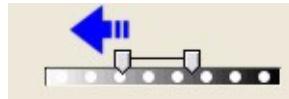
In the Video window, check the quality of detection.

Example 1 – The subject is brighter than the background. Only the whiter area of the subject is detected.

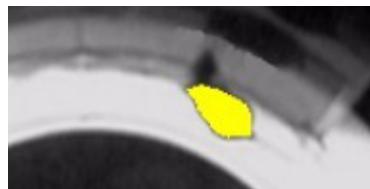


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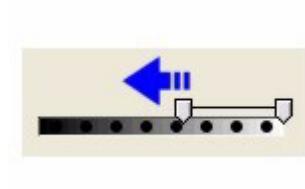
→ Move the **Bright Contrast** slider to the left to increase the range towards values of lower contrast between subject and background.



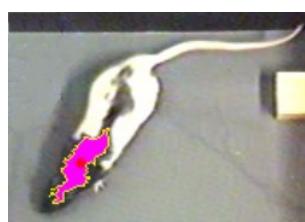
Example 2 – The subject is darker than the background. Its body is detected only partially in the area of lower contrast.



→ Move the **Dark Contrast** slider to the left to increase the range towards lower values of contrast between subject and background.



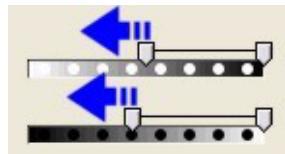
Example 3 – The subject is brighter and darker than the background. Only the darker areas of the black fur are detected.



→ Move the **Bright Contrast** slider to the left to increase the range towards less contrast between the subject's white areas and the gray background.

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Then, move the **Dark Contrast** slider to the left to increase the range towards less contrast between the subject's black areas and the background.



- 8 Move the sliders until the subject (or the part which is of interest) is detected fully, and the noise is minimized. Check that the subject is properly detected in all parts of the arena by playing back different parts of the video file, or by waiting for the live animal to move.



It is important that the complete animal's body is detected for optimal tracking. Proceed with the Contour adjustments (see page 249) to optimize body detection.

Detection method: Dynamic subtraction

How does the Dynamic subtraction method work?

Like with Static subtraction (see page 235), the program compares each sampled image with a reference image, with the important difference that the reference image is updated regularly. This compensates for temporal changes in the background.

With Dynamic subtraction, the reference image is updated at every sample. You specify the percentage contribution of the current video image to reference image.

Configuring Detection Settings

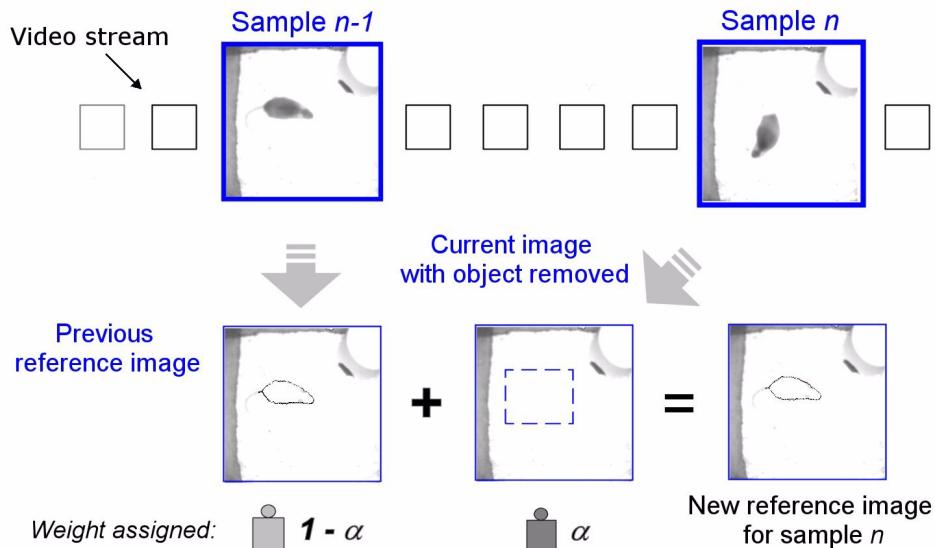


Figure 8.9. In the Dynamic subtraction detection method, the Reference image is updated at each sample. The starting reference image is the one you specify by clicking the **Grab from Video**, **Grab from Camera**, or **Grab from Other** button in the Reference Image window (see page 247), otherwise it is the first frame analyzed (not shown in the picture). For the general sample n , the reference image is obtained by summing the reference image of the previous sample $n-1$ and the current image n where the area around the subject estimated from the previous sample has been removed. The current image with subject removed is given the weight α that you specify (see the procedure), while the previous reference image is given the weight $(1-\alpha)$. Because of the way it is determined, each reference contains information on a number of past images, depending on the value of α . See the text for more information.

Procedure

- 1 In the Method section of **Detection Settings** window, select **Dynamic subtraction**.
- 2 In the **Detection** section, click the Reference Image **Settings** button. Create reference images without animals, following the procedure under **Reference image** on page 242.
- 3 From the **Subject is ... than background** list, select one of the options from the list, depending on the color of the subject you want to track (see step 6 at page 237 for details).

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- 4 Move the slider next to **Current frame weight** or enter the value in the appropriate field to specify how the reference image is updated (range **0-100%**):



- In typical situations, a value between 1-5 gives a good result.
- Select a low value if you want to have a large number of past images to contribute to each reference image. As a result, changes in the background are diluted over many images. Choose a low value when the background changes slowly.
- Select a high value if you want to have a small number of past images to contribute to each reference image. As a result, changes in the background are captured over short time. Choose a high value when the background changes rapidly, for example, when the subject is very active and moves the bedding material around.
- If you select **0**, the reference image is not updated. This is the same as using Static Subtraction.
- If you select **100**, each sample gets its own reference image with no contribution by the past images.
- Changing the Current frame weight does not affect the processor load significantly.



To find the optimal Current frame weight, set a value and carry out one or more trials. Evaluate if the tracking was satisfactory. If not, increase or decrease the setting by 20% and try again.



It is important that as much of the animal's body is detected for good tracking. Proceed with the Contour adjustments (see page 249) to optimize body detection.

Reference image

Under Detection, click the **Settings** button next to **Reference Image**. You now see two video images. The image on the left is the Reference Image that is used at the start of the track. The image on the right is the Reference Image that is

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continuously updated during tracking.

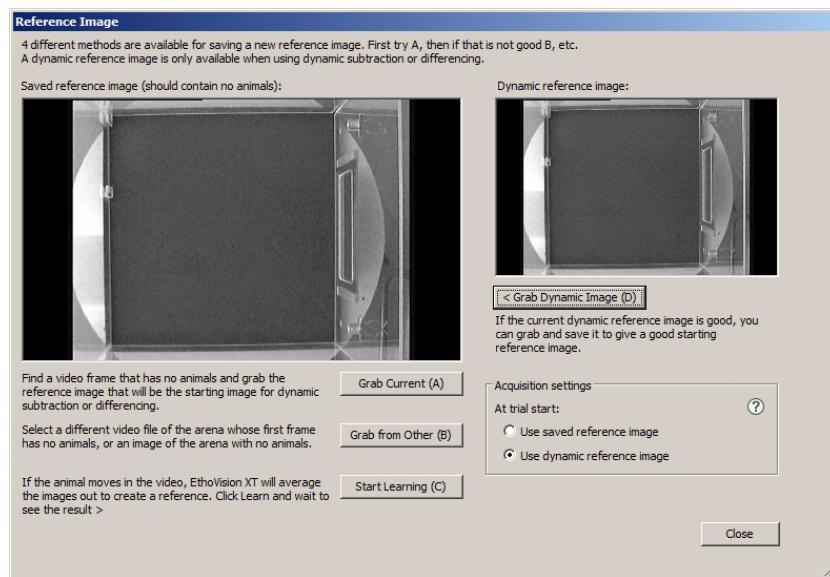


Figure 8.10. The **Reference Image** window for dynamic subtraction and tracking from video file. If you track live, the text in this window is slightly different but the options are the same. Follow the procedure in consecutive order until both images are without animals.

The aim is to obtain reference images that do not contain images of the animals you want to track. To do so, follow the instructions below in consecutive order. If A fails, move on to B, if that fails move on to C etc.

- 1 **Grab Current (A)** - Scroll through the video until you find an image without animals. If you track live, make sure that there are no animals in the arena. Click **Grab Current (A)**. This image will be the initial reference image. Skip Steps 2-4 and click **Close**.
If your video does not contain images without animals, continue with option 2. Also continue with option 2 if you track live and you cannot start with an empty arena.
- 2 **Grab from other (B)**- You may have a video with an identical background as the one in the video you track from, but without animals. Or you may have an image of a background without animals. If this is the case, click **Grab from Other** and select this video file or image file. If you select a video file, the first

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frame of this file will be used as an initial reference image. If you select an image file, this has to have the same resolution as the video file you use for tracking. Browse to this file and click **Open**. Skip steps 3 and 4 and click **Close**. If you do not have such video or image, proceed with option 3.



By default, the reference images are stored in the folder **Bitmap Files** of your experiment. If the background has not changed, you can use these images as reference images in other experiments.

- 3 **Start learning (C)** - With this option an average image of the entire video will be made. If the animals are moving, learning will average out the pixels of the animals, resulting in an initial reference image without animals.
If you track live, you have to click **Start Learning**, and subsequently click **Stop Learning** as soon as you have obtained an initial reference image without animals.
If this step results in a satisfying initial reference image, skip step 4 and click **Close**. If not, proceed with step 4.
- 4 **< Grab Dynamic Image (D)** - If options 1 to 3 do not result in a satisfying initial reference image, using the current updated reference image as the initial reference image may solve the problem. Click **< Grab Dynamic Image (D)** below the dynamic reference image.

Acquisition settings - If you run a number of consecutive trials, you may want to choose which image to use as initial reference image.

- Use saved reference image** - Use this option if the background remains constant between the different trials.
- Use dynamic reference image** - Use this option if the background changes between the different trials.



Grabbing the reference image is optional with the Dynamic Subtraction method. If you do not do that, EthoVision XT takes the first sample or video frame available and considers that as the first reference image.

If you are tracking from video files, you must play the video forward whilst making dynamic subtraction settings. This is because the program needs to update the reference image. Do not skip through the file, since the reference image will then not be correctly made.

How is the reference image updated?

A video stream is composed of a number of video images (frames). During data acquisition, EthoVision XT analyzes one every x images according to the sample rate specified (see page 223). When analyzing the sample (image) n, the reference image is obtained by summing up the gray scale values of each pixel from two images:

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- The reference image made of pixels which have an average value of previous images.
- The current image, where a square area around the subject detected in the previous sample has been removed. This provides a rough estimate of the current background.

The gray scale values are summed up according to the formula:

$$\text{Reference}_{i,n} = (1-\alpha) * \text{Reference}_{i,n-1} + \alpha * \text{Current}_{i,n}$$

for each pixel i , where:

- $\text{Reference}_{i,n}$ = Gray scale value of pixel i in the reference image of sample n .
- $\text{Reference}_{i,n-1}$ = Gray scale value of pixel i in the reference image of sample $n-1$.
- $\text{Current}_{i,n}$ = Gray scale value of pixel i in sample n where a square area around the subject previously detected has been removed.
- α = Current Frame weight.

The Current Frame weight determines the relative weight of the two components of the new reference image.

Because the above formula is recursive, that is, each value of $\text{Reference}_{i,n}$ is also a function of the previous sample, the value of α determines the number of past images that contribute to the reference image for the sample n . The lower α , the more past images contribute at least partially to the current reference image.



The extent to which each past image contributes to the current reference image is a power function of $1-\alpha$. The older an image relative to the current one, the smaller its contribution to the reference image.

- Example – If $\alpha=20\%$, then $1-\alpha=80\%$. The first video image contributes by 80% to the second sample, by $80\%^2=64\%$ to the third sample, then by $80\%^3=51\%$ to the fourth sample, etc. At the 21th sample, the contribution by the first image gets below 1%.

Detection Method: Differencing

How does the Differencing method work?

Like with Dynamic subtraction, the Differencing method updates the reference image over time. Differencing makes a statistical (probabilistic) comparison between each pixel in the reference image and the pixels of the current image.

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The statistical comparison uses the variance in the contrast between the current and reference image to calculate the probability that each pixel is the subject.

In most cases, the Differencing method works better than the other two subtraction methods to detect the subject.



The Differencing method takes more processor load than the subtraction methods. Therefore, when using Differencing, make sure your computer meets the system requirements as specified on page 36.

Procedure

- 1 In the **Method** section of the **Detection Settings** window, select **Differencing**.
- 2 In the **Detection** section, click the Reference Image **Settings** button. Create reference images without animals, following the procedure under **Reference image** on page 247.
- 3 From the **Subject is ... than background** list, select one of the options from the list, depending on the color of the subject you want to track (see step 6 at page 237 for details).
- 4 Next, if necessary, adjust the position of the **Sensitivity** slider and change the option selected in the **Background Changes** list.\



The **Sensitivity** slider determines what difference in contrast from the background is seen as the animal. For an image with good contrast, there is no need to change the slider. For images with less contrast, adjust the position of the slider to the right or the left until the subject is properly detected.

In the **Background Changes** list you can select options that reflect how fast the background changes. For example, a cage with bedding might change a lot because of animals kicking around the bedding material. If this case, to prevent changes in the background to interfere with detection, select 'Medium fast' or faster. Usually, 'Medium slow' works just fine.



It is important that as much as possible of the animal's body is detected for good tracking. Proceed with the Contour adjustments (see page 249) to optimize body detection.

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Reference image

Under **Detection**, click the **Settings** button next to **Reference Image**. You now see two video images. The image on the left is the Reference Image that is used at the start of the track. The image on the right is the Reference Image that is continuously updated during tracking.

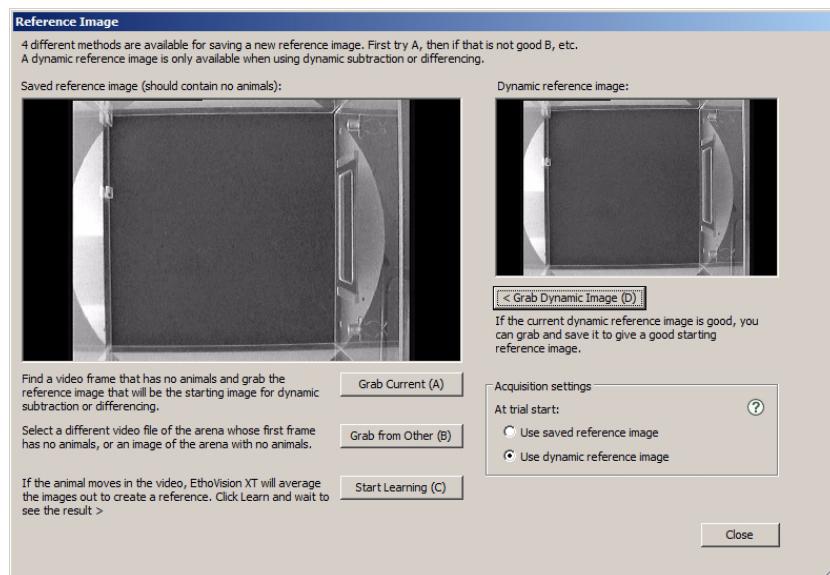


Figure 8.11. The **Reference Image** window for differencing and tracking from video file.

If you track live, the text in this window is slightly different but the options are the same. Follow the procedure in consecutive order until both images are without animals.

The aim is to obtain reference images that do not contain images of the animals you want to track. To do so, follow the instructions below in consecutive order. If A fails, move on to B, if that fails move on to C etc.

- 1 **Grab Current (A)** - Scroll through the video until you find an image without animals. If you track live, make sure that there are no animals in the arena. Click **Grab Current (A)**. This image will be the initial reference image. Skip Steps 2-4 and click **Close**.
If your video does not contain images without animals, continue with option 2. Also continue with option 2 if you track live and you cannot start with an empty arena.

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- 2 **Grab from other (B)**- You may have a video with an identical background as the one in the video you track from, but without animals. Or you may have an frame of a background without animals. If this is the case, click **Grab from Other** and select this video file or image file. If you select a video file, the first image of this file will be used as an initial reference image. If you select an image file, this has to have the same resolution as the video file you use for tracking. Browse to this file and click **Open**. Skip steps 3 and 4 and click **Close**. If you do not have such video or image, proceed with option 3.



By default, the reference images are stored in the folder **Bitmap Files** of your experiment. If the background has not changed, you can use these images as reference images in other experiments.

- 3 **Start learning (C)** - With this option an average image of the entire video will be made. If the animals are moving, learning will average out the pixels of the animals, resulting in an initial reference image without animals.
If you track live, you have to click **Start Learning**, and subsequently click **Stop Learning** as soon as you have obtained an initial reference image without animals.
If this step results in a satisfying initial reference image, skip step 4 and click **Close**. If not, proceed with step 4.
- 4 **< Grab Dynamic Image (D)** - If options 1 to 3 do not result in a satisfying initial reference image, using the current updated reference image as the initial reference image may solve the problem. Click **< Grab Dynamic Image (D)** below the dynamic reference image.

Acquisition settings - If you run a number of consecutive trials, you may want to choose which image to use as initial reference image.

- Use saved reference image** - Use this option if the background remains constant between the different trials.
- Use dynamic reference image** - Use this option if the background changes between the different trials.



Grabbing the reference image is optional with the Dynamic Subtraction method. If you do not do that, EthoVision XT takes the first sample or video frame available and considers that as the first reference image.

If you are tracking from video files, you must play the video forward whilst making dynamic subtraction settings. This is because the program needs to update the reference image. Do not skip through the file, since the reference image will then not be correctly made.

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How is the reference image updated?

The Differencing method uses a gaussian distribution of all pixels in a frame. EthoVision XT keeps a running average of the mean μ and the variance σ^2 of the gray value of each pixel to detect unlikely pixels. These pixels are considered to be the subject.

The mean of the gray values is summed up according to the same formula as for Dynamic subtraction (see page 244).

The variance of the gray values is summed up according to the following formula:

$$\text{Variance}_{i,n} = (1-\alpha) * \text{Variance}_{i,n-1} + \alpha * (\text{Current}_{i,n} - \text{Reference}_{i,n})^2$$

for each pixel i , where:

- $\text{Variance}_{i,n}$ = Variance of gray scale value of pixel i in the reference image of sample n .
- $\text{Current}_{i,n}$ = Mean gray scale value of pixel i in sample n where a square area around the subject previously detected has been removed.
- $\text{Reference}_{i,n-1}$ = Mean gray scale value of pixel i in the reference image of sample $n-1$.
- α = Current Frame weight.

The Current Frame weight determines the relative weight of the two components of the new reference image (see the example on page 245).

Contour Adjustments - Erosion / Dilation

Before you start setting the Contour Adjustments

 It is important that the complete body of the animal is detected (indicated by the 'noise' color in the video window). If even after setting the Contour adjustments you do not achieve this, go back to the appropriate Detection method and adjust the contrast to improve body detection.

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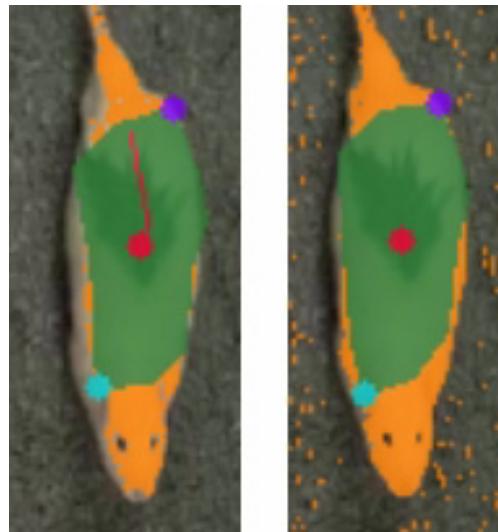


Figure 8.12. The picture on the left shows a sub-optimal result of body detection (part of the right side of the body is not detected). The picture on the right shows the result when the contrast settings are optimized; now the complete body is detected. The color of the body contour at this stage is orange (=noise) because the model parameters have not been configured yet.

Why use Contour Adjustments?

- **To give a smooth contour for accurate modeling and to remove individual pixels of noise** – For this purpose, **Erode first, then dilate** is selected by default.
- **To eliminate the detection of thin objects such as the rat's tail** – Select **Erode first, then dilate**. A reason for why you may want to eliminate the animal's tail is that when the animal sits still and its tail moves, it adds to distance moved.
- **To remove indentations in the shape of the subject**, such as those caused by the cage bars, or to 'join up' the stripes on the animal's body (for wasps, fish etc.) – Select **Dilation and Erosion**, and **Dilate first, then erode**. This removes indentations in the shape of the subject, giving a smoother outline, or ensures that EthoVision XT detects them as one animal.

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- **To deal with occlusions of the animal's body** – If you use nose-tail tracking (Advanced Model-based) with rodents, optimize the **Shape Stability** (see page 260).
- **To deal with two animals touching** – When two animals touch, EthoVision loses the separate shapes. By optimizing the Modelling effort (see page 261), EthoVision can determine which part of the large body fill belongs to which animal.

Contour erosion

The Erosion function reduces the subject's area by setting the contour pixels of the subject to the background value. The detected subject appears smaller in the **Video** window.

To apply Erosion, select **Erosion** and from the list select the thickness of the layer of pixels to be removed, expressed in number of pixels (Minimum =**1**, Maximum =**10**).

Figure 8.13.A shows the subject as detected by EthoVision with no filtering. After applying **Erosion**, a layer of pixels is removed from the contour (Figure 8.13.B). Figure 8.13.C shows the pixels that were removed.

Contour dilation

The Dilation function increases the subject's surface area by setting the background pixels adjacent to the subject's contour to the subject value. Therefore, the detected subject appears larger in the **Video** window.

To apply Dilation, select **Dilation** and from the list select the thickness of the layer of pixels to be added, expressed in number of pixels (Minimum =**1**, Maximum =**10**).

Figure 8.13.A shows the subject as detected by EthoVision XT with no filtering. After removing the rat's tail with the Erosion function (Figure 8.13.B), a layer of pixels is added back using Dilation (Figure 8.13.D), restoring the original size of the subject.

Combining dilation and erosion

Select both **Dilation** and **Erosion** if you want to apply the two filters together. From the **Order** list, select one of the following:

- **Erode first, then dilate** – A layer of pixels is removed, then added to the contour.
- **Dilate first, then erode** – A layer of pixels is added, then removed.

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Use Erode first, the dilate when you use the Advanced Model-based nose-tail tracking method because in this case the tail can negatively affect tracking. When you use one of the other nose-tail tracking methods, make sure the tail stays visible.

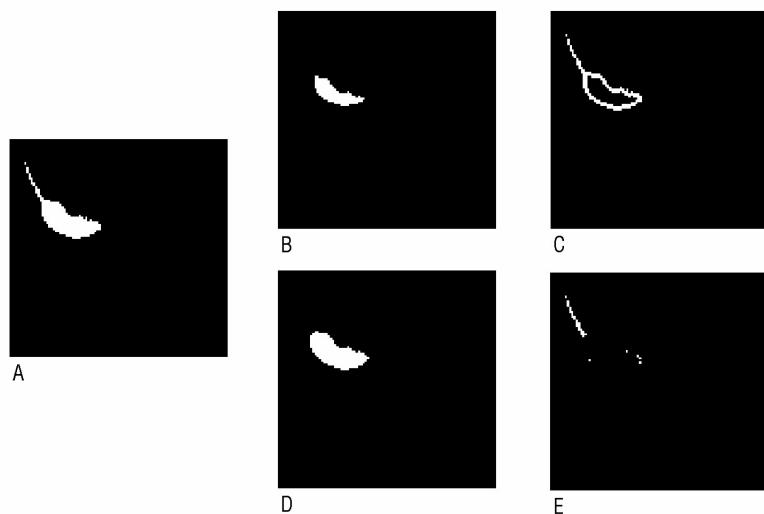


Figure 8.13. **A** – An example of a rat detected by EthoVision XT without any filtering applied. **B** – The same animal, after applying the Erosion filter on. **C** – The layer of pixels removed by Erosion. **D** – The same animal when first Erosion and then Dilation are applied. **E** – The net result of Erode first, then dilate: the pixels corresponding to the rat's tail are removed.

Subject size



Before you set the Subject size, make sure all animal body contours are detected properly and, for multiple animals, the animals do not touch each other.

The Subject size settings use the result of the body detection to model the body size of the animals. This prevents objects like droppings or large reflections from being detected during tracking. Please note that the term size here means surface area in video pixels, not length or screen pixels. Enlarging the Video window does not change the subject's size in video pixels.

Configuring Detection Settings

Setting the Subject size for a single animal

- Set the **Detected subject size** using the **Minimum** and **Maximum subject size** when you want to carry out Center-point detection or Nose-tail detection with either the Shape-based (XT4) or Model-based (XT5) detection method. The Detected subject size sets the absolute limits of the size that is possible to be detected as a subject.
- Set the **Modeled subject size** when you want to carry out Nose-tail detection using the Advanced Model-based (XT6) detection method. The Modeled subject size is the size of the model that the program will try to fit to the detected subject.

Setting the Subject size for multiple animals

Set the **Modeled subject size** when you want to track multiple animals. To set the Subject size:

- 1 In the **Subject size** section, click the **Edit** button.

In the **Subject Size** window, in the figure at the top, the thin red contour represents the current size of what EthoVision XT assumes is the animal shape.

➔ If you want to set the **Detected subject size**, proceed with step 2.

➔ If you want to set the **Modeled subject size**, proceed with step 3.



Click the info button for more information about setting the subject size.

- 2 Set the **Minimum** and **Maximum subject size** (represented by a green contour):

● **Maximum subject size** – The largest surface area (in pixels) that is detected as the subject. Objects bigger than the Maximum subject size, for example, the experimenter's arm, are detected as noise and not tracked. Decrease the **Maximum subject size** until its thick green contour surrounds the thin red contour by a fair margin.

● **Minimum subject size** – The smallest surface area (in pixels) that is detected as the subject. Objects smaller than the Minimum subject size, such as droppings or disturbed sawdust, are detected as noise and not tracked. Increase the **Minimum subject size** until its thick green contour is smaller than the thin red contour by a fair margin.



The two sliders are interdependent. So, after you have set the Minimum subject size, when you next change the Maximum subject size, the slider

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for the Minimum subject size also moves (although the size in pixels stays the same).

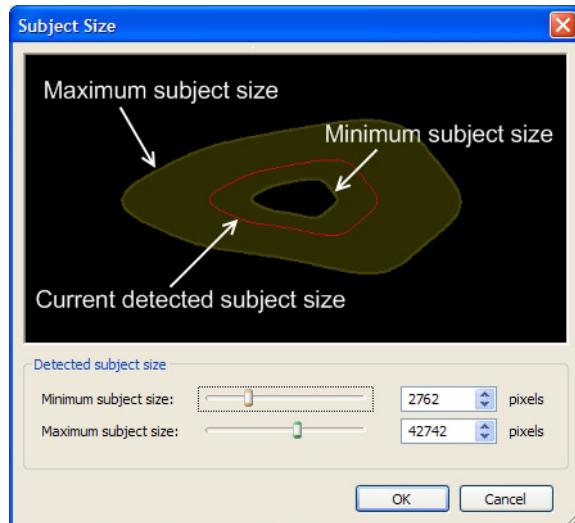


Figure 8.14. The Subject size window with the current detected subject size, Minimum and Maximum subject size.

- 3 In the Modeled subject size group, select **Apply settings to all subjects** if your multiple animals have similar sizes.



The **Modeled subject size** settings are only available when you use multiple subjects or the Advanced Model-based (XT6) nose-tail detection.

- 4 Select one of the subjects to model the subject size for, by clicking the name of the subject.
- 5 Next, adjust the modeled subject size (under Average - pixels) to the detected subject size (under Current - pixels):

You do this by clicking the **Grab** button. Keep clicking the **Grab** button until the modeled (Average) subject size equals the detected (Current) subject size.

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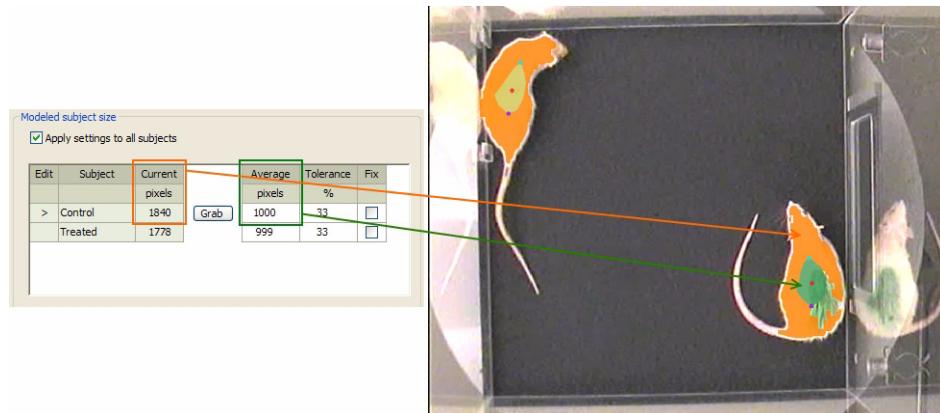


Figure 8.15. Part of the **Modeled subject size** group in the **Subject size** window (left) and the **Video** window. In the table, **Current** shows the current detected subject size in pixels, **Average** shows the modeled subject size in pixels. The arrows point to the visual feedback you get about the current and average subject size in the **Video** window.

When the modeled (Average) subject size equals the detected (Current) subject size, this becomes visible:

- In the **Modeled subject size** group: the Average subject size now equals or is larger than the Current subject size (see the table in Figure 8.16.).
- In the **Video** window: the modeled subject size now completely overlaps with the current subject size (see the Video window in Figure 8.16.).
- In the picture at the top of the Subject size window: the bold yellow contour represents the modeled subject size. This now coincides with the detected subject size indicated by the thin red contour (see Figure 8.14.).

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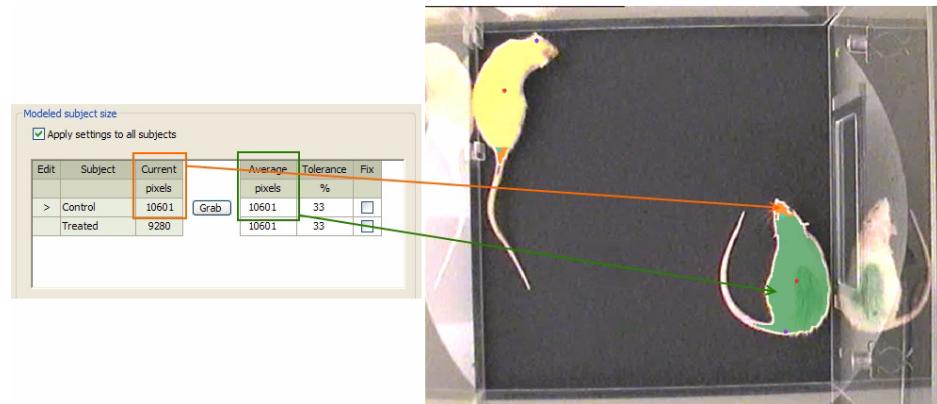


Figure 8.16. Part of the **Modeled subject size** group in the **Subject size** window (left) and the **Video** window. The modeled (Average) subject size is now adjusted to the detected (Current) subject size. Compare the table and video window in this figure with those in Figure 8.15.

6 You can now set the **Tolerance**. Click the corresponding cell and enter a value.

The Tolerance determines the deviation of the average subject size. When the Current detected size deviates more from the Average subject size than the Tolerance, then the object is not considered to be the subject anymore and EthoVision starts making an educated statistical guess about the body contour of the animal.



This is visible in the video window by a wobbling marker-color area. When this happens when animals do not touch, you should increase the **Tolerance**.

7 Select the **Fix** check box for each subject.

8 You can now proceed to set the **Maximum noise size**, **Shape stability** and **Modeling effort**.

Tips for setting the Subject Size



- Make sure you do not set the Tolerance too small; it is better get a wrong body size/shape than a wrong location of the animal.
- It is better to set your Average subject size slightly bigger than the actual subject size, especially when you carry out nose-tail tracking.

Configuring Detection Settings

- If you want to carry out Live tracking with multiple similarly-sized animals, it is recommended to first introduce one animal into the arena and make the Subject Size settings for this animal.
- If the subject size changes a lot between trials, it is recommended to create new Detection Settings for this new size.

Using a scan window

When **Use scan window** is selected, Ethovision XT finds the subject, 'follows' it and searches only the area immediately around it in the following video image. Therefore, the scan window moves with the subject.



Only select **Scan Window** after you have finished configuring the Detection Settings. **Scan window** should not be selected while you configure the Detection Settings.

Why use a scan window?

Use a scan window for two purposes:

- **To reduce problems with reflections** – If a reflection occurs outside the scan window (for example, waves in a water maze), this is ignored, resulting in fewer detection errors. However, make effort to improve lighting to eliminate reflections (see page 60).
- **To increase the sample rate without missing samples** – With a scan window, your computer processes data from a small proportion of the video image. This reduces the average processor load, so you can increase the sample rate, if necessary, without missing samples (remember that the higher the processor load, the more likely samples are skipped).

Losing the subject – When the subject disappears from the scan window, EthoVision XT scans the whole arena to find the subject again, and then repositions the scan window over that new location.



For user of previous EthoVision versions – The size of the scan window is automatically determined by the program and changes during acquisition according to the subject size. Therefore, you do not need to specify it.

8.3 Working with Nose-tail base detection

Overview

When you set an experiment for Nose-tail base detection, EthoVision XT analyzes the contour of the area detected as subject at each sample, and assigns Nose-point and Tail-base to two specific pixels of the contour. Furthermore, it determines the direction the animal is supposed to point to (Head direction).

- **Nose- and tail-base points** – The two points are detected independently through one of two complex algorithms. The nose-point is found in all cases, except when the center-point is not found either. The tail-base may not be found in a few cases if detection is good.
- **Head direction** – Once the nose-point has been found, two points are determined along the contour lying at a specific distance from the nose-point. The Head direction is the line dividing equally the angle formed by the center and those additional points.

Notes



- You can have EthoVision detect the nose- and tail-base points of your subjects when you have upgraded to the **Multiple Body Point Module**. To do so, upgrade your hardware key (see page 53). To set an experiment to Nose-tail base detection, in the Experiment Settings select **Center-point, nose-point and tail-base detection** (see page 102).
- The **Head direction to zone** is quantified as a dependent variable, and is expressed in units of rotation (see page 539).

Methods of Nose-Tail detection

In EthoVision XT 6.0, three methods for nose-tail base detection are available:

- **Shape-based (XT 4)** – This detection method analyzes the contour of the area detected as subject at each sample to assign the nose-point and tail-base. Make sure in the detection settings that the tail is fully detected. With this method it may be possible to track 'non-rodent' shapes but the method is not designed for it.

Configuring Detection Settings

- **Model-based (XT 5)** – This detection method analyzes the varying shape of the contour of the area detected as subject and builds up a 'rodent model'. It is more robust than the Shape-based method because it does not require the nose and tail to be visible: it can 'predict' the position of the nose and the tail based on previous samples. Make sure in the detection settings that the tail is fully detected.
- **Advanced Model-based (XT 6)** – This detection method teaches the animal shape and how it moves in the first 15 frames and continually updates its statistics. Therefore, it can handle severe shape distortions, such as, for example, when the animal's body is occluded or when multiple animal's touch. However, it requires a lot of computer performance.

This is the only method available when you track multiple animals with nose-tail detection. It is the preferred methods for rodents. For accurate detection of the tail-base, make sure the tail itself is removed from the body contour with Erode and Dilate (see page 251).

Which of the three methods should I use?

- When you want to track other animals than rodents, we recommend you use the **Shape-based (XT 4)** method.
- When you want to track a single rodent without occlusions or without difficult tracking conditions, we recommend you use the **Model-based (XT 5)** method.
- When you track rodents that can be occluded, for example, by bars or other objects in the cage, we recommend you use the **Advanced Model-based** method and to track **From video file**.
- When you want to track multiple rodents using Marker assisted tracking, EthoVision automatically selects the **Advanced Model-based (XT 6)** method. In this case, we recommend you track **From video file**.

Maximum noise size



Maximum noise size is only available if you have chosen the Advanced Model-based (XT6) nose-tail detection method.

You set the Maximum noise size in the Subject size window:

- 1 Go to the **Advanced** section by clicking the little down-arrow at the bottom-right of the Subject size window.

- 2 Set the **Maximum subject noise**. The value should be lower than the minimum subject size and but high enough to remove noise from the video image.

Shape stability



The Shape stability setting is only available if you have chosen the Advanced Model-based (XT6) nose-tail detection method.

The Shape stability setting is used when you track animals whose body can be occluded by, for example, cage bars or part of the body of another animal. When this happens, the animal's body consists of two separate objects that are close together.

You set the Shape stability in the Subject size window:

- 1 Go to the **Advanced** section by clicking the little down-arrow at the bottom-right of the Subject size window.
- 2 The **Shape stability optimized for** slider has two extreme settings:
 - **Occlusions** – When you set the slider close to **Occlusions**, EthoVision considers separate objects that are close together part of one animal.
 - **Noise** – When you set the slider close to **Noise**, EthoVision considers separate smaller parts not part of the animal.

The figure below shows the animal model as a result of applying the two extreme Shape stability settings.

Configuring Detection Settings

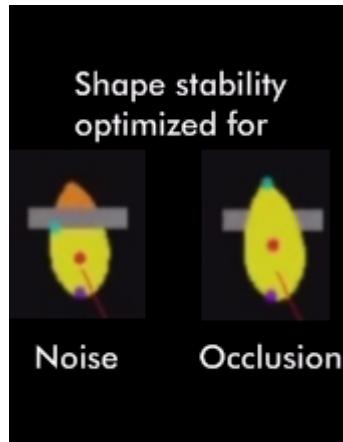


Figure 8.17. An example of the result of the two extreme Shape stability settings. 'Noise' shows that the front of the animal, on the other side of the bar, is not considered to be part of the animal. 'Occlusion' displays the animal body as a whole.

When you are not sure which setting to select, leave Shape stability at the default value of '620'.

Modeling effort

The Modeling effort setting is used when two animals touch and EthoVision loses the separate shapes. At this point, EthoVision tries to determine which part of the big 'merged' body fill belongs to either animal. This costs a lot of processing load.

The **Modeling effort optimized for** slider has two extreme settings:

- **Performance** – When you set the slider close to Performance, EthoVision is only allowed a short time to determine which part of the 'merged' body fill belongs to which animal. Therefore, Modeling quality is low.
- **Modelling** – When you set the slider to **Modelling**, EthoVision is allowed a longer time per frame to determine which part of the 'merged' body fill belongs to which animal. Therefore, Modelling quality is good, but this costs a lot of processor load.

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We recommend to select **Modelling** only when you have a computer that exceeds the minimum system requirements.

When you are not sure which setting to select, leave Modeling effort at the default value of '500'.

How to optimize Nose-Tail detection

Because of the way the nose- and tail base points are found, nose-tail base detection is much depending on the quality of the video image and the experimental setup. Before using this feature, please check the following guidelines:

Conditions related to the Arenas

- **Light** – Light conditions must be equal across the arena. Try to remove shades, light spots and reflections. For proper detection, the subject's body contour must be kept as constant as possible across the whole arena.
- **Subject/background contrast** – The color of the subject and of the background must be contrasting enough to get a full and clear body contour.
- **Video quality** – Noise and interference reduce the proportion of samples which are correctly detected.
- **Noise reduction** – The **Pixel smoothing** function (see page 230) can sometimes help getting a more appropriate body contour. However this is of little use if the video has too much noise or too little contrast.
- **Areas hidden to the camera view** – When the animal enters or exits areas hidden to the camera (for instance, a shelter), nose-point and tail-base are wrongly assigned.

Conditions related to the Subjects

- **Subject's apparent size** – The subject must be large enough to get a constant body contour. Small animals and large arenas pose detection problems with nose- and tail-base points.
- **Subject's color variation** – For hooded rats, the light flanks and dark head must contrast with the background, otherwise detection of body contour is sub-optimal, although the Differencing detection method (see page 245) can help.

Configuring Detection Settings

- **Water maze** – Tracking nose- and tail-base points in a water maze is impossible because the tail-base is under the water, and it is not possible to obtain a proper body contour.
- **Subject's behavior** – Immobile animals are hard to track because their body contour differs from that of a mobile animal. Nose-points are therefore hard to detect.

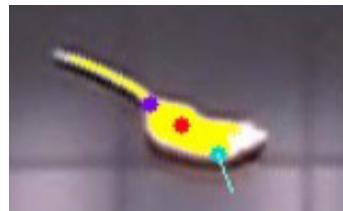
Experiment Settings

- **Detection methods** – We recommend to track From video files if you use the Advanced Model-based (XT 6) method.
- **Sample rate** – As high as possible (25 or 29.97 samples/s). For Nose-tail tracking in combination with Marker assisted tracking, you should use a sample rate of 12.5 or 14.98 samples/s.
- **Tracking live** – When tracking requires high processor load, it may result in many missing points. Tracking from video files is preferred (see below), especially when you use the Advanced Model-based (XT6) method.
- **Tracking from video files** – Keep the **Detection Determines speed** option selected.
- **Missing tail-base points** – The high percentage of missing tail-base points is an indication of poor detection. The higher this percentage, the greater the probability that the nose-point is not placed in the correct location. To estimate the proportion of missing tail-base points, run some test trials and visualize the Sample list (see Chapter 12). You can quantify this by selecting Number of samples as a statistic for a dependent variable such as Velocity for the nose point.

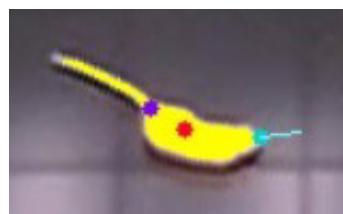
In practice...

The contour of the pixels detected as subject is crucial for proper detection of nose- and tail-base points. If only part of the subject is detected, EthoVision may swap the pixels assigned as nose-point and tail-base. Or the nose-point is not placed on the subject's nose tip (for clarity, the **nose point** is shown together with the **Head direction**; see page 264 for how to view this on the screen):

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Select a wider range of gray scale values (see page 234 or page 238) or adjust the sensitivity (see page 246) to increase the number of pixels detected as subject. When you use the methods Shape-based (XT4), or Model-Based (XT-5), make sure that the tail is fully detected. As a result, the nose- and tail-base points are detected correctly:



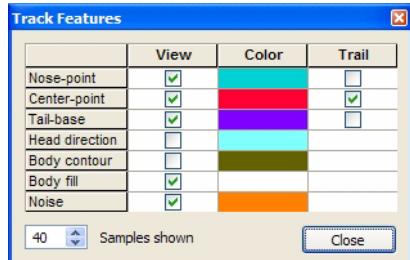
8.4 Customizing the Detection Settings screen

To achieve optimal subject detection, you need proper feedback about the effect of your settings on the quality of detection. EthoVision offers you a number of statistics for this purpose.

Customizing the track features

- 1 Open the Detection Settings (see page 211).
 - 2 Click the **Show/Hide** button on the component tool bar and select **Track features**.
- Result – The **Track Features** window appears.

Configuring Detection Settings



3 Choose the color and trail of the features of the body you want to view.

- **Nose-point** – To check that the nose tip is detected correctly (see page 258 for details).
- **Center-point** – To check that the center-point of the subject is detected correctly.
- **Tail-base** – To check that the base of the tail is detected correctly (see page 258 for details).
- **Head direction** – To estimate at what the subject is sniffing. Select this option especially with novel object and orientation tests.
- **Body contour** – To check that the subject's contour (or the part which should be found) is detected.
- **Body fill** – To check that the subject's body (or part of it) is detected. For example, in a Porsolt forced swim test where it is important to measure the change in the animal's shape to estimate its mobility.



If you do not select a color for Body fill, the body contour will be shown as noise.



- **Noise** – To view the pixels that match the criteria for subject detection (depending on the detection method), but other than those detected as subject.
- Some of the options above are not available if your experiment is set to **Only center-point detection** in the Experiment Settings (see page 96).
- The center-point is the point whose X,Y coordinates are the arithmetic mean of the X,Y coordinates of all pixels detected as subject. For more information on how the nose- and tail-base points are calculated, see page 258.
- We recommend to keep **Noise** selected. This way you can see which parts of the video image have gray scale values similar to those of the subject(s) to be detected.

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- 4 If you have selected to view the body points' Trail, choose the number of **Samples** you want to be shown at a time.

- 5 Check in the **Video** window the appearance of the track features. When you are satisfied with the options selected, close the **Track Features** window by clicking the button in its top right corner. Next, continue with the procedure below.

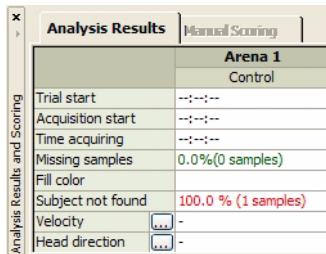
 Displaying track features can use a lot of processor power and reduce the maximum possible sample rate if you are tracking live.

Showing the detection statistics in the Analysis Results pane

The detection statistics are displayed in the Analysis Results pane, which are, by default, displayed at the bottom of the screen. If the Analysis Results pane is not displayed, do the following:

Click the **Show/Hide** button on the component tool bar of the Detection Settings screen and select **Analysis Results and Scoring**.

Result – The Analysis Results and Scoring pane appears.



Analysis Results		Manual Scoring
Arena 1		
Control		
Trial start	--:--:--	
Acquisition start	--:--:--	
Time acquiring	--:--:--	
Missing samples	0.0% (0 samples)	
Fill color		
Subject not found	100.0 % (1 samples)	
Velocity	[...]	-
Head direction	[...]	-

Detection statistics include:

- **Subject not found** – The percentage and number of samples in which the subject was not found. This information is useful to check the quality of detection in general. When a subject is not found, it means that EthoVision XT processed the image, but did not find anything matching the current Detection Settings.



After acquisition you can view the proportion of samples in which the subject was not found in the Trial list with the System Variable 'Subject not found'. If this column is hidden, click **Show/Hide** on the tool bar and select **Variables**. Select the variable **Subject not found**.

Configuring Detection Settings

- **Missing samples** – The percentage and number of samples that were skipped. This information is useful to check whether the sample rate you specify (see page 223) can be handled by your computer. See page 227 for tips on how to increase the maximum sample rate handled by the PC.



After acquisition you can view the proportion of missing samples in the Trial list with the System Variable 'Missing samples'. If this column is hidden, click **Show/Hide** on the tool bar and select **Variables**. Select the variable **Missing samples**.



The percentages of missed samples and samples where the subject is not found are usually displayed in green. When the values are above the set threshold, they are highlighted in red. To change the thresholds, click the **Show/Hide** button on the component tool bar and select **Feedback**. In the Feedback window, change the values under **Thresholds warning colors**.

You can also change the properties of the behavioral variables shown in the Analysis Results pane. For more information, see page 276.



Head direction and Elongation are only displayed if you have the Multiple Body Points add-on installed on your PC and you have selected **Center-point, nose-point and tail-base detection** in the Experiment Settings.

9

Acquiring Data

This chapter is about:

- **The Data Acquisition screen** – An overview of the objects you see when you start the Acquisition module, and their function.
→ See the next page
- **Customizing your Data Acquisition screen** – To specify, for example, whether you want to view the contour of the animal being tracked, and in which color.
→ See page 279
- **Acquiring the data** – You can acquire data in four ways:
 - Track from the live camera.
 - Track live, and record video at the same time.
 - First record video to a file, then track from that file.
 - Track from a video file recorded with software other than EthoVision XT.
→ See page 284



If you plan to use hardware devices like a pellet dispenser or the PhenoTyper, see also the EthoVision XT Trial and Hardware Control Manual, which you can find on your installation DVD.

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9.1 The data acquisition screen

To view the data acquisition screen, do one from the following:

- In the Experiment Explorer, in the Acquisition folder, click **Acquisition**.
- From the **Acquisition** menu, select **Open Acquisition**.

Video window

The **Video window** shows the video signal from your camera (after being digitized by the frame grabber), or the content of the digital media file currently selected, depending on what you selected as **Video source** (see page 98).

The video image is always shown independent of whether you are acquiring data (with some exceptions, see page 279).

The following items can be shown superimposed on the **Video window**:

- **Overlay text** – You can display the arena name, acquisition status (see below) or timer.
If no trial has been planned (see page 117), the **Video window** shows **No Trial Planned**.
- **Arena Features** – You can display Arenas, Zones and Points.
- **Track Features** – You can display body points, head direction, body contour, body fill, and noise.

You can choose to only show some of the elements listed above. To customize the **Video window**, see page 279.

Is the video image absent?



- If you are tracking live, check that the video camera is powered on and connected to the PC. If you have the Picolo Diligent board, or the Picolo U4 H.264 board installed on the PC, make sure that the input connector chosen on the board corresponds to the video source channel selected in the Experiment Settings.
- Click the **Show/Hide** button in the components tool bar and make sure that **Video Source** is selected.
- If you work with video files, click the **Video** button in the **Acquisition Method** window and select the file you require.

Acquiring Data

Acquisition status

You can check the status of acquisition in the **Video** window, below the arena name:



- **Ready for start** – Displayed until data recording starts.
 - **In progress** - Displayed as long as recording lasts.
 - **The timer** – Displayed as long as recording lasts.
 - **Acquired** – Displayed briefly when the trial stops.
 - **No Trial Planned** – Displayed when no trial is planned to the trial list. To acquire data, add a trial (see page 272), or in the Trial List change the trial status.
 - **To Skip (in orange)** – Displayed when the arena was skipped within a trial, that is, the value of the **Acquisition status** variable for that arena in the Trial List was changed from **Planned** to **To Skip** (see page 305).
 - If you choose to save video only and then acquire data (see page 290), when saving video the Acquisition status is the following:
 - **Ready for start** – Displayed until you start saving video.
 - **Generating video**– Displayed as long as video recording lasts.
- i** ● If you apply Trial Control with Start and Stop conditions, see the notes on page 295.
- To customize the **Video** window, see page 279. For more information on Trial Control, see Chapter 7.

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Acquisition Control window

Showing/hiding the Acquisition Control window

To show or hide this window, click the **Show/Hide** button  in the component tool bar and select **Playback Control** to show/hide the **Acquisition Control** window.



Figure 9.1. The **Acquisition Control** window if you track from video files.

The **Acquisition Control** serves two functions:

- Starting and stopping trials, and adding/skipping trials in your list.
- Positioning video files on the frame you require, when you track from video files (see page 290 and page 293).

Data acquisition functions



- **Add Trial** – Adds a trial to your **Trial list**. As soon as recording starts (see below), data are stored in that trial. You can only add a trial when at least another one is already acquired.
- **Start Trial** – Starts the current trial. If you choose to save video, then acquire data (see page 290), it also starts recording the video.
- **Stop Trial** – Stops the current trial. The data are saved automatically in the **Data Files** folder of your experiment. If you choose to save video, then acquire data (see page 290), this button also stops recording the video.
- **Skip Trial** – Skips the current trial, so you can acquire data for the next one. When you click this button, the status of that trial in the **Trial List** is set to **To Skip** (see page 305).



The name of the current trial is indicated in the **Acquisition Method** window (see page 274).

Acquiring Data

If you apply Trial Control's **Start** and **Stop** conditions, recording starts and stops when the defined Start and Stop conditions are met. See page 295 for more information.

Playback functions

The following buttons are available to control the playback of your video. They are only available when your experiment is set to acquire data from video files, not during actual acquisition (that is, after the trial has started).



Clicking the buttons below does not result in acquiring data!



- **Jump to begin** – Rewinds the media file backwards.



- **Step frame backward** – Moves the media file one frame back with each click of the button. To use this function, first pause the video (see below).



- **Pause** – Pauses the video while continuing to display the current video frame in the **Video** window.



- **Play forward** – Plays the media file at the normal play speed.



- **Step frame forward** – Moves the media file one frame forward with each click of the button. To use this function, first pause the video (see above).



- **Jump to end** – Advances the media file to the end.



- **Reset background** – Updates the background image using the current video frame.

Detection determines speed



In EthoVision 3, this option was known as '**As fast as possible**'.

When you select **Detection determines speed**, and during acquisition the media file is played, the images are analyzed at the maximum speed that the processor can handle. EthoVision varies the speed depending on how much data it has to process at each frame.

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We recommend to keep this option selected, so that video frames are not missed with high processor load.

Detection determines speed is only available when the Trial List contains planned trials, or you have added a trial with the **Add Trial** button.

Why use Detection determines speed?

- **To track faster when EthoVision XT can process images at a speed higher than the regular playback speed** – If you select this option, the program tracks at a speed higher than normal (1x). For example, when you use a fast detection method such as Gray scaling.
- **To ensure that no sample is missed when EthoVision XT can only process images at a speed lower than the regular playback speed** – If you select this option, when EthoVision cannot keep up with the regular playback speed it tracks at a speed lower than normal (1x) in order not to miss any sample. For example, when you track in many arenas and with a high sample rate combined with the Dynamic subtraction detection method.

Acquisition Method window

This window differs depending on the video source you use, either **Live tracking** or **Offline tracking (from video file)** (see page 98).

Live tracking

- **Live tracking** – To acquire data from the live image (see page 287 for the procedure).
 - **Save video** – Select this box if you want to save the live video to a MPEG-4 video file using the Picolo Diligent board or an H.264 video file using the Picolo U4 H.264 board. Note: If **Save video** is not available, check that **Save video file** is selected in **Video Settings** window (see page 98).
- **Save video only, track later** – To record the live footage to a video file using the Picolo Diligent board or the Picolo U4 H.264 board, without gathering the track data (see page 290 for the procedure). You can then acquire the data when it is most convenient for you, for example after you have adjusted your detection settings.
- **Track saved video file** – To acquire data from a video file previously recorded using the **Save video only** option (see above). This option is only available if

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you save a video file but no data have been acquired yet from it (see page 290).

- **Redo tracking** – To repeat tracking from one of the video files recorded with either **Live Tracking** (with the **Save video** option selected) or **Save video only, track later**. This option is only available if you have acquired data using one of those two methods. See page 306 for information on how to redo trials.
- **Show Independent Variables** – To open the **Independent Variables** window and enter the values of the Independent Variables for the trial you are about to carry out. This window only contains the variables you selected in the Trial list (see page 117).
- **Edit Independent variables after acquisition** – To update the Independent variables after the trial has stopped.



The number of the current trial to be acquired is shown next to the option.

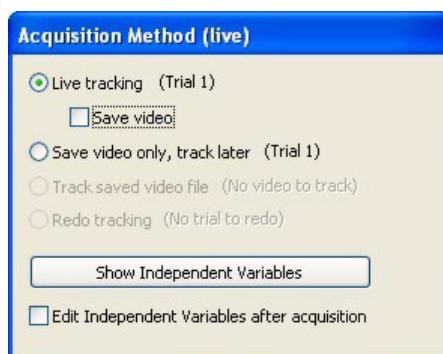


Figure 9.2. The **Acquisition Method** window for live tracking

Offline tracking (from video files)

- **Video** – Click this button to select a new video file. The content of the video is shown in the **Video** window.



Use this button to change video file before starting the next trial.

- **Track from file (Trial number)** – Shows the name of the trial to be acquired. If no trials are planned in the **Trial List**, it shows **No trials planned**.

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- **Show Independent Variables** – Click this button to open the **Independent Variables** window and enter the values of the Independent Variables for the trial you are about to carry out. This window only contains the variables you selected in the Trial list (see page 117).
- **Edit Independent variables after acquisition** – To update the Independent variables after the trial has stopped.

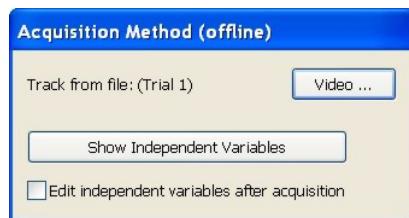


Figure 9.3. The **Acquisition Method** window for offline tracking

Analysis Results pane

The **Analysis Results** pane shows real time information on each subject detected in the arenas. By default this window is docked and located at the bottom of the EthoVision XT screen.

By monitoring that information you can check if detection is optimal. You can then adjust detection settings if necessary (see Chapter 8) and change the properties of the dependent variables.

The screenshot shows the 'Analysis Results' pane with two tabs: 'Analysis Results' (selected) and 'Manual Scoring'. The main area is titled 'Arena 1' and contains a table with the following data:

	Control	Treated
Trial start	2009-04-02 14:09:56	
Acquisition start	2009-04-02 14:09:56	
Time acquiring	0:00:03	
Missed samples	0.0% (0 samples)	0.0% (0 samples)
Fill color		
Subject not found	9.4% (3 samples)	15.6% (5 samples)
Velocity	4.767 cm/s	-
Head direction	80.29 deg	-110.1 deg

Figure 9.4. The **Analysis Results** pane with two subjects in one Arena.

Notes



- You can reduce processor load, for example when tracking from many arenas, by closing the **Analysis Results** pane. **Do this by clicking the Show/Hide button and then de-selecting Analysis Results and Scoring. Do not use the Close button on the Analysis Results pane.**
- In EthoVision XT 8, the **Trial Information** pane that was available in EthoVision XT 4/5 is included in the **Analysis Results** pane.

Information

- **Trial start** – Shows the time the current trial has started. If the current trial has not started yet, **Trial start** shows '---'.
- **Acquisition start** – Shows the time that tracking has started. If the current trial has not started yet, **Acquisition start** shows '---'.



If you track from video files, **Trial start** is the time that the video was recorded. **Acquisition start** is the video time when acquisition has started.

- **Time acquiring** – The time elapsed since the start of data recording for a subject in a specific arena.
- **Missed samples** – The percentage and the number (in parentheses) of samples missed up to the current sample. **Missed samples** statistics are shown in red when the percentage exceeds the value set in the **Feedback** () .



Click the ellipsis button next to this variable to set its threshold value.

After acquisition you can view the proportion of missing samples in the column **Missed Samples** in the Trial list. If this column is not visible, click the **Show/Hide** button on the component tool bar, select **Variables** and select the check box in front of **Missed Samples**.

- **Fill color** – Shows the color associated to each subject. You can assign a new color in the **Subject identification** settings (see page 216). If your experiment is set to tracking of one subject per arena, **Fill color** shows '-'.
- **Subject not found** – The percentage and the number (in parentheses) of samples in which the subject was not found up to the current sample (see also page 266).

Subject not found statistics are shown in red when the percentage exceeds the value set in the **Feedback** window (see page 266).



Click the button next to this variable to set its threshold value.

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After acquisition you can view the proportion of samples in which no subject was found in the column **Subject not found** in the Trial list. If this column is not visible, click the **Show/Hide** button on the component tool bar, select **Variables** and select the check box in front of **Subject not found**.

- **Velocity** – The velocity calculated for the current sample (see page 501).



Click the button next to this variable to specify the body point for which velocity is calculated. By default, center-point is selected.

- **Head direction** – The angle formed by the Head direction segment at the current sample (see page 512).

- **Mobility** – The Mobility state of the subject at the current sample.



Click the button next to this variable to specify the thresholds of the change in subject area that determine whether the subject is Immobile, Mobile or Highly mobile (see page 530).

- **Elongation** – The Elongation state of the subject at the current sample.



Click the button next to this variable to specify the thresholds of the elongation index that determine whether the subject is Contracted, Normal or Stretched (see page 528).

Notes



- **Time acquiring** can differ per arena. If you apply Trial Control with Start and Stop conditions for each arena (see page 178), the actual data recording starts and stops independently in each arena.
- Head direction, Mobility and Elongation and the body points for Velocity are only displayed if you have the **Multiple body points** add-on installed on your PC. For Velocity and Head direction, the experiment must be set to **Center-point, nose-point and tail-base detection** in the Experiment Settings.

9.2 Customizing your data acquisition screen

Customizing the Video window

The **Video** window is always displayed in the acquisition screen. Use the mouse to move and resize the **Video** window. Note: While you resize the **Video** window, the image's aspect ratio is kept constant.

Showing/hiding the Video source

To show or hide the Video source, click the **Show/Hide** button in the component tool bar and select or deselect **Video source**.

Showing/hiding the overlay text

- 1 Click the **Show/Hide** button on the component tool bar and select **Text features**.
- 2 Select **Hide Information** or **Show Information**.
- 3 Select one of the following:
 - **Per arena** or **Per Trial**.
- 4 Select whether to show the name, acquisition status and/or elapsed time. Furthermore you can select the text color and opacity.

Customizing the detection statistics

The detection statistics **Missing samples** and **Subject not found** are displayed in red in the Analysis Results pane when their value exceeds a certain threshold value. Below this threshold the text is displayed in green.

To set these threshold values:

- 1 Click the **Show/Hide** button in the component tool bar and select **Feedback**.
- 2 Under **Threshold warning colors**, set the threshold above which **Missing samples** and **Subject not found** are displayed in red in the Analysis Results pane.



If you do not want to display the **Missing samples** and **Subject not found** in different colors, de-select the checkboxes.

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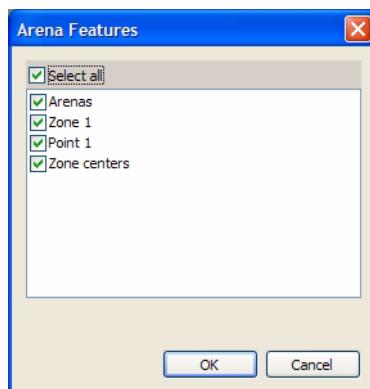
Customizing the track features

You can specify which track features (for example, the body points, or the body contour) to have displayed overlay during acquisition:

- 1 Click the Show/Hide button on the component tool bar and select **Track Features**.
- 2 Select the features you want to display and their colors and opacity.

Customizing the Arena Features

- 1 Click the **Show/Hide** button on the component tool bar and select **Arena Features**.
- 2 In the Arena Features window (see figure below), select the Arena Features you want to display.



In the example in Figure 9.5., the four quadrants of an open field have been selected for display.

Acquiring Data

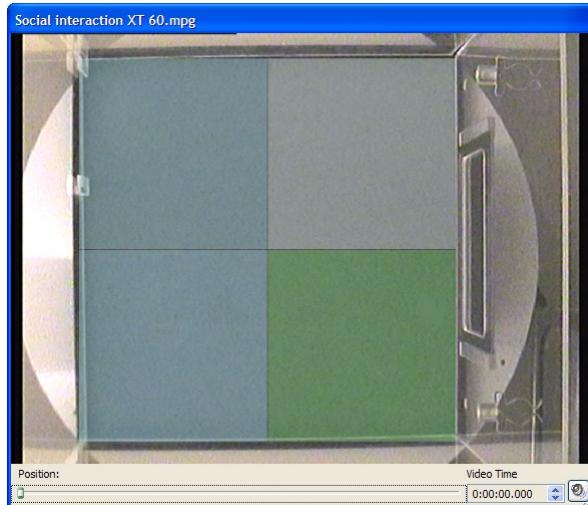


Figure 9.5. The Video window with four quadrants of an open field displayed.



The arenas and zones are highlighted in the colors selected in the currently active Arena Settings. If you want to change the color of a zone or arena, open those Arena Settings, and change the fill color.

Showing and hiding the Video Source

To show the Video source, click the **Show/Hide** button on the component tool bar and select **Video Source**.

If you choose not to display the video source, you can still display the center-point and other features of the subject to monitor the tracking (see page 280).



Displaying the video footage requires significant processor power. If viewing the video footage during acquisition is not necessary in your experiment, de-select **Video Source** to reduce processor load and consequently increase your maximum attainable sample rate (see page 224).

Customizing the format of the video file position

The current position of the media file is shown below the video image as elapsed time or number of frames. To choose between the two formats, right-click in the

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middle of the **Video** window and select either **Position in Time** or **Position in Frames**.



Customizing the Analysis Results pane

Showing/hiding panes

To show the **Analysis Results and Scoring** pane, click the **Show/Hide** button in the component tool bar and select **Analysis Results and Scoring**.



You can reduce processor load during acquisition by hiding the **Analysis Results pane**. Do this by clicking the **Show/Hide** button and then de-selecting **Analysis Results and Scoring**. Do not use the **Close** button on the **Analysis Results pane**.

Moving and docking a pane

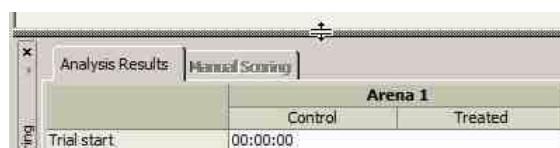
You can move the **Analysis Results and Scoring** pane to any position: click the pane's title bar (on the left side of the pane) and drag it to the desired position.

Letting a pane float on the screen

Press and hold **Ctrl** while you dragging the pane to the desired position.

Resizing

Place the mouse pointer over the pane's margin and drag it to the desired position.



Re-establishing a pane

If one of the panes is missing, click the **Show/Hide** button in the component tool bar and check that the corresponding check box is selected.

Customizing independent variables entry

Why edit the independent variables during acquisition?

Each trial can be associated with one or more values of user-defined independent variables (see page 105). For example, at the start of a trial you may want to enter the ID number of the animal currently used. Or, at the end of the trial you may want to specify whether the animal had epileptic seizures during the test. You can specify when in the acquisition procedure you enter such data.

Entering independent variable values before acquisition

Do the following **before** starting a trial (that is, before clicking the green **Start trial** button):

- 1 In the **Acquisition Method** window, click the **Show Independent Variables** button.
Result – The **Independent Variables** window appears.
- 2 Enter the values of the variables in the corresponding cells, then click **OK** when finished.

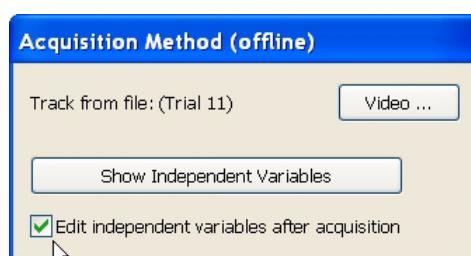
Now you are ready to start the trial (see page 284).



If the **Show Independent Variables** button is not available, click the **New trial** button in the **Acquisition Control** window.

Entering independent variable values after acquisition

- 1 In the **Acquisition Method** window, select the **Edit Independent Variables after acquisition** option.



- 2 Carry out the trial (see page 284).

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- 3 When the trial stops, the **Independent Variables** window appears. Enter the values of the variables in the corresponding cells, and click **OK** when finished.

Entering independent variable values before and after acquisition

It is sometimes necessary to enter values of independent variables both at the start and at the end of a trial. To do so, make sure that you keep the **Edit Independent Variables after acquisition** option selected, and click the **Show Independent Variables** button before you start each trial.

You can update independent variables at any time except for when a trial has started. To update independent variables, from the **Setup** menu select **Trial List**.

9.3 Acquiring data

Four methods to acquire data



We assume at this point of the manual that you have adjusted your Arena, Trial Control and Detection Settings, and that EthoVision XT can detect the subjects correctly. If this is not the case, see Chapter 8.



Please turn off the screen saver and power save options of your computer before you start acquiring data.

Choose the method that best suits your setup and experimental protocol:

1 - Acquiring data live

The animals are tracked as they move in your experimental setup. You do not use pre-recorded video.

Advantages – Tracking takes place as the test is carried out. This method requires no disk space for video storage.

Disadvantages – You do not have a video backup, so if you need to repeat data acquisition you have to repeat the test itself.



To apply this method, make sure that **Live Tracking** is selected as **Video Source** in the Experiment Settings.

→ See page 287

Acquiring Data

2 - Acquiring data live and recording video simultaneously

The animals are tracked as they move in your experimental setup. At the same time, EthoVision XT records video to an MPEG-4 or H.264 video file, depending on the encoder board.

Advantages – You have a video backup that you can use to acquire data once again if needed. Moreover, you do not need additional hardware on your PC since video is recorded via the encoder board.



To apply this method, you must have a frame grabber and encoder board installed on your PC. Make sure that **Live Tracking** is selected as **Video Source** in the Experiment Settings.

➔ See page 288

3 - Recording video with EthoVision XT, then acquiring data

Depending on the encoder board you have, EthoVision XT records video to an MPEG-4 or H.264 video file as the animals move in your experimental setup. Afterwards, open the video file and acquire the data.

Advantages – You benefit from having a video backup, and you are free to track data later, for example after adjusting detection settings.



To apply this method, you must have the Picolo Diligent board installed on your PC. Make sure that **Live Tracking** is selected as **Video Source** in the Experiment Settings.

➔ See page 290

4 - Acquiring data from video files recorded with other programs

Use an external program, like the Media Recorder, to record video to a file while the animals move in the experimental setup. Later on, acquire data from that video file.

Advantages – You can use a wide array of hardware and software to record video. You can also reuse already digitized video files. See Appendix B for details of which video formats are supported.



To apply this method, make sure that **From video file** is selected as **Video Source** in the Experiment Settings.

➔ See page 293

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Advantages of using video files

- **Creating a video backup** – A backup copy of the video footage is very handy since it allows you to acquire data for a trial once again, if needed. For example, if you realize that the detection settings were not optimal when you acquired the data. To redo tracking, see page 306.
- **More reliable tracking** – When you track from a video file, you can let EthoVision XT take as much time as it needs to analyze each sample, independent of the actual frame rate. For example, if the detection method used requires a lot of time per sample, acquisition may be slower than real time, but you ensure that no sample is missed. For more information, see page 273.
- **Quicker tracking** – In many cases EthoVision XT can analyze samples at a rate faster than the actual sample rate. This means that acquisition from video files is faster than real time.



If you use a frame grabber and encoder board, a track acquired from the resulting video file is identical to one acquired with live tracking.

See also Appendix B for more information on video files.

Important notes for all acquisition methods



- **Adding a trial** – You must add a new trial if there are no planned trials. In this case, the **Video** window shows **No Trial** and the **Add Trial** button is available in the **Acquisition Control** window.



- **Skipping a trial** – The trial to be acquired is indicated by the trial number next to option you select in the **Acquisition Method** window. If you want to skip one or more trials and acquire a trial further in the list, click the **Skip Trial** button in the **Acquisition Control** window one or more times. You can always acquire data for a skipped trial in a later session.

- **Starting/Stopping data recording automatically** – If you apply Trial Control with Start and Stop conditions, data recording starts and stops when those conditions are met in the specified arena. In all other cases, it starts when you start the trial and stops when you stop the trial. See page 295 for more information.

- **Screen update** – Under intensive processor work, for example when tracking in many arenas, the program updates the screen irregularly. For example, the area detected as subject may look shifted relative to the position of the animal. This is due to the lower priority that display gets in such conditions. It does not affect detection or any measurement.

Acquiring Data

- If your experiment is set to **Use of trial control hardware** with devices such as a pellet feeder, when starting a trial you may get a message "Cannot start acquisition - IO interface not connected". Before starting a trial, make sure that the IO box is connected to the EthoVision computer, and all devices are connected to the IO box. For more information, see the Trial and Hardware Control Manual.

Acquiring data live

Before starting

- 1 Make sure that the video camera is connected to your PC's frame grabber board, and that **Live Tracking** is selected as **Video source** in the Experiment Settings.
- 2 Open the Acquisition module: from the **Acquisition** menu, select **Open Acquisition**. Make sure that the detection settings you want to apply are active (see Chapter 8).
- 3 If you want to acquire physiological data together with track data, make sure that the physiological data recording device is connected to the EthoVision PC and switched on, and that **Enable DAQ co-acquisition** is selected under **Video source** in the Experiment Settings. See also page 299.
- 4 In the Acquisition module, select **Live Tracking** in the **Acquisition Method** window.

1 – Add a new trial



If no trials are planned, click the **New trial** button in the **Acquisition Control** window or press **Ctrl+F3**.

2 – Start the trial

- 1 Optional – Click **Show Independent Variables** in the **Acquisition Method (live)** window and enter the values of the Independent Variables for that trial, then click **OK**.



You can customize the entry of Independent Variables values. See page 283.

- 2 Do one of the following:



- From the **Acquisition** menu, select **Start Trial**.

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- Click the **Start trial** button in the **Acquisition Control** window.
- Press **Ctrl+F5**.

Result – The trial starts.

- 3** Release the animal in the arena.

As soon as data recording starts, the status shown as overlay text on the **Video** window changes to the elapsed time.

- 3 – Stop the trial**

- 1** Do one of the following:



- From the **Acquisition** menu, select **Stop Trial**.
- Click the **Stop trial** button in the **Acquisition Control** window.
- Press **Ctrl+F6**.

- 2** If you have specified to edit independent variables after acquisition (see page 283), the **Independent Variables** window appears. Edit the values where needed, then click **OK**.



Effect on the status variables in the Trial List – For the trial just acquired, the **Acquisition status** is **Acquired**. The **Video file status** is **Skipped** (this is of little importance when acquiring data live without saving video).

- 4 – What next?**

Remove the animal from the arena and repeat the procedure with the next trial. This is indicated next to **Live tracking** in the **Acquisition Method** window.

Acquiring data live and recording video simultaneously

Before starting

- 1** Make sure that (a) the video camera is connected to two inputs of your PC's frame grabber and encoder board (video signal is split to two inputs via a T-connector) (see page 46), and (b) that **Live Tracking** is selected as **Video source** in the Experiment Settings. The **Save video** option must also be selected under **Settings**.

Acquiring Data

- 2 If you want to acquire physiological data together with track data, make sure that the physiological data recording device is connected to the EthoVision PC and switched on, and that **Enable DAQ co-acquisition** is selected under **Video source** in the Experiment Settings. See also page 299.
- 3 Open the Acquisition module: from the **Acquisition** menu, select **Open Acquisition**. Make sure that the detection settings you want to apply are active (see Chapter 8).
- 4 In the Acquisition module, select both **Live Tracking** and **Save video** in the **Acquisition Method** window.

1 – Add a new trial



If no trials are planned, click the **New trial** button in the **Acquisition Control** window.

2 – Start the trial (record data and video)

- 1 Optional – Click **Show Independent Variables** in the **Acquisition Method (live)** window and enter the values of the Independent Variables for that trial, then click **OK**.



You can customize the entry of Independent Variables values. See page 283.

- 2 Do one of the following:



- From the **Acquisition** menu, select **Start Trial**.
- Click the **Start trial** button in the **Acquisition Control** window.
- Press **Ctrl+F5**.

Result – The status shown as overlay text on the Video window changes to the elapsed/remaining time. Video is being recorded to a file on the hard disk.

- 3 Release the animal in the arena.

3 – Stop the trial

- 1 Do one of the following:



- From the **Acquisition** menu, select **Stop Trial**.
- Click the **Stop trial** button in the **Acquisition Control** window.
- Press **Ctrl+F6**.

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- 2 If you have specified to edit Independent Variables after acquisition (see page 283), the **Independent Variables** window appears. Edit the values where needed, then click **OK**.



Depending on the board, the video file that has just been created is stored as MPEG-4 or H.264 file on your PC. It is named as the trial name (Trial 1.mpg, Trial 2.mpg, etc.).

Effect on the status variables in the Trial List – For the trial just acquired, the **Acquisition status** is **Acquired**. The **Video file status** is **Generated**.

4 – What next?

Remove the animal from the arena and proceed with the next trial.

Recording video, then acquiring data

Before starting

- 1 Make sure that (a) the video camera is connected to two inputs of your PC's frame grabber and encoder board (video signal is split to two inputs via a T-connector), and (b) that **Live Tracking** is selected as **Video source** in the Experiment Settings. The **Save video** option must also be selected under **Settings**.
- 2 If you want to acquire physiological data together with video, make sure that the physiological data recording device is connected to the EthoVision PC and switched on, and that **Enable DAQ co-acquisition** is selected under **Video source** in the Experiment Settings. See also page 299.
- 3 Open the Acquisition module: from the **Acquisition** menu, select **Open Acquisition**. Make sure that the detection settings you want to apply are active (see Chapter 8).
- 4 In the Acquisition module, select **Save video only, track later** in the **Acquisition Method** window.

1 – Add a new Trial



If no trials are planned, click the **New trial** button in the **Acquisition Control** window.

2 – Record video

- 1 To start video recording, do one of the following:

Acquiring Data



- From the **Acquisition** menu, select **Start Trial**.
- Click the **Start trial** button in the **Acquisition Control** window.
- Press **Ctrl+F5**.

Result – The status shown as overlay text in the Video window changes from **Save video only - Waiting to start to Save video only - Generating**.



When you record video, Trial Control is not applied, except for the maximum trial duration. This means that you can for example have video recording stopped automatically after 10 minutes.

2 To stop video recording, do one of the following:



- From the **Acquisition** menu, select **Stop Trial**.
- Click the **Stop trial** button in the **Acquisition Control** window.
- Press **Ctrl+F6**.

Depending on the board, the video is saved as an MPEG-4 or H.264 file on your PC. It is named as the trial name (Trial 1.mpg, Trial 2.mpg etc.). Remove the animal from the arena. You can now proceed with the next test (go back to step 1 or 2 above) or track the data from the video just saved (step 3 below).



Effect on the status variables in the Trial List – For the trial corresponding to the video just saved, the **Acquisition status** is **Postponed**. The **Video file status** is **Generated**.

3 – Start the trial

- 1 Make sure that the video file is positioned at its beginning or, in any case, at the point where you want to start data acquisition.
- 2 Optional – Click **Show Independent Variables** in the **Acquisition Method** window and enter the values of the Independent Variables for that trial, then click **OK**.



You can customize the entry of Independent Variables values. See page 283.

3 Select **Track saved video file** in the **Acquisition Method** window.



Optional – Select **Detection determines speed** in the **Acquisition Control** window to make sure that samples are not missed. See page 273 for the advantages of applying this option.

The trial name shown next to **Track saved video file** refers to the first saved video in your Trial List that you still have to track from.

4 Do one of the following

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- From the **Acquisition** menu, select **Start Trial**.
- Click the **Start trial** button in the **Acquisition Control** window.
- Press **Ctrl+F5**.

Result – The status shown as overlay text on the **Video** window changes to the elapsed time. The video is played forward and tracking takes place.

4 – Stop the trial

1 Do one of the following:



- From the **Acquisition** menu, select **Stop Trial**.
- Click the **Stop trial** button in the **Acquisition Control** window.
- Press **Ctrl+F6**.

Acquisition stops automatically when the video file has reached the end.

2 If you have specified to edit Independent Variables after acquisition (see page 283), the **Independent Variables** window appears. Edit the values where needed, then click **OK**.

 Effect on the status variables in the Trial List – For the trial just acquired, the **Acquisition status** is **Acquired**. The **Video file status** is **Generated**.

5 – What next?

- Proceed with the next trial if required.
- If you wish to discard the track data and re-do tracking from that video, see **Redo a trial** on page 306.
- When you save more videos in a run (step 2 above) without tracking (steps 3 and 4), you notice a difference between the trial name shown next to **Save video only, track later** and that next to **Track saved video** in the **Acquisition Method** window. This is because at that moment there are fewer trials with track data than videos saved on your PC. In the example below, video files have been saved for the first five trials. The **Save video only, track later** option prompts you to save the sixth video file. If you select **Track saved video file**, the program prompts you to track data from the first trial with no video, in this case Trial 1.



Acquiring data from video recorded with other programs

Before starting

- 1 Please first read sections “Using consumer camera models” on page 48, “Digital cameras” on page 25, and “Video file formats supported by EthoVision XT” on page 601.
- 2 Make sure that **From Video File** is selected as **Video source** in the Experiment Settings.
- 3 Co-acquisition of physiological data is not possible when you track data from a video file. See also page 299.
- 4 Open the Acquisition module: from the **Acquisition** menu, select **Open Acquisition**. Make sure that the detection settings you want to apply are active (see Chapter 8).

1 – Record video

Put the animals in the arena and record video to a digital media file using the appropriate hardware and software.



Please see the video recording software's user manual for information on how to record video. For information on the video file formats that can be used with EthoVision XT, see Appendix B.

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2 – Add a new Trial



If no trials are planned, click the **New trial** button in the **Acquisition Control** window.

3 – Load the video file

If the video file in the Video window is not the one you want to use for tracking, do the following:

- 1 Click the **Video** button in the **Acquisition Method** window.

Result – The **Open** window appears. The window lists the video files of MPEG format stored in the folder that was opened last.

- 2 Navigate to the folder where the video file is stored, and click the file name in the list that appears. Next, click **Open**.



To have files of all formats listed, select **All files** from the **Files of type** list.



To check that the correct video file has been loaded, view its content by clicking the **Play forward** button in the **Acquisition Control** window.

4 – Start the trial

- 1 Make sure that the video file is positioned at its beginning or, in any case, at the point where you want to start data acquisition.
- 2 Optional – Click **Show Independent Variables** in the **Acquisition Method** window and enter the values of the Independent Variables for that trial, then click **OK**.



You can customize the entry of Independent Variables values. See page 283.

- 3 Optional – Select **Detection determines speed** in the **Acquisition Control** window to make sure that samples are not missed (see page 273 for more information).

- 4 Do one of the following



- From the **Acquisition** menu, select **Start Trial**.
- Click the **Start trial** button in the **Acquisition Control** window.
- Press **Ctrl+F5**.

Result – The status shown as overlay text on the **Video** window changes to the elapsed time. The video is played forward and tracking takes place.

5 – Stop the trial

1 Do one of the following:



- From the **Acquisition** menu, select **Stop Trial**.
- Click the **Stop trial** button in the **Acquisition Control** window.
- Press **Ctrl+F6**.



If Trial Control is active and contains Stop conditions, acquisition stops automatically, depending on whether and when the defined conditions are met. See page 295 for more information.

Acquisition stops automatically when the video file has reached the end.

2 If you have specified to edit Independent Variables after acquisition (see page 283), the **Independent Variables** window appears. Edit the values where needed, then click **OK**.



Effect on the status variables in the Trial List – For the trial just acquired, the **Acquisition status** is **Acquired**. The **Video file status** is **External**.

6 – What next?

- Proceed with the next trial if required.
- If you wish to track data once again from that video, add a trial and repeat the procedure. You cannot re-do a trial (page 306) obtained with this acquisition method.

Working with Trial Control



The **Trial and Hardware Control Manual** contains extensive information on designing and applying trial control to your trials. You can find this guide in the **Documentation** folder on your installation DVD.

Trial vs. track

See the important difference between trial and data recording on page 203.

Applying Trial Control

To apply Trial Control settings to a trial, make sure that the Trial Control Settings profile containing the rule with required conditions is highlighted in blue in the Explorer under **Trial Control Settings**. To do so, right-click the Trial Control profile and select **Set as Current**.

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Starting data acquisition

- If you use the default **Trial Control** profile, data acquisition starts 1 second after the animal has been detected in the arena.
- If you have defined one or more conditions to trigger the Action box **Start track**, data acquisition starts as soon as the conditions are met.
- If conditions to start data acquisition are never met within an arena, data are not recorded for that arena. A track file is created, but contains no track data.

Stopping data acquisition

- If you use the default **Trial Control** profile, data acquisition stops when you give the Stop command (by clicking the **Stop** button or pressing **Ctrl+F6**).
- If you have defined one or more conditions to trigger the Action box **Stop track**, data acquisition stops as soon as the conditions are met.
- If conditions to stop data acquisition are never met within an arena, data are recorded indefinitely unless you have specified a **Maximum trial duration** in the Trial Control (see page 199).

Using Trial Control in a multiple-area setup

A Trial Control rule in **Trial Control Settings** applies to all arenas:

- **Multiple Starts of data acquisition** – Once a trial starts, data acquisition starts independently for each arena.
- **Multiple Stops of data acquisition** – Data acquisition stops independently for each arena.

Example – A condition has been defined so that data recording stops when the animal is detected for 10 cumulative minutes (**Duration**). Data recording stops at different **Elapsed** times, because when the animals enter the shelter they are not detected, therefore the 10-minute threshold is reached at different times in the four arenas.



Figure 9.6. A screenshot taken to the Video window for a multiple-arena setup. The Elapsed time is the time since the start of the actual recording in the arena. Acquisition stops first in Arena 3 at 10 min 46 sec, then in Arena 1 15 seconds later. In Arena 2 and 4 data acquisition has not stopped yet, because the animals have been in the shelter for longer, therefore the cumulative time of 10 minutes has yet to be reached.

If you select **Current Duration** instead of **Duration**, recording stops at 10 minutes for all arenas.

9.4 Acquiring external data

What is external data?

In EthoVision XT, external data is any data set acquired with a separate Data Acquisition (DAQ) system (a data recording device connected to a computer) and stored or exported as an ASCII file. External data include physiological data (EEG, ECG, blood pressure etc.), and environmental data (temperature, humidity etc.).

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You must have the **Physiological inputs** add-on in order to use this functionality.

When do I need this information?

You need this information when your DAQ system is physically connected to the EthoVision PC, and you want to have physiological and track data synchronized automatically.



If your DAQ system is not physically connected to the EthoVision PC, you can import and then have data synchronized manually. See **Importing physiological data** in Chapter 10.

Procedure overview

- 1 Connect the EthoVision PC to the DAQ system and make sure that this is connected to its dedicated computer.
- 2 Specify external data co-acquisition in EthoVision XT (see below).
- 3 Carry out a trial as usual (see page 284).
- 4 Import the external data files into EthoVision XT and associate them with a trial, an arena, or a subject. See Chapter 10.

Notes



- You can only co-acquire external data when you track data live. This means:
 - In the Experiment Settings, select **Live tracking** as **Video Source**.
 - In the Acquisition module, select either **Live Tracking** or **Save video only, track later** in the **Acquisition Method** window depending on the acquisition method you choose (see page 284).
- EthoVision XT does **not** record physiological data. It is the dedicated computer that saves those data in the form of ASCII files. These data files must be imported in EthoVision XT in order for you to view the external data together with the track data.
- During the trial, you do not see the external data on the EthoVision screen.

Specify external data co-acquisition in EthoVision XT

You can only follow this procedure if your EthoVision computer is connected to the DAQ device through a COM or a USB port.

- 1 In the computer dedicated to the DAQ device, check that the external data are received properly.
- 2 In the EthoVision PC, open the experiment and from the **Setup** menu select **Experiment Settings**.
- 3 Under **Video source**, make sure **Live Tracking** is selected and select **Enable DAQ co-acquisition**. Click the **Settings** button.

Result – The **DAQ Hardware Settings** window appears.

- 4 Under **Predefined Settings**, select the **DAQ Hardware Settings** profile corresponding to the DAQ device you are currently using. Alternatively, create a new one (see page 314).
- 5 Click **OK**.
- 6 Start the Acquisition module and carry out trials as usual (see page 284).
Result – The synchronization pulses are sent from the EthoVision PC to the DAQ device. You should see such pulses on the screen of the DAQ computer.
- 7 The physiological data are stored in the computer dedicated to the DAQ device. Import those data into EthoVision XT. For the detailed procedure see Chapter 10.



The information of the synchronization signal used to acquire external data is stored in the external data file. When you import the data into EthoVision XT, if the sync signal has been correctly identified the data set is marked by a clock icon.



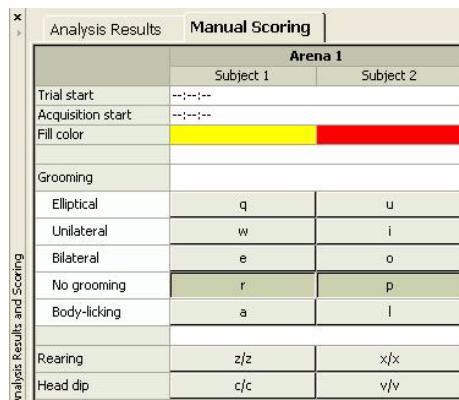
9.5 Scoring behaviors manually

Before you can score behaviors manually during a trial, you must first define them (see page 120).

If you have defined behaviors, these are listed in the Analysis Results and Scoring panel of the Acquisition screen.

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Click the **Manual scoring** tab to list the behaviors and the corresponding buttons (with key, if defined) for each arena and subject. You can use the fill color as a reference to identify animals.



Analysis Results			Manual Scoring
	Arena 1		
	Subject 1	Subject 2	
Trial start	--:--:--	--:--:--	
Acquisition start	--:--:--	--:--:--	
Fill color	Yellow	Red	
Grooming			
Elliptical	q	u	
Unilateral	w	i	
Bilateral	e	o	
No grooming	r	p	
Body-licking	a	l	
Rearing			
	z/z	x/x	
Head dip	c/c	v/v	

Mutually-exclusive behaviors are shown under their group name:



Grooming
Elliptical
Unilateral
Bilateral
No grooming
Body-licking



If at least one trial is set to Planned in your Trial List, the button of the behavior set as **Initially active** (page 122) is highlighted (see **No grooming** in the picture above), indicating that when you start the trial this is recorded automatically as initial behavior.

Each Start-Stop behavior is shown on one line:



Rearing	r/r
---------	-----



If you do not see the **Analysis Results and Scoring** pane, click the **Show/Hide** button and select **Analysis Results and Scoring**.

Before you start the trial, the buttons for the behaviors currently active are highlighted (in the examples above, **No grooming**).

Acquiring Data

To score behaviors:

- 1 If you track from video, make sure that the **Detection Determines Speed** option is **not** selected. This prevents video from playing faster than normal, which makes scoring more difficult.
- 2 Start the trial.
- 3 To score a behavior for a specific subject, arena and behavior, do one of the following:
 - Press the key for that combination.
 - Click the button corresponding to that combination.



Result – The button of the scored behavior is highlighted.

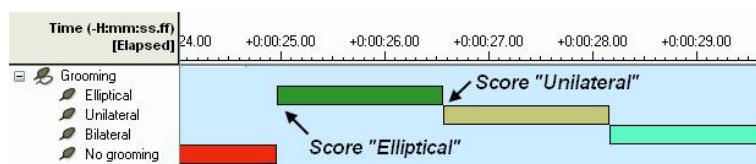
For mutually-exclusive behaviors, the button of the previous behavior is reset.

- 4 When the subject no longer shows that behavior, do the following:
 - For Mutually-exclusive states, score the behavior that is active next.
 - For Start-Stop states, press the stop key or click the button for that behavior.
- Result** – The button of the behavior is reset. For mutually-exclusive behaviors, the button of the new behavior is highlighted.
- 5 When finished, stop the trial.

Notes

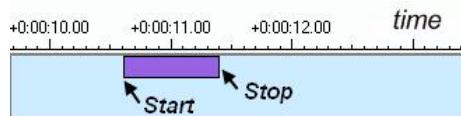


- Data can only be recorded after tracking has started. If you start the trial and tracking starts sometime later as a result of Trial control conditions setting some delay, any scoring action between the start of the trial and the tracking is not recorded.
- Scored behaviors are states with a duration that can be represented as follows:
- Mutually-exclusive



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- Start-Stop



- If you want to change the initial state of a subject, do so in the **Manual Scoring Settings** (see page 126).
- When observing multiple subjects simultaneously, to easily recognize the buttons of a specific subject look at the color of the body fill for that subject in the Video window and locate that color in the **Manual Scoring** tab. The column marked with that color contains the recording buttons for that subject.
- You cannot pause recording behaviors even when you track from a video file.
- If you press keys at a higher frequency than the sample rate you set in the Trial protocol window, only the behaviors active at the start of each sample are recorded to the data file.
- To modify the coding scheme, stop the trial and click **Manual Scoring Settings** in the Experiment Explorer. Please note that scored behaviors cannot be deleted (only after trials have been deleted).
- For more efficient data scoring you can use the X-keys programmable keyboards. You can for example press and hold a key as long as the behavior lasts. Contact Noldus for more information.

9.6 How to...

Get an overview of your trials

From the Trial List



- 1 From the **Setup** menu, select **Trial List**, or click **Trial List** in the Experiment Explorer.
- 2 To view the status of your trials, click the **Show/Hide** button on the component tool bar. In the **Show Variables** window, make sure that **Acquisition status** and **Video file status** are selected, then click **OK**.

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Data are organized in a hierarchical fashion: **Trials** contain **Arenas**, which on their turn contain the single track files of individual **Subjects**.

Label				System	System	System
		Start time	Track	Acquisition status		
Trial	Arena	Subject	No.			
Trial 9	Arena 1	Subject 1	9	19-Oct-04 14:53:54.0	Track filet0008a...	Acquired
	Arena 2	Subject 1	10			Skipped
	Arena 3	Subject 1	11		Track filet0008a...	Acquired
	Arena 4	Subject 1	12		Track filet0008a...	Acquired
Trial 10	Arena 1	Subject 1	13	19-Oct-04 14:53:59.8	Track filet0009a...	Acquired
	Arena 2	Subject 1	14		Track filet0009a...	Acquired
	Arena 3	Subject 1	15		Track filet0009a...	Acquired
	Arena 4	Subject 1	16		Track filet0009a...	Acquired

Figure 9.7. The hierarchical organization of data in the Trial List. Each row in the table corresponds to a tracked subject. For Trial 9, Arena 2 was set to To Skip before acquisition. After carrying out the trial, the status of Arena 2 is set to Skipped. You cannot acquire data for this arena, since the trial has been acquired.

See page 103 for information on the **Status** variables.

Note the difference between **To Skip** and **Skipped**:

- **To Skip** – The status of a trial or arena for which data acquisition is momentarily skipped before acquisition (that is, you have clicked the **Skip trial** button when that trial was highlighted in the **Acquisition Method** window). You can set the trial/arena back to **Planned** to acquire data (see page 305).
- **Skipped** – The status of an arena in a multi-area setup that was set to **To Skip**, after the corresponding trial was carried out. You cannot acquire tracks from arenas marked with **Skipped** for that specific trial, because the trial itself has been acquired already (in Figure 9.7., see the status of Arena 2 for Trial 9 and that of other arenas). To collect data for that arena/subject, add a new trial.

As track plots

To visualize all trials, do one of the following:

- In the Experiment Explorer, in the Analysis folder, under Results, click **Track Visualization**.
- From the **Visualize** menu, select **Plot Tracks**.

To visualize a specific trial, do one of the following:

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- In the Experiment Explorer, in the Analysis folder, under Results, click **Integrated Visualization** and select a trial in the component tool bar. See Chapter 13.
- From the **Visualize** menu, select **Plot Integrated Data** and select a trial in the component tool bar. See Chapter 13.

View the settings used to acquire data

For example, view the Detection Settings used to acquire Trial 25.

- 1 In the Explorer, click **Trial List**.
- 2 Click the **Show/Hide** button on the component tool bar and select **Detection settings** in the **Show Variables** window.



Settings profiles are marked with a lock icon, meaning that they cannot be edited. To edit a settings profile, make a copy.

Correct tracking errors

Sometimes EthoVision XT tracks a reflection or an inanimate object instead of the animal moving in the arena. This generally results in wrong data when calculating statistics. If that occurs, you can edit the tracks, for example remove the wrong samples and filling the gaps in the tracks with interpolated data.



For information on how to edit tracks, see Chapter 11.

Good detection settings do not generally result in tracking errors. Before editing tracks, make sure that the lighting conditions and the detection settings are optimized.

Carry out trials in an order different from that specified in the Trial List

You carry out your trials in the order specified in the Trial List (see page 117). However, if you want to acquire data in a different order, for instance carry out

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Trial 7 before Trial 6, you can use the Skip Trial function to skip Trial 6 and let EthoVision XT acquire data for Trial 7.

When you skip a trial, you skip all tracks that are expected for that trial. For example, if you have a setup with one animal in each of four arenas, the four expected tracks are skipped. To skip tracks for some arenas, not others in the same trial, see **Acquire data for some arenas, not others in the same trial** below.

How to skip a trial

To skip the current trial:

- 1 Make sure that the **Acquisition Method** window shows the name of the trial you want to skip. Click the **Skip Trial** button in the **Acquisition Control** window.

Result – The **Acquisition Method** window shows the next trial.

- 2 Carry out the trials as usual (see page 284).



The name of the current trial is indicated in the **Acquisition Method** window (see page 274). If you want to skip for example Trial 6, make sure that the **Acquisition Method** window shows **Trial 6** next to the Acquisition method you have chosen.

To skip a trial other than the current one in the **Trial List**:

- 1 In the Experiment Explorer, click **Trial List**. Click the **Show/Hide** button on the component tool bar, select **Variables** and make sure **Acquisition status** is selected in the **Show Variables** window.
- 2 Locate the trial you want to skip, and select **To Skip** from the list under **Acquisition status** for that trial.

How to carry out a skipped trial

When you wish to carry out a trial that was skipped, do the following:

- 1 In the Experiment Explorer, click **Trial List**. Click the **Show/Hide** button on the component tool bar, select **Variables** and make sure **Acquisition status** is selected in the **Show Variables** window.
- 2 Locate the trial and change its **Acquisition status** from **To Skip** to **Planned**. Make sure that you do this for all the arenas you want to use in that trial (in the case of multiple-area setups, there are two or more rows per trial).
- 3 Go to the Acquisition module and carry out the trials as usual.

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When the trial has been carried out, its **Acquisition status** changes from **Planned** to **Acquired**.

Acquire data for some arenas, not others in the same trial



This information is useful when you have a multiple-arena setup, and you want to acquire data for some arenas or animals, not others.

Example – For Trial 32, the animal to be tracked in Arena 2 is not available. Nevertheless, you want to track the animals in the other Arenas 1, 3 and 4.

How to skip tracking for a specific arena/subject

- 1 In the Experiment Explorer, click **Trial List**. Click the **Show/Hide** button on the component tool bar, select **Variables** and make sure **Acquisition status** is selected in the **Show Variables** window.
- 2 Locate the trial for which you want to skip arenas, and change the Acquisition status for those arenas from **Planned** to **To Skip**.
- 3 Carry out the trials as usual.

Result – The tracks are acquired for the arenas whose **Acquisition status** was **Planned**. Their status is now **Acquired**. For the skipped arenas, the **Acquisition status** changes from **To Skip** to **Skipped**.



You cannot acquire data for arenas and subjects whose Acquisition status is **Skipped**. This is because the trial itself has been acquired (see step 4 above). To acquire data for those arenas and subjects, add a new trial and acquire data anew.

Redo a trial

What does 'Redo a trial' mean?

Re-doing a trial means record video or track data once again for that trial. It applies to the following recording methods:

- **Live tracking** (with the **Save video** option selected; page 288);
- **Save video only, track later** (page 290);

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- **Track from video files recorded with programs other than EthoVision** (page 293).



With this procedure you also remove the data scored manually and the trial control event data (including hardware device data) recorded during a trial.

When do I need this information?

You need this information when you want to discard track data and or video saved with one of the methods above, and you want to acquire data and video again for that trial.

Example 1 – You have acquired a trial and realize that you have acquired the data with the wrong detection settings, or the tracks obtained from that trial contain too many missing samples.

Example 2 – You have acquired video using EthoVision for Trial 12 and realize that something went wrong during the test. You want to record new video and assign it to Trial 12.

Procedure

We assume at this point that you have carried out a trial and a video file corresponding to that trial has been saved.

- 1 In the **Acquisition Method** window, click the **Redo Trial** button.
- 2 In the **Redo Trial** window, select the trial that you want to redo.



- 3 Select one of the following options:
 - **Clear tracks** – To delete the tracks obtained from that trial.

This is the only option available if you track live without saving video, or from a video file recorded with a program other than EthoVision.

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- **Clear tracks but re-use the video** – To delete the tracks, not the video file associated with those tracks. You can then track again from that video file.
- **Clear tracks and video** – To delete the tracks and the associated video and redo the trial anew.
- **Clear video** – To clear a trial that has video only. You will then record new video and track data. This is the only option available if you do your trials by saving the video first.

4 Click **OK**.

5 In the **Acquisition Method** window, select the method to acquire the trial. Each method shows the number of the trial that will be acquired when starting.



The options available here depend on the tracking method set in your experiment and whether you have trials planned other than the one you want to re-do (heretofore named *Trial x*).

- If you normally do **live tracking** without saving video, the only option available is **Live tracking (Trial x)**. The program prompts you to carry out Trial x that you have just cleared.
- If you do live tracking and save the video:
 - If you have cleared the tracks only, not the video file, then choose **Track saved video file (Trial x)** to re-do tracking for Trial x that you have cleared. You can also choose one of the other options available to carry out other planned trials (if present).
 - If you have cleared the tracks *and* the video file, choose one of the options to re-do Trial x that you have cleared.
- If you save the video and track later, choose one of the options available for re-doing Trial x (**Live tracking** or **Save video only, track later**). You can

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also choose **Track saved video file** to carry out other planned trials (if present).

- If you track from video recorded with other programs, the only option available is **Track from file (Trial x)**.
- 6 If necessary, click **Video** and select the video file to track from.
- 7 Start the trial as usual.



- The trial name next to **Redo tracking** refers to the first trial in your list that was set to redo in step 2 above.
- Effect on the status variables in the Trial List – The Acquisition status is set back to **Acquired** for the trial you have just done.
- If you track from video recorded with programs other than EthoVision, to delete a video you must do so manually.
- In the Trial List, the **Acquisition status** variable for that trial changes from **Acquired** back to **Planned**. If the video file has been cleared, the **Video file status** variable changes to **Planned**. If the video file has not been cleared, Video file status stays **Generated**.

Renaming a trial

Trials are given default names depending on the order they are added in your Trial List: Trial 1, Trial 2, etc. If you want to rename your trials, edit the **Trial name** variable in the Trial List.

- 1 In the Experiment Explorer, click **Trial List**.
- 2 In the **Trial List**, make sure that the **Trial name** column is visible (this is not the first column in your table!). If this is not the case, click the **Show/Hide** button on the component tool bar and select **Variables**. In the **Show Variables** window, make sure that **Trial name** is selected, then click **OK**.
- 3 Locate the trial you want to rename. Click the cell corresponding to the Trial name column and enter the new name.

You can rename trials regardless of their acquisition status (Planned, Acquired, etc.).

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Deleting a trial

Deleting a trial means that all the tracks and external data files (if these have been imported in EthoVision XT) are removed from your PC. To delete a trial, do one of the following:

- In the Trial List, right-click a Trial and select **Delete Trials**.
- In the Trial List, right-click a trial in the **Trial name** column and select **Delete**.
- In the Experiment Explorer, in the Acquisition folder, under Acquired Trials, right-click a trial and select **Delete**.



- If you have tracked from video files generated with EthoVision XT, you are asked whether you want to keep the video file associated with the trial or delete this file too (see the next section).
- If you have tracked from video files generated with programs other than EthoVision, the video file associated with the trial is kept on your PC.
- To delete multiple trials, select the trials in the first column of the Trial List, then right-click and select **Delete Trials**.
 - To select adjacent trials, drag across the row headings, or select the first trial name, hold down **Shift** and select the last trial name.
 - To select non-adjacent trials, select the first trial name, hold down **Ctrl** and select the other trial names.
- You can also delete acquired trials in the Explorer: right-click the trial name in the **Acquired trials** folder and select **Delete**.
- In a multiple-area experiment, you cannot delete tracks acquired in some arenas within a trial. You can only delete all tracks, that is, the entire trial.
- If you delete a trial and then add a new one, this is not given the name of the deleted trial. For example, if you have 10 trials in your Trial List and you delete Trial 4, when adding a new trial this is named Trial 11.

Rename and delete a generated video file

Video files generated within EthoVision XT (see page 288 or page 290) are given the same name as the corresponding trial: Trial 1.mpg, Trial 2.mpg, etc. **If you**

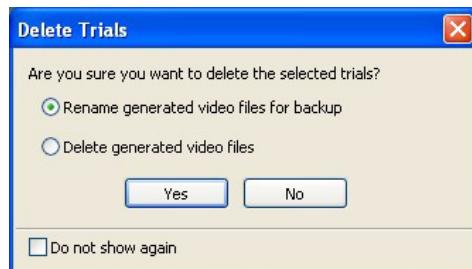
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want to rename or delete a video file, you must first delete the corresponding trial.

If you want to rename a video file without deleting its trial, make a copy of the video file with Windows Explorer and give this copy the new name.

- 1 From the **Setup** menu select **Trial List**, or double click **Trial List** in the Explorer.
- 2 In the **Trial List**, right-click the row corresponding to the video you want to delete and select **Delete**.

Result – A message appears:



- 3 Do one of the following:
 - Choose **Rename generated video files for backup** if you want to rename the file and delete the trial.
 - Choose **Delete generated video files** if you want to delete the video file and the trial.

In any case the tracks and the external data files associated with that trial will be deleted!
- 4 Click **Yes** to confirm.

Use a remote control to start and stop acquisition

You can use a remote control with “Page-up and Page-down functionality” (such as those typically used for PowerPoint presentations) to start and stop data acquisition:

- 1 Close EthoVision XT.
- 2 On the installation DVD, in the folder **Utilities**, double-click the **remote-control.reg** file. Click **Yes**.

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- 3 You can now use the remote control.
- 4 Connect the remote control to your computer and open EthoVision XT. Signals from the remote control will only be recognized:
 - While acquiring data.
 - If the trial is not finished yet.
 - When you are not entering independent values.
 - When you are not browsing through the video (using playback functions).
- 5 To start a trial: use the page-up button on the remote control.
- 6 To stop a trial, use the page-down button on the remote control.

9.7 What next?

- To import external data, see Chapter 10.
- To correct tracking errors, see Chapter 11.
- To select data for analysis/visualization, see Chapter 12.
- To visualize data, see Chapter 13.
- To calculate statistics, see Chapter 14.

10

Physiological Data

This chapter is about:

- **Physiological Data** – You can import external data into EthoVision XT. Usually this is **physiological data**, such as ECG, EEG or blood pressure data, but it can also be environmental data (e.g., room temperature, humidity). These data are acquired with an external Data AcQuisition (**DAQ**) system and stored on this external system.
→ see the next page
- **Synchronization** – You can automatically synchronize physiological data and tracking data in EthoVision XT. To do this, you connect the EthoVision XT computer to the external DAQ system with a synchronization cable. When you subsequently simultaneously acquire tracking data and physiological data, EthoVision XT sends out a synchronization signal, containing time code information, to the external DAQ system. After data acquisition, the physiological data is imported into EthoVision XT and the tracking and external data are automatically synchronized, using the time code information. When the EthoVision computer and the DAQ system cannot be connected, you can manually synchronize tracking data and physiological data during import.
→ See page 334
- **Visualization** – You can plot the tracking data and the physiological data in EthoVision XT for visual inspection.
→ See page 340

10.1 Working with physiological data

Working with physiological data in EthoVision XT involves three basic steps:

- 1 When possible, connect the EthoVision XT computer to one of the input channels of the external DAQ system, and select **Enable DAQ co-acquisition** in the **Experiment Settings** in EthoVision XT (see below).
- 2 Next, you acquire tracking data, while the DAQ system is acquiring its physiological data.
- 3 After data acquisition, import the physiological data file(s) and assign them to a **Trial**, **Arena**, or **Subject** (see page 336). Tracking data and physiological data are automatically synchronized in case you used DAQ co-acquisition. If not, you can use manual synchronization.



The physiological data are saved on the computer dedicated to the DAQ device, not on the EthoVision XT computer. However, if you want to use automatic synchronization, you must connect the EthoVision XT computer to the DAQ device in order for EthoVision XT to send its **synchronization signal** (see below).

Enabling DAQ co-acquisition in EthoVision XT

- 1 In EthoVision XT, open a new experiment: From the **File** menu, select **New Experiment**, type in the name and click **OK**.
- 2 Open the **Experiment Settings** (see Chapter 5).
- 3 In the **General** group, in **Tracking Source**, select **Live-tracking** and the **Enable DAQ co-acquisition** box. Click the **Settings** button.
- 4 Under **Predefined Settings**, select the item corresponding to your DAQ system, or create a new one (see below).
- 5 Click **OK**.
 - When you open **Experiment Settings** in an existing experiment in which you already have acquired data, the **Experiment Settings** are read-only (settings are greyed out).
 - You can only co-acquire physiological data when you carry out **Live Tracking**.



Physiological Data

Creating a new DAQ Hardware Settings profile

- 1 In the **DAQ Hardware Settings** window, click the **New** icon.
- 2 A new field appears at the bottom of the list of predefined profiles (see Figure 10.1.). Type a name for the new profile.

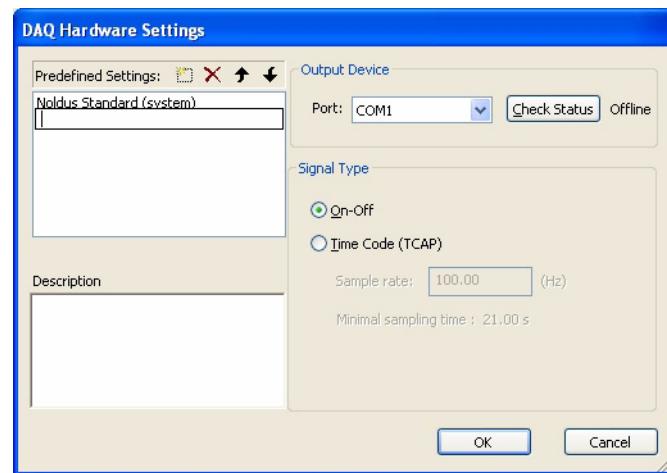


Figure 10.1. The **DAQ Hardware Settings** window. A default profile Noldus Standard (System) is already present.

- 3 In the **Output device** group, select one of the COM ports from the list. This COM port is used to connect the EthoVision XT computer to the DAQ system.



EthoVision XT comes with a USB-to-COM converter if your computer does not have a COM port (some laptops). This converter automatically selects **COM5** on the EthoVision XT computer.

Click the **Check Status** button to check the availability of the port.

- 4 In the **Signal Type** group, select:

- **On-Off** if the external signals are sampled at low rate (less than 10 Hz) and without interruption (thus no scheduled sampling).
- **Time Code (TCAP)** in all other cases. Enter the **Sample rate** (in Hz) with which you sample data on your DAQ system.

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If you want to sample data at a rate higher than 2 KHz, set 2000 Hz.

Depending on the **Sample rate**, the **Minimal sampling time** changes; this indicates the minimum time the synchronization signal should be acquired on the DAQ system for synchronization to work properly.

See below for more information On-Off and TCAP signals.

- 5 Click **OK**.

Deleting a DAQ Hardware Settings Profile

In the **DAQ Hardware Settings** window, select a **Profile** and click the **Delete** icon or press <**DEL**> on your keyboard.

Changing the order of DAQ Hardware Settings Profiles

In the **DAQ Hardware Settings** window, select a **Profile** and click the **Move Up** / **Move Down** button or press <**ALT-arrow down**> / <**ALT-arrow up**> on your keyboard.

Adding a description to a DAQ Hardware Settings Profile

In the **DAQ Hardware Settings** window, select a **Profile**, click in the **Description** box and type in text. Click on another **Profile** and click **Yes** to save the **Description**.

On-Off vs. Time Code (TCAP) synchronization signal

In order to synchronize physiological data and EthoVision XT tracking data, you can send out a synchronization signal with time information (SyncOut) from the EthoVision XT computer to the DAQ device. This signal is sampled by the DAQ computer and after acquisition imported into the EthoVision computer as a home coming signal where it is compared with the original synchronization signal.

On-Off and **Time Code (TCAP: Time Code Auxiliary Parity)** are two types of signals that reflect two ways of synchronizing physiological data (for example, heart rate) with tracking data.

- **On-Off** – This signal coincides with the start and stop of data acquisition in EthoVision XT. The difference in offset between the synchronization signal and reference signal is calculated at the start and at the end of the recording. In order for synchronization to be successful, the DAQ recording must start before starting acquisition in EthoVision XT, and end after stopping acquisition in EthoVision XT.

Physiological Data



A dialog in EthoVision XT informs you to start and stop data acquisition on your DAQ device.

Furthermore, the DAQ recording should not be stopped during acquisition in EthoVision XT, which means that you cannot use the On-Off signal when performing scheduled sampling (that is, starting and stopping DAQ acquisition at regular intervals).

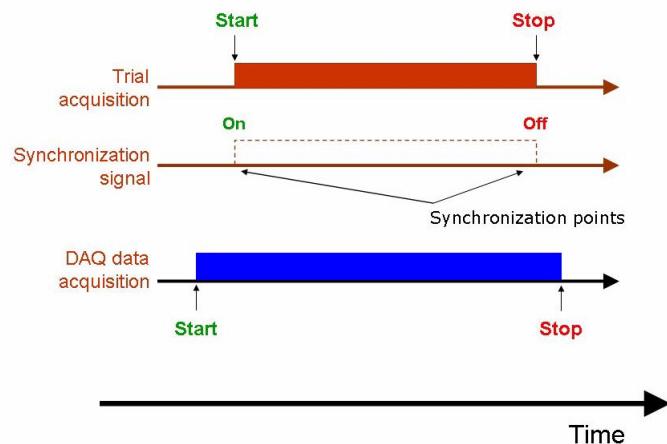


Figure 10.2. The relationship between Trial acquisition, the On-Off synchronization signal from EthoVision XT and acquisition on the external DAQ system.

- **Time Code** – A series of bits called a *frame* is sent from the EthoVision XT computer to the external DAQ device (see Figure 10.3. below). Each frame contains the current date and time and additional information. The difference in offset between the synchronization signal and the reference copy is calculated for several time points during acquisition (by default, for 10 time points), while the gain is calculated through regression analysis of offset values obtained at different points in time (that is, different frames). Therefore, synchronization of tracking and physiological data is more accurate using the **Time Code** signal because it is done for more than two offset points and not just at the start and stop of acquisition as with the **On-Off** signal.

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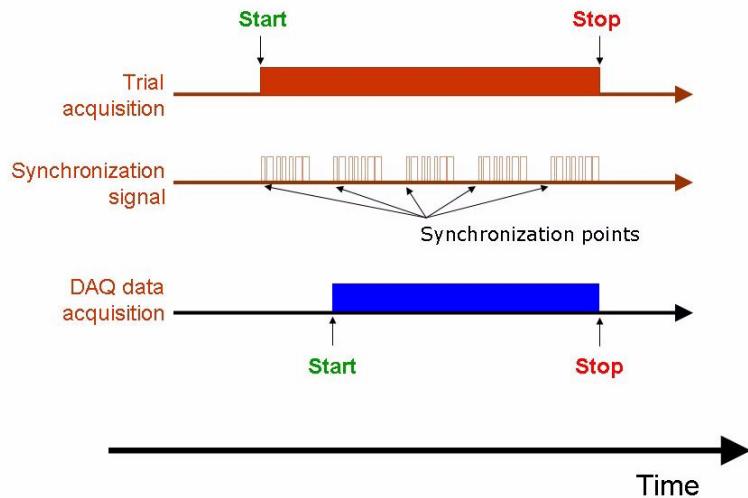


Figure 10.3. The relationship between Trial acquisition, the Time Code synchronization signal from EthoVision XT and acquisition on the external DAQ system.

10.2 Carrying out Live Tracking with DAQ co-acquisition

- 1 Make sure the EthoVision XT computer and the DAQ device are connected through a synchronization cable, if possible.
- 2 Set EthoVision XT to **enable DAQ co-acquisition** (see page 314).
- 3 Carry out **Live Tracking** in EthoVision XT (see page 287, page 288 or page 290) while simultaneously acquiring physiological data with the external DAQ system.
- 4 Stop tracking in EthoVision XT and stop acquisition on your DAQ device.

10.3 Importing Physiological Data

To import physiological data you need to link it to an acquired **Trial** or to an **Arena** or a **Subject**. It is not necessary that these external data were scored simultaneously and/or synchronously with the tracking data.



You can only import physiological data that was sampled with a constant sample rate and is stored in ASCII format. This physiological data file must at least contain 15 data lines. It should preferably contain a header with information describing the data sets. This information about the data set, such as data set name, date and time information, sample rate, is typically stored in the header section of a DAQ export file.



You can only import external data into an acquired Trial. This is indicated in the Trial List by 'Acquired' in the System Variable Acquisition Status column.

A physiological data file can contain one or more data sets. For example, you test three rats, each in its own arena, and for each rat you have acquired ECG data. The resulting physiological data file contains three ECG data sets. In EthoVision XT you now have the option:

- to link the complete physiological data file (i.e. all three data sets at once) to a **Trial** or an **Arena**.
- to link one or more data sets from a physiological data file to a **Trial** or an **Arena**.

How to link a complete physiological data file at once

1 Do one of the following:

- In the **Trial list** window, right-click a **Trial**, **Arena** or **Subject** and select **Import Data...**.
- In the Experiment Explorer, under the **Acquired trials** item, right-click **External data** and select **Import**.

2 Next, you can either:

- Select a predefined **Import Profile**. Select a predefined Import Profile from the drop-down list under **files of type**. Next, locate the physiological data file, select the filename and press **Open**.

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- Create a new Custom Import filter (see page 321).

How to link one or more specific data sets

- 1 From the **Setup** menu, select **Import External Data**.
- 2 Select a predefined Import Profile from the drop-down list under **files of type**. Next, locate the physiological data file, select the filename and press **Open**. A second **Import External Data** window (see Figure 10.4.) shows one or more **Data Set Names**. The **Trial list** also appears.

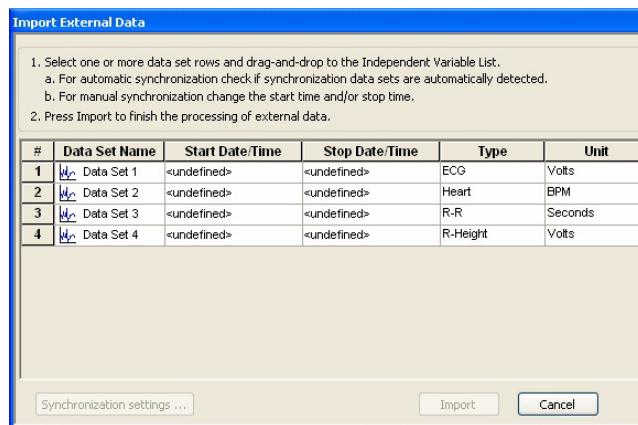
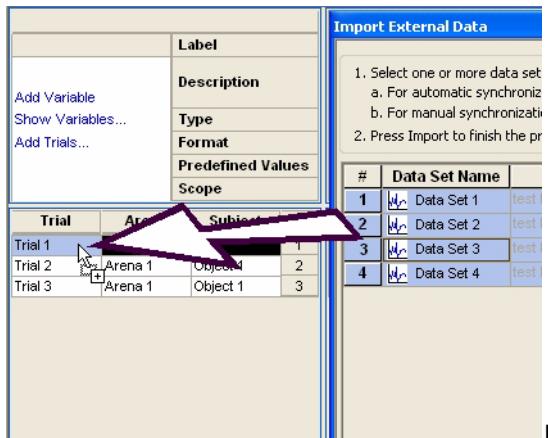


Figure 10.4. The **Import External Data** window with four data sets.

- 3 To add physiological data to a Trial, select one or more **Data Set** rows and drag-and-drop it to the **Trial list**; you can choose to drag-and-drop external data to a **Trial**, **Arena** or a **Subject** (see figure below).

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- Click the **Import** button in the **Import External Data** window to finish import of physiological data.



EthoVision XT offers predefined **Import Profiles** for a number of DAQ systems (e.g., DSI Dataquest A.R.T., Polar Precision Performance software). If your DAQ system is not in the list, you can create a **Custom Import Profile** (see page 321).

Creating a new Custom Import Profile

- From the **Select** menu, click **Import External Data**.
- At the bottom of the **Import External Data** window, select **Custom Import Profiles**. The **Import Profiles** window opens. Click the **Create New** button.
- In the **Import Profile Definition** window, click the **Browse** button in the **Select sample file** section at the top of the window to select a physiological data file, locate and select this file and press **Open**.
- EthoVision XT automatically detects header and data information in the physiological data file. This information is visible in the **File content** section of the **Import Profile Definition** window (see Figure 10.5.).

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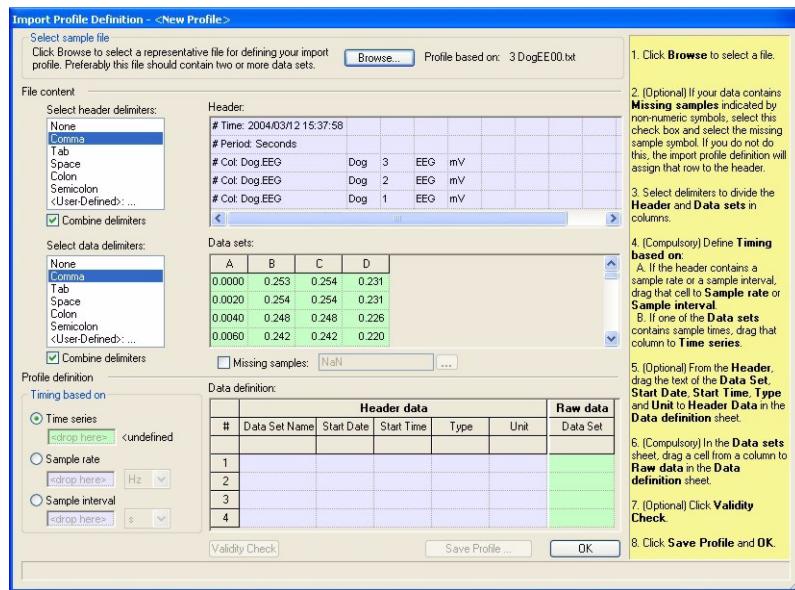


Figure 10.5. The **Import Profile Definition** window.

- 5 EthoVision XT uses the comma as the default delimiter to separate text in the header and data sets. However, if text that should be in separate columns is still in one column, you can separate the text by selecting the appropriate delimiters from the list under **Select header delimiters** and **Select data delimiters**.

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The figure consists of two screenshots of the 'Import Profile Definition' window. Both screenshots show a 'Select data delimiters:' dropdown menu with options: None, Comma, Tab, Space, Colon, Semicolon, and <User-Defined>: In the first screenshot (labeled 1), the 'None' option is selected, and the 'Data sets:' section displays a single column labeled 'A' containing five rows of data: 0.0000, -0.172, -0.173, -0.157; 0.0020, -0.186, -0.188, -0.170; 0.0040, -0.196, -0.197, -0.178; 0.0060, -0.199, -0.199, -0.181. In the second screenshot (labeled 2), the 'Comma' option is selected, and the 'Data sets:' section displays four separate columns labeled A, B, C, and D, each containing the same five rows of data.

Select data delimiters:		Data sets:			
	None	A			
1	None	0.0000	-0.172	-0.173	-0.157
1	Comma	0.0020	-0.186	-0.188	-0.170
1	Tab	0.0040	-0.196	-0.197	-0.178
1	Space	0.0060	-0.199	-0.199	-0.181
2	None	A	B	C	D
2	Comma	0.0000	-0.172	-0.173	-0.157
2	Tab	0.0020	-0.186	-0.188	-0.170
2	Space	0.0040	-0.196	-0.197	-0.178
2	Semicolon	0.0060	-0.199	-0.199	-0.181
2	<User-Defined>: ...				

Figure 10.6. The **Data sets** section of the **Import Profile Definition** window. 1 - All data sets are in one column. 2 - This figure shows the same data, but now with the Comma delimiter selected; the data sets are now in separate columns.



Some DAQ software enables you to select the type of delimiter when saving the DAO data to an ASCII export-file. In that case you select the same delimiter in the **File content** section of the **Import Profile Definition** window. A semicolon is advised as delimiter.



If you select a comma as delimiter, make sure that in Windows **Regional and Language Options** the comma is **not** used as the Decimal symbol.

- **Windows XP** – In the **Control Panel**, open **Regional and Language Options**. In the **Regional Options** tab, in the **Standards and formats** group, select **English (United States)** or another country that uses the point as the Decimal symbol from the drop-down menu.
- **Windows 7** – In the **Control Panel**, open **Region and Language**. In the **Formats** tab, select **English (United States)** or another country that uses the point as the Decimal symbol from the drop-down menu.

- 6 Now you can assign the information from the **Header** and the **Data Sets** in the **File content** section to the appropriate cells in the **Profile Definition** section.



Under **Data definition** in the **Profile definition** section there are two parts. The lilac part is labeled **Header Data** where you enter **Header** information from the lilac cells in the **File content** section. The green part

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is labeled **Raw data** where you enter **Data sets** from the green cells in the **File content** section.

First you set the sample rate with which the external data were acquired by the DAQ system.

- i If the Header contains the sample rate, select the **Sample rate** button under **Timing based on** in the **Profile definition** section. Drag the sample rate to the **Sample rate box** and select the appropriate unit (Hz or kHz) from the drop-down list.
- ii If the Header contains the sample interval, select the **Sample interval** button in the **Profile definition** section. Drag the sample interval to the **Sample interval box**. Select the unit of time from the drop-down list (see Figure 10.7.).

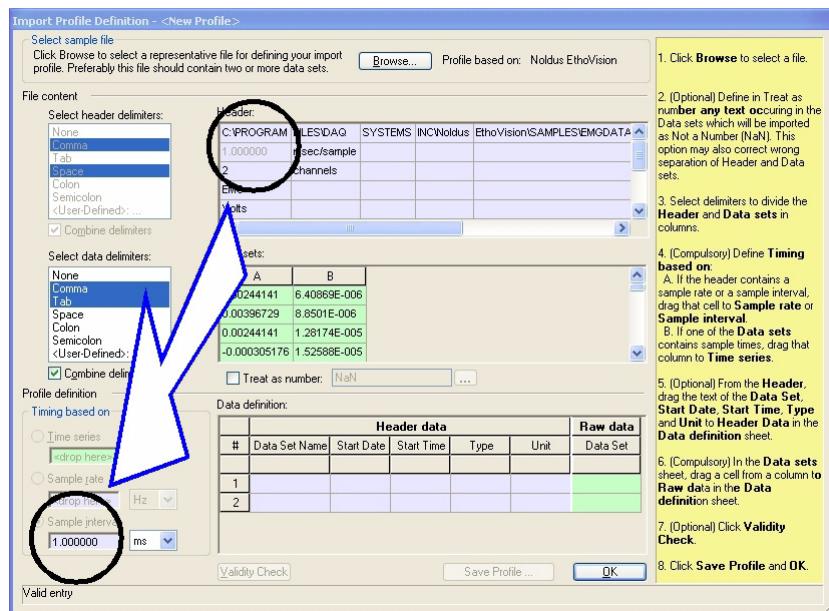


Figure 10.7. The **Import Profile Definition** window. Example of information about sample rate in the Header that is assigned to the **Sample interval** box in the **Timing based on** group.

- iii If the Header does not contain information on sample rate, select the **Time series** button under **Timing based on**. Under **Data sets** in the **File content** section, select one of the green cells in the

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column with time stamps and drag this to the **Time series** box (see Figure 10.8.). Next, select a time format (you can choose between **Numerical value** or **Date/time format**).



The column with time stamps now appears greyed. You can drag-and-drop only one column at a time.

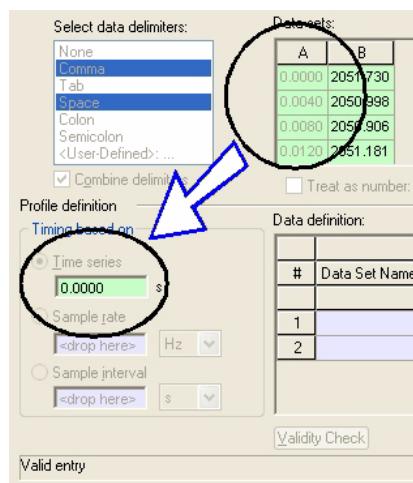


Figure 10.8. Part of the **Import Profile Definition** window. Example of the information about sample rate in the Header that is assigned to the **Time series** box in the **Timing based on** group.

7 Next you can define the **Data set(s)**.



A **Dataset** consists of time-ordered values of a variable, e.g. EEG, heart rate, body temperature. A data file can contain more than one **Data set**.

If your **Data set** contains **Missing samples** indicated by non-numeric symbols, you need to specify this symbol first:

- Select the **Missing samples** checkbox.

- i Type in the non-numeric symbol in the **Missing samples** box or click the **Browse** button next to it to select one of the predefined symbols.



A missing sample is converted to a ‘zero’. As a result, it is plotted as a ‘zero’ in the Visualization.

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- ii Drag one of the green cells under **Data Sets** in the **File content** section to one of the empty cells in the **Raw data - Data Set** column. As a result, the letter of the original column appears in the cell and the column under **Data Sets** in the **File content** section is greyed.

EthoVision XT assumes that your header and data set info are ordered in a regular way in your external data file (e.g., left-right, with/without empty cells in between).

- iii Drag the first two columns with data to the first two rows of the **Raw data** column in the **Data definition** group. Next, the **Validity Check** button becomes active. When you click this **Validity Check** button, EthoVision automatically assigns the other columns from the **Data sets** group to the remaining rows in the **Data Definition** group.

Example - your external data file contains four **Data Sets** in columns A, C, E and G. Columns B, D, F are empty. When you drag columns A and C to the first two rows in the **Raw Data** column and next click the **Validity Check** button, EthoVision automatically assigns columns E and G to rows 3 and 4, thereby taking into account the empty columns between **Data Sets**.

Data definition:					
#	Header data				Raw data
	Data Set Name	Start Date	Start Time	Type	Unit
1					A
2					B
3					C
4					D

Validity Check **Save Profile ...** **OK**

Figure 10.9. The Data definition sheet in the **Import Profile Definition** window. In this example, the **Raw Data** column shows the letters of the columns with data sets from the **Data sets** sheet.

- 8 Next, you can assign information to the **Header Data** part of the **Profile definition** section.

- i **Dataset Name** - you can drag the name of the **Data sets** under **Header** (the top lilac window) in the **File content** section to the **Dataset Name** column under **Data definition** (the bottom lilac window) in the **Profile definition** section.

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The **Dataset Name** in the original location is greyed. You can return the **Dataset Name** to its original cell by selecting the **Dataset Name** in the **Data definition** field and pressing **delete**.

The screenshot shows the 'Import Profile Definition' window with two main sections: 'Header' and 'Data definition'.

Header:

P-M				
R-R	Interval			
Seconds				
R-Height				
Volts				

Data definition:

#	Data Set Name	Start Date	Start Time	Type	Unit	Raw data
1	ECG				Volts	A
2	HeartRate				BPM	B
3	R-Interval				Seconds	C
4	R-Height				Volts	D

A blue arrow points from the 'Data Set Name' column in the 'Data definition' table to the 'Dataset Name' field in the 'Header' section, indicating the mapping between them.

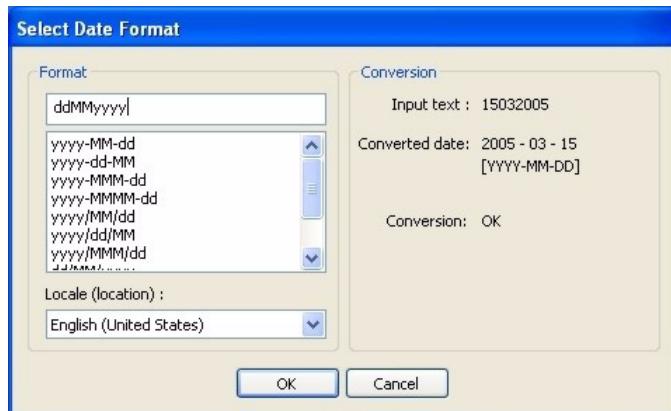
Figure 10.10. The **Import Profile Definition** window. Example of the data set names in the Header that are assigned to the **Data Set Name** column in the **Data definition** section

- ii **Start Date** - select the date under **Header** in the **File content** section and drag it to the **Start Date** column under **Data definition**.

If the date matches one of the predefined formats, EthoVision XT automatically selects one in the **Select Date Format** window. In that case it says **Conversion: OK** in the **Conversion** section. Click **OK** to proceed.

You can also define a date format yourself. For example, your date in the header is formatted as: 15032005 (March 15 2005). This date format does not match a predefined format. In the format box, type '**dd**' for the numbers representing 'day', '**MM**' for month and '**yyyy**' for 'year'. Behind **Converted date** is now the correct date and **Conversion** shows **OK**. Click **OK** to set the format.

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iii Start time - select the start time under **Header** in the **File content** section and drag-and-drop it to the **Start time** column under **Data definition**.

In the **Select Time Format** window a predefined format is selected automatically. Click **OK** if this is the right one. If not, type in a new format in the **Format** box. Click **OK**.

iv Type - drag-and-drop the **type** of measurement from the **Header** part of the **File content** section to the **Type** column in the **Data definition** part, in the **Profile definition** section.

v Unit - drag-and-drop the **unit** of measurement from the **Header** part to the **Unit** column in the **Data definition** section.

9 When all the information is in the **Data definition** sheet, click the **Save Profile As** button.



Type a profile name in the **Profile Name** box of the **Save Import Profile** window. You can add a description of the import profile in the small **Description** window. Click **OK**.

This import profile is stored with the extension *.eip.

10 Click **Close** to close the **Import Profile Definition** window.

In the **Import Profiles** window the newly created import filter is now in the list of import profiles

11 Close the **Import Profiles** window by pressing the **Close** button.

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- 12 In the **Import External Data** window, the new import profile is now selected in the list of **Files of type**. If not, click the drop-down button and select the newly created import profile.
- 13 Locate the external data file, select it and press **Open**. The **Import External Data** windows opens. Follow the instructions starting from 2 (page 320) to import the external data.

Editing a Custom Import Profile

If you want to import an external data file that is very similar to, but not exactly the same as, another data file for which you already have an **Import Profile**, you can edit the existing **Import Profile**.



When you edit an Import Profile, the new Import Profile replaces the previous one, even if you save it under a different name.

- 1 In the **Import External Data** window, click **Custom Import Profiles**.
 - 2 Select the **Import Profile** from the list in the **Import Profiles** window and click **Edit**.
-
- In the **Select Sample File** group you see the original sample file behind **Profile based on**.
- 3 Click **Browse** in the **Select Sample File** group to select the new external data file and click **Open**.
 - 4 Follow the instructions 4-8 under *Create a new Custom Import Profile* above.
 - 5 Click **Save Profile As** when you are finished filling in the **Data definition** sheet.
 - 6 Type in the name for the **Import Profile** and click **OK**. **Close the Profile Definition** window.
 - 7 **Close the Import Profiles** window. Make sure you select the right **Import Profile**.
 - 8 Select the external data file and click **Open** to finish import.

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10.4 Predefined Import Profiles

EthoVision comes with a number of predefined import profiles for the import of physiological data files from the following suppliers of DAQ systems (software package between brackets):

- DSI (Dataquest® A.R.T. version 2.3 and higher).
- Polar (Polar Precision Performance version 4.01 and higher).

DSI

The Data Sciences Dataquest® A.R.T. system is used for the collection of telemetric data.

Below you see an example of part of a Dataquest file with EEG data.

```
#;Time;;5-7-2004;14:42:36;;
#;Period;;Seconds;;
#;Col;;Dog.EEG;DogX;3;EEG;mV
#;Col;;Dog.EEG;DogY;2;EEG;mV
#;Col;;Dog.EEG;DogZ;1;EEG;mV
0.0000;-0.225;-0.225;-0.205;;
0.0020;-0.226;-0.225;-0.205;;
0.0040;-0.223;-0.221;-0.201;;
0.0060;-0.219;-0.218;-0.198;;
0.0080;-0.219;-0.218;-0.199;;
0.0100;-0.222;-0.222;-0.203;;
0.0120;-0.226;-0.226;-0.205;;
0.0140;-0.223;-0.224;-0.203;;
0.0160;-0.217;-0.216;-0.197;;
0.0180;-0.213;-0.212;-0.194;;
0.0200;-0.218;-0.217;-0.198;;
```

Figure 10.11. The header and first part of the data of a DSI DataQuest physiological data file.

Figure 10.12. shows the **Import Profile Definition** window in EthoVision XT with the same DataQuest file.

The header contains information about date & time, the unit of time at which the samples were taken (seconds), and information about the channels. In this example, the EEG from three dogs was measured. The unit was millivolts (mV).

The header of the DataQuest A.R.T. file contains no information about the sample rate of the DAQ system. The sample rate is calculated based on the first column with sample times of the data part of the file. In this case the samples are taken at

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an interval of 0.002 seconds. This corresponds to a sample rate of 500 Hz.

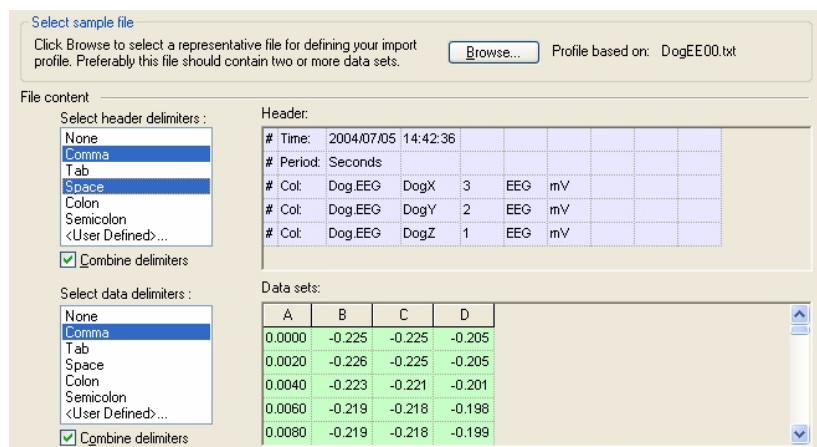


Figure 10.12. The **Import Profile Definition** window with the information from the DSI DataQuest A.R.T. file assigned to the **File Content** section.

Polar (Polar Precision Performance software)

The Polar system is used for monitoring heart rate. After data acquisition the data can be transferred to a computer (not available with all Polar systems). The data is stored in a HRM file. These HRM files can be directly imported into EthoVision XT.



EthoVision XT cannot be connected to the Polar system. Therefore, Polar heart rate data and EthoVision tracking data always need to be synchronized manually.



Interbeat interval data cannot be imported into EthoVision. See "Interbeat interval data" on page 333 for more details.

To facilitate manual synchronization of Polar heart rate data and tracking data you can do the following:

- **If the Polar software is running on the EthoVision XT computer –** Synchronize the Polar watch with the clock of the EthoVision XT computer.

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Please refer to the Polar Precision Performance software Reference Manual for a description of how to do this. Please note that this is not possible for all Polar systems.

- **If the Polar software is not running on the EthoVision XT computer –** Synchronize the EthoVision XT computer and the Polar computer (see page 336 for a description of how to do this). Synchronize the Polar watch with the clock of the Polar computer.

The settings on the Polar receiver determine what header information is written to the HRM file. Please refer to your Polar manual for a detailed description of the settings.

Below you see an example of part of a Polar HRM file.

```
[Params];
Version;105
Monitor;1
Mode;0
Date;20011209
StartTime;22:21:13.0
Length;0:12:54.8
Interval;5
Upper1;250
Lower1;10
Upper2;250
Lower2;10
Upper3;250
Lower3;10
ActiveLimit;0
MaxHR;193
RestHR;70

[HRData];
185;
188;
188;
183;
178;
```

Figure 10.13. The header and first part of the data of a Polar HRM file.

Figure 10.14. shows an example of the **Import Profile Definition** window in EthoVision with the same Polar file.

The header contains information about date and time, the length of the recording period (00:12:54:08) and the sample interval (5 sec). It also contains information about settings of the upper and lower limit of the heart rate.



Depending on the settings of the receiver, the HRM file can contain additional information. This does affect subsequent import of HRM files acquired with different type of receivers.

The HRData column show the mean heart rate (in beats per minute) over a 5-

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second period.

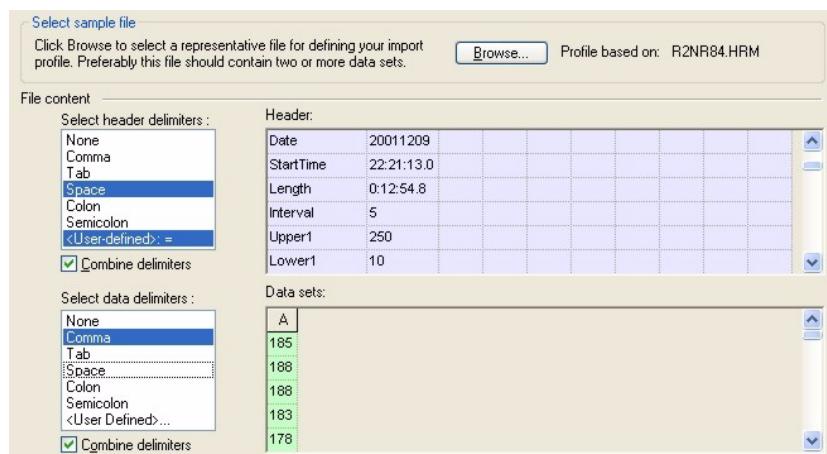


Figure 10.14. The **Import Profile Definition** window with the information from the Polar HRM file assigned to the **File Content** section.

Interbeat interval data

Interbeat interval data obtained with a Polar system have the same format as HRM data. It is therefore in principle possible to import these data with the import profile for Polar data. However, EthoVision then uses the sample rate of the Polar system for the time information. In fact, the time information should be obtained from the intervals between two heartbeats. Therefore interbeat intervals imported with the import profile for Polar data are meaningless, since the time information is incorrectly imported.

Interbeat intervals by definition do not have a constant sample rate. Therefore, that data cannot correctly be imported as external data.

10.5 Synchronization of tracking data and physiological data

You have two options if you want to synchronize tracking data and physiological data:

- **Automatic synchronization** – This requires EthoVision XT to send out a synchronization signal to the external DAQ system during live tracking (see Chapter 9). The information in the synchronization signal is then used to synchronize the tracking and physiological data upon import of the physiological data into EthoVision XT.
- **Manual synchronization** – If it is not possible to connect the EthoVision XT computer and the external DAQ system, you can manually synchronize the tracking data and the physiological data upon import of the physiological data into EthoVision XT.



Synchronization of physiological data and tracking data takes place before import of the physiological data. After import, synchronization of tracking and physiological data is not possible.

Automatic synchronization

For automatic synchronization, do the following:

- 1 Import the physiological data that was simultaneously acquired during live tracking into EthoVision XT (see page 320 for a description of how to import physiological data).
- 2 In the **Import External Data** window, you see one or more '**Normal Data Sets**' (indicated by a diagram icon) and one or more '**Sync Data Sets**' (indicated by a clock icon).

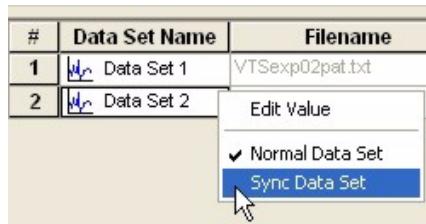


The **Link Variable** cell for a **Sync Data Set** shows which Trial was acquired simultaneously with the physiological data.

A **Sync Data Set** is automatically detected when you use the **Time Code (TCAP)** synchronization signal. When you use the **On-Off** signal, it may be necessary to select **Sync Data Set** from the context menu to tell EthoVision XT that the data set contains the synchronization signal:

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In the **Import External Data** window, right-click the **Data Set** that you want to change to Sync Data Set and select **Sync Data Set**.



- 3 If necessary, adjust the **Synchronization Settings** (this step) or proceed to point 4.

The **Synchronization Settings** button in the **Import External Data** window becomes active, when **Enable DAQ co-acquisition** is selected in the **Experiment Settings**. In the **Synchronization Settings** you can set values for a number of parameters that determine how the information from the synchronization signal is extracted from the imported physiological data file.



The information in the synchronization signal is sampled by the DAQ system. During import into EthoVision XT some digital signal processing takes place to deal with noise, signal distortion, spikes etc.

Click on **Synchronization Settings...** to open the **Synchronization Settings** window. Here you can set values for the following parameters (default values between brackets):

- **Number of offset values** (10) - corresponds to the number time points in the synchronization signal that is used to calculate offset points. This only applies to the Time Code signal. The offset values are used for the calculation of average offset and gain. A higher value increases the accuracy of synchronization, but also increases calculation time.
- **Smoothing factor** (1) - corresponds to the number of sample points over which an average signal level is calculated. A higher value reduces spikes on the signal, but also reduces the time accuracy of the synchronization. The Smoothing Factor is used for both On-Off and Time Code signal.
- **Signal-to-noise-ratio** (50) - determines the tolerance for detecting high-low signal transitions. A higher value increases this tolerance, but also increases the chance of detecting false transitions.
- **Number of samples prescan TCAP** (180000) - corresponds to the maximum number of sample points used for auto-detection of a Time Code signal in the DAQ samples. For example, 180 seconds at 1 kHz or 30

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minutes at 100 Hz. This setting only applies when you started your DAQ system before you started tracking in EthoVision XT.

Click in the boxes to set a value for each of the parameters. Click **Defaults** if you want to return to the default values or click **OK** to finish.

- 4 In the **Import External Data** window, select one or more **Data Sets** you want to link, click on one of the **Data Sets** with the left mouse-button, keep the left mouse-button pressed and link the **Data Sets** with a drag-and-drop action to the corresponding **Trial**, **Arena** or **Subject**.

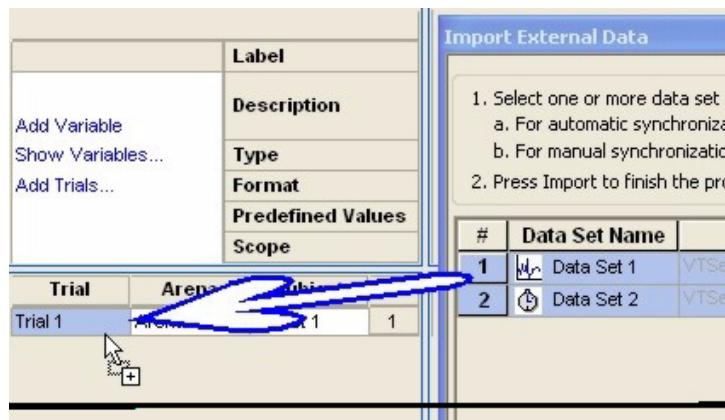


Figure 10.15. Example of Data Set 1 and Data Set 2 (containing the synchronization signal) that are assigned to **Trial 1**.

- 5 Click **Import** to finish linking the **Data Sets**.

Manual synchronization

You can manually synchronize physiological and tracking data during import of physiological data into EthoVision XT:

- **Shifting the offset** – This means that the complete physiological Data Set is shifted in time. The duration of the physiological Data Set remains unchanged. Shifting is done by changing the Start Date/Time of a Data Set.

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- **Stretching/shrinking the physiological data set** – This means that the duration of the physiological Data Set is changed to match the duration of an acquired Trial. Stretching the physiological Data Set is done by changing the **Start Date/Time** and/or the **Stop Date/Time**. This way you can either stretch or shrink the duration of the Data Set.



Manual synchronization of physiological data and tracking data is accurate to within one second.

When do I need to Shift the offset?

For example, you started acquisition on your DAQ system 10 seconds after you started acquisition in EthoVision XT. During import, you shift your physiological data set 10 seconds to match the offset of the Trial (see page 339).

When do I need to Stretch/Shrink?

You need stretching/shrinking in the scenario that you carry out acquisition during a prolonged period of time during which the clocks of the EthoVision XT computer and the DAQ computer start running 'out of sync' (you started with synchronized clocks (see below how to synchronize computer clocks).

Example – You carry out tracking for 24 hours and after this period you notice that the DAQ computer is 10 seconds ahead of the EthoVision XT computer. This means you need to shrink the physiological data set by changing the **Stop Time** of the data set to 10 seconds earlier (see page 339).

How can I facilitate manual synchronization?

You can facilitate manual synchronization by synchronizing the clock of the EthoVision computer and the computer on which the external DAQ system is running, before acquisition. You can use one of the following methods:

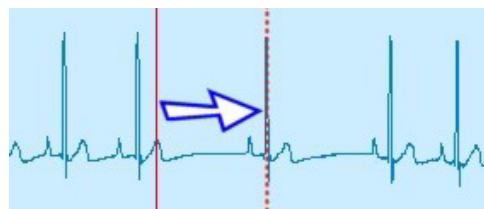
- **The computers are situated next to each other** – Double-click the clock in the Task bar at the bottom of the screen on both computers. In the **Date & Time** tab, set both clocks to the same time.
- **The computers are in different rooms** – Use a third clock (for example, a watch or an online clock, when connected to the Internet) to set both clocks to the same time (see description above).
- If, for some reason, it is **not possible to synchronize the computer clocks** there is a more advanced method to facilitate manual synchronization.



This method only works when the physiological data file contains information about date and time of acquisition.

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- 1 Acquire tracking data in EthoVision XT and physiological data on the DAQ system and make sure on both systems a synchronization event is recorded.
For example, you can use a relay to create a peak on your DAQ system and simultaneously switch on a light which is visible in the video.
- 2 Import the physiological data into EthoVision XT (see page 319 for a description of how to do this).
- 3 Visualize the data by selecting **Plot Integrated Data** from the **Visualize** menu (see Chapter 13). Go to the position in the video where the swiping action occurs. Note down the time stamp.
- 4 In the physiological data window, move the hairline to the position where the peak, caused by the swiping action, occurs. Note down the corresponding time.



Sync example – The swiping action in the video occurred at 0:00:02. The corresponding peak in the physiological data occurred at 0:00:03 in the physiological data plot, so 1 second later.

- 5 In the **Trial list** window, remove the physiological data:
 - i Select the columns that contain the physiological data sets.
 - ii Right-click of these columns and select **Delete Variable**.
- 6 Change the start time of the physiological data file by doing one of the following:
 - Open the physiological data file (for example, with Notepad) and change the start time.

Import the physiological data file. In the **Import External Data** window, you can change the start time of the physiological data set (see below **How do I Shift...?**)

Sync example – The difference in offset was 1 second (with the physiological data starting later than the tracking data). This means that the start time of the physiological data set should be set to 1 second earlier.
- 7 If you have edited the physiological data file, re-import it into EthoVision XT.

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Manual synchronization of tracking data and physiological data is accurate to within one second.

How do I Shift a physiological Data Set?

- 1 Import the physiological data that was simultaneously acquired during live tracking into EthoVision XT (see page 320 up to step 2 for a description of how to import physiological data with a predefined **Import Profile** or see page 321 for how to import with a new **Custom Import Profile**).
- 2 In the **Import External Data** window, double-click the **Start Date/Time** cell of the physiological Data Set you want to shift.
- 3 In this cell, click the year, month, day, hours, minutes or seconds and change them by using the arrow-up/arrow-down on your keyboard, the up/down arrow at the right side of the cell or just type in the desired value.



When you change the **Start Date/Time** of a Data Set, the **Stop Date/Time** also changes. As a result the duration remains the same.



- 4 Finish import (go to step 4 on page 336).

How do I Stretch/Shrink a physiological Data Set?

Stretching or shrinking can be used in the unlikely case that during acquisition the clock of the external DAQ system starts running out of sync with the clock of the EthoVision computer. This probably only occurs when live tracking is done for a prolonged period of time. For example, in case the DAQ computer clock starts slowing down during acquisition, you need to manually increase the duration of the physiological Data Set.

- 1 Import the physiological data that was simultaneously acquired during live tracking into EthoVision XT (see page 320 until step 2 for a description of how to import physiological data with a predefined **Import Profile** or see page 321 for how to import with a new **Custom Import Profile**).
- 2 To stretch the **Import External Data** window, double-click the **Stop Date/Time** cell of the physiological Data Set you want to stretch.
Shift the physiological data set if necessary (see above).

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- 3 In this cell, click the year, month, day, hours, minutes or seconds and change them by using the arrow-up on your keyboard or the up-arrow at the right of the cell.

You can shorten the physiological Data Set, by changing the **Stop Date/Time** to earlier.

- 4 Finish import (go to step 4 on page 336).

10.6 Visualizing physiological data

You can visualize physiological data together with video and acquired tracks by **Plotting Integrated Data**.

Before you display Integrated Visualization, make sure that the Data profile specifying the data you want to plot is highlighted in blue. If you want to visualize tracks according to the values of a dependent variable, make sure that that dependent variable is specified in the active Analysis profile. To edit a profile, click it in the Experiment Explorer and make the necessary changes.

- You can only visualize data from one trial at a time.
- See page 465 for a description of Plotting Integrated Data.

11

Editing Data

This chapter is about:

- **Why edit data?** – Shows a few examples of why you may want to edit your tracks. It also explains important terms.
→ See the next page
- **Customizing your screen before editing** – Includes a few tips for improving the clarity of your track plots.
→ See page 344
- **Selecting samples to edit** –
→ See page 349
- **Swapping data points (subjects, nose-point and tail-base)** –
→ See page 355 and page 356
- **Deleting points** –
→ See page 363
- **Moving and interpolating points** –
→ See page 365
- **Managing your Restore points** –
→ See page 373

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11.1 Why edit data?

Sometimes EthoVision XT tracks a reflection instead of the subject, or confuses the nose-point and tail-base. If that occurs, you can edit your tracks to correct these errors.

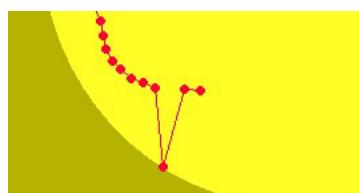


The Edit Tracks function in EthoVision XT is only intended for minor corrections of a few points. If you have many errors, there is a problem with your experimental setup, trial control or detection settings, and you should correct those problems rather than try to manipulate the raw data. For example, if EthoVision XT tracks the arm of the operator who released the animal in the water maze, use the **Trial Control** function (Chapter 7) to start data recording a few seconds after the program has detected the subject.

Examples

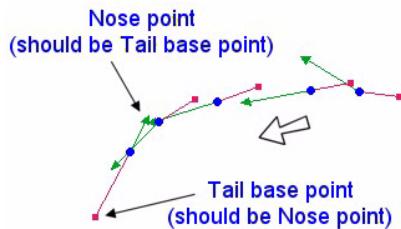
Example 1 – Wrong points due to reflections

In a water maze experiment, EthoVision XT tracks a reflection next to the maze's border. The track shows sharp changes in direction.



Example 2 – Nose-point and tail-base are exchanged

In an open field experiment, the rat's nose-point is sometimes detected as tail base, and vice versa. The following picture shows the last sample with the two points inverted (triangles are nose-points, squares are tail-base points; the thick arrow indicates the direction of movement).



Important definitions

Samples and points



Throughout this chapter, **Sample** refer to the whole set of coordinates found by EthoVision XT at any sampling time. This means that:

- If your experiment is set to **Only center-point detection**, a sample is the subject's center point (a pair of X, Y coordinates).
- If your experiment is set to **Center-point, nose-point and tail-base detection**, a sample includes the center, nose-point and tail-base (three pairs of coordinates).

Within a sample, one or more points can be missing. Missing points are shown in a different color (see page 344).

Restore points

When you edit a track, the original track is always kept for backup purposes. While you are editing, you may want to keep a side copy of the changes before you go on with editing. A **Restore point** is a copy of the track that includes the last edits. If you make more changes after setting the Restore point, you can always go back to the Restore point if you are not satisfied with those changes.

For example, you plan to interpolate two points, but are not sure that you like the result.

- 1 Make a Restore point.
- 2 Make the necessary changes.
- 3 Judge the result and decide whether to keep it or revert to the Restore point.



Making a Restore point is not the same as saving your data!

For more information on Restore points, see page 373.

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11.2 Customizing your screen before editing

The track-editing screen

To open the track-editing screen, do one of the following:

- From the **Acquisition** menu select **Edit Tracks**.
- In the Explorer, open the **Acquired Trials** folder, right-click the trial you want to edit and select **Edit**.

In the track-editing screen you can view the following objects (see Figure 11.1.):

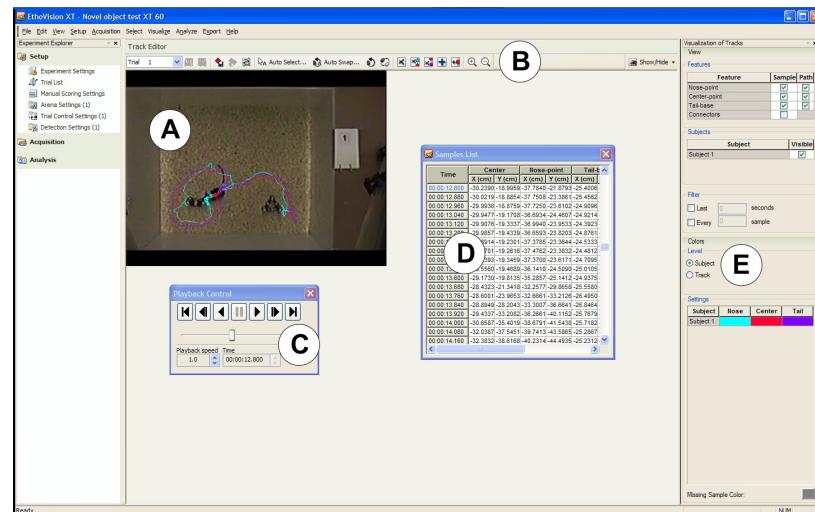


Figure 11.1. The track-editing screen.

- **A** – The track area, where you can view:

- The background – This can be of uniform color, the captured image of the arenas from the Arena Settings, or the content of the video file (see page 349).
- Arenas and zones as overlay objects.
- Tracks (samples and paths).

Editing Data

- **B** – The component tool bar that contains:
 - The **Trial Navigation** section, for selecting the trials to be edited.
 - The **Recovery** section, for managing Restore points and reverting to the original data if needed.
 - The **Toolbox** section, for selecting and editing data points.

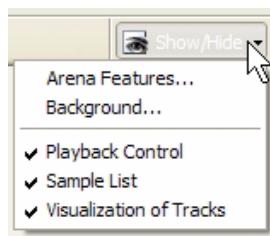


The component tool bar also contains the view settings under the **Show/Hide** button.

- **C** – The **Playback Control** window.
- **D** – The **Samples List** window, listing the coordinates of the data points and the surface area of the subjects tracked.
 - The **Time** column shows the time elapsed since the start of the trial. If data recording started after the start of the trial or ended before the end of the trial, the Samples List shows asterisks (*) for the samples not collected before the start and after the end of data recording.
 - The current time (also shown in the **Playback Control** window) is marked in blue.
 - Missing points are listed with coordinates and surface area '-'.
- **E** – The **Track Plot Settings** pane, for customizing the tracks (see page 444).



To display the **Playback Control** window, the **Samples List** and the **Track Plot Settings** pane, click the **Show/Hide** button on the component tool bar and make sure they are selected.



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Visualizing the tracks

To visualize the track you want to edit, select the trial name from the list on the component tool bar.



 To switch from one trial to the previous or next, click the **Previous Trial** button or the **Next Trial** button on the component tool bar, respectively.

 You can visualize one trial at a time.

Default visualization

-  To play back tracks, use the buttons in the **Playback Control** window. Set the play back speed you require (see page 462).
-  To visualize the whole set of samples, clear the check boxes under **Filter** in the **Track Plot Settings** pane and click the **Jump to end** button in the **Playback Control** window.

Colors

By default, **Subject** is selected under **Level**, that is, the track color does not change between tracks of the same subject. If you select **Track**, the color of tracks can change between tracks, according to the value of the variable selected under **Variable** for those tracks.

See page 454 for more information on how to specify the colors of tracks.

Improving readability of tracks

This section contains a few tips that makes it easier to locate the data points that you want to edit.

Zooming in and out the track plots

To zoom in and out, click the **Zoom in**  or **Zoom out**  button on the component tool bar, respectively.

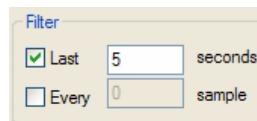


If the data points are out of view after zooming in, use the scrollbars to find them.

Visualizing part of the track

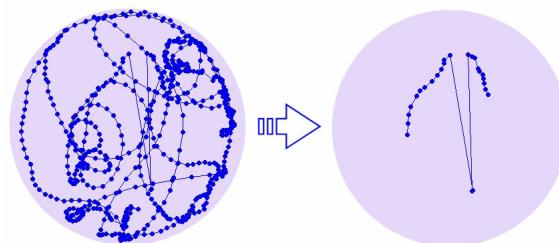
You can have the track visualized partially. This option is handy when samples are overlapping and you want to focus on a specific time fragment of the track.

To visualize a few seconds of the track before the current sample, select **Last** under **Filter** in the **Track Plot Settings** pane and enter the time interval.



The samples acquired in the last five seconds are displayed.

Partial Track not selected **Partial Track 5 Seconds**



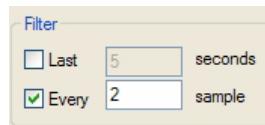
The current sample is highlighted in blue in the **Samples List**.

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Visualizing one sample every n-th

You can have one sample every n-th visualized at a given moment. This option is handy when the space between consecutive samples is small. This usually happens with high sample rates or slow moving animals.

To show one sample every n-th, click **Every** under **Filter** in the **Track Plot Settings** pane and enter **n** in the corresponding field.



n can only be integer. If you select **0**, only the current sample point is shown. If you select **1**, this is the same as not selecting the option above (all samples are displayed).

Hiding unnecessary track features

The track plots can look very complex due to the presence of different features (data points and connectors). To remove unnecessary features, clear the corresponding box under **Sample** or **Path** in the upper part of the **Track plot Settings** pane under **Features**.

Features		
Feature	Sample	Path
Nose-point	<input type="checkbox"/>	<input type="checkbox"/>
Center-point	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Tail-base	<input type="checkbox"/>	<input type="checkbox"/>
Connectors	<input type="checkbox"/>	<input type="checkbox"/>

Customizing the missing sample points

Missing sample points are always visualized in the track plot. You can change the color of missing points to distinguish them more easily from the actual data. The current color for missing points is indicated next to **Missing Samples Color** at the bottom of the Colors tab in the **Track Plot Settings** pane. To change the color for missing points, click this button and select the new color in the **Color** window.



Editing Data



Remember that *within a sample*, nose-point and tail-base can be missing independently. For example, the nose-point may be missing, while the corresponding tail-base may not. However, if the center-point is missing, then the other two points are too.



If the experiment is set to **Only center-point detection** or **Color marker tracking**, or you do not have the **Multiple body points** add-on installed on your PC, the term *sample* coincides with the only point (center).

Customizing the background



- **Video image** – Click the **Show/Hide** button on the component tool bar and select **Background**. In the Background window, you can choose between the following:
 - **Plain** – The background is uniform. Click the button next to it and choose the color you require. Click **Other** to select among more colors.
 - **Captured image** – The background image captured in the Arena Settings that was used to acquire the data. This image does not change as you play the tracks back.
 - **Video file** – The video footage from the video file used to acquire the data. The video file is synchronized with the track as you play it back.



Select **Video file** every time you want to compare the track data with the orientation and behavior of the animal. For example, when you want to verify that the nose point and the tail base point have been detected properly.

- **Arenas** – Click the **Show/Hide** button on the component tool bar and select **Arena Features**. Select the arena features you want to have displayed superimposed on the background.

11.3 Selecting samples

To edit a sample, you first need to select it. There are several ways to select samples:

- **Manual selection** (see below) – To select a single sample or one or more ranges of samples.

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- **Automatic selection** (see page 353) – Let EthoVision find the samples that match a specific criterion.



Important note – If you select one point within a sample, all body points of that sample are automatically selected, no matter whether they are actual data or missing points.

Selecting samples manually

Selecting a single sample

Do one of the following:

- **Click the sample:**

- Hover the mouse on a point belonging to the sample. The mouse pointer changes to a four-headed arrow.



- Click the point. The sample is highlighted.



To cancel the selection, press **Esc**.



Do not drag the mouse while the cursor is a four-headed arrow, because this results in moving the sample point.

- **Choose the sample from the Sample List:**

- Make sure that **Sample List** is selected under the **Show/Hide** button on the component tool bar.
- In the **Samples List** window, click any cell corresponding to the sample you want to select. Scroll down the list if necessary to locate the sample.

Result – The line is highlighted in green.

Editing Data

Time	Center		Nose-point		Tail-t
	X (cm)	Y (cm)	X (cm)	Y (cm)	X (cm)
00:00:04.160	-16.9207	-13.4620	-26.4352	-17.1586	-8.74145
00:00:04.240	-20.3022	-15.1462	-30.3151	-19.4325	-11.6177
00:00:04.320	-23.9711	-15.8536	-33.8699	-20.5142	-15.8162
00:00:04.400	-26.5096	-16.8636	-35.7907	-21.2851	-18.6364
00:00:04.480	-28.9394	-18.8429	-36.9440	-23.2754	-21.6950

- iii Right-click the row and select **Jump to Time** to display the selected samples on the plot.



If you click the **Time** column, you select all subjects for that sample.

Selecting a range of samples

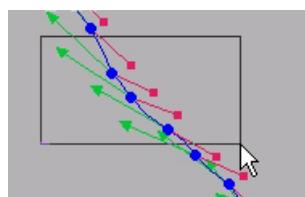
Do one of the following:

- **Select the area that includes the samples.**

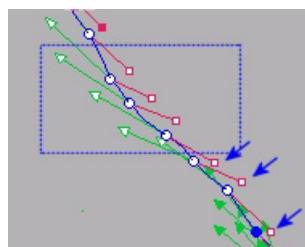
Use this method to quickly select samples in a specific area (for example, a specific quadrant in the water maze).

This method is not handy if you want to select one subject in a trial with multiple subjects. Rather use the **Sample List** (see the next page).

- i Press and hold your left mouse button, and drag to draw a rectangle. All samples within this rectangle are selected.



- ii Release the mouse button. The selected samples are highlighted.



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To cancel the selection, press **Esc**. To select another sample, repeat steps **1** and **2**.



It can happen that body points lying outside the original rectangle are selected, because they belong to samples for which at least one point is included in the rectangle. In the example above, some tail-base and center-points outside the rectangle are selected (see the points indicated by the arrows) because their corresponding nose-points and/or center points lie within the rectangle.

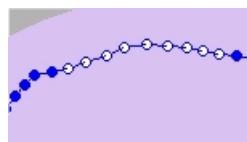
● Select the first and last sample points

- i Click the first sample of the range you want to select.

Result – The sample is highlighted.

- ii Press and hold down **Shift**, and click the last sample of the range you want to select.

Result – The sample range is highlighted.



To cancel a range selection, press **Esc**. To select another range, repeat steps **1** to **2**.



You cannot select multiple sample ranges with this method.

● Choose the sample range from the Samples List

Use this method when the ones described above do not allow you to easily select a range because of the high density of sample points, or when you want to select.

- i Make sure that **Samples List** is selected under the **Show/Hide** button.
- ii In the **Samples List** window, click a cell corresponding to the first sample of the range. The line is highlighted in green.
- iii Press and hold the left mouse button, and drag down to the last sample you want to select. Release the button when ready.

Result – The selected lines are highlighted in green. Right-click one of the rows and select **Jump to Time** to display the selected samples in the plot.

Editing Data



To select *all subjects* in your trial, click the cell containing the time of the first sample of the range, then drag down to the last sample you want to select.

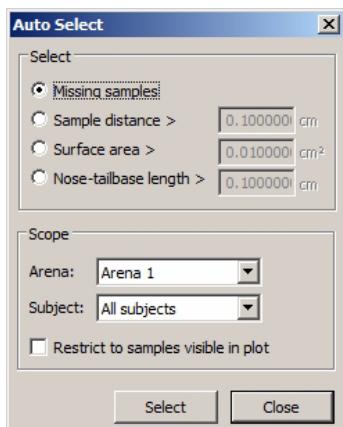
Selecting samples automatically

Use this method to easily find:

- Missing points.
- Samples separated by a distance greater than a specific value. For example, to find points lying far from the normal path (page 342).
- Samples with Surface area greater than a specific value.
- Samples with the distance between nose-point and tail-base greater than a specific value.

To select samples automatically:

- 1 In the component tool bar, select the **Auto Select** button Auto Select... .
- 2 In the **Auto Select** window that appears, select the option you require under **Select**.



For more information, See the notes at the end of the procedure.

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3 Under Scope:

- Select the arena you want to search. If you want to find samples in all arenas, select **All arenas**.
- Select the subject you want to search. If you want to find samples in all subjects, select **All subjects**.
- Select **Restrict to samples visible in plot** if you want to select only the samples currently displayed in the track plot that match the criterion specified under **Select**. The other samples displayed are ignored.

4 Click **Select**.

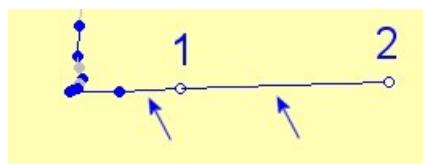
Result – The samples matching the criterion are highlighted in the track plot. In the **Samples List** window, the corresponding rows are highlighted in green.

To make a new selection, change the settings under **Select** and click the **Select** button. When you are satisfied with the selection, click **Close**.

Notes



- If you choose **Sample distance**, EthoVision XT selects all the non-missing points separated by a distance greater than the threshold value you specify. This is not the same as selecting samples on the basis of the per-sample distance moved. In the following example:



Sample 1 and 2 have been selected automatically because their distance is greater than the specified threshold. Sample 1 is selected no matter what the distance moved is for that sample (for clarity, distance moved is indicated by the arrows).

- Sample distance is always calculated for the center-point, not nose-point or tail-base.
- The **Nose-tailbase length** option is only available if your experiment is set to **center-point, nose-point and tail-base detection**. **Nose-tailbase length** is the sum of the distance between nose-point and center-point, and between center-point and tail-base point.

Editing Data

- If you select the **Restrict to samples visible in plot** option and then play back the track, the samples in the remaining part of the track that match the criterion under **Select** are not selected.

De-selecting samples

To de-select samples, do one of the following:

- Press **Esc**.
- Click anywhere on the background image that is not a point.
- Right-click anywhere on the background image.

What next?

Once you have selected samples or ranges, see the next sections depending to the kind of editing you want to do.

11.4 Swapping subjects

Aim

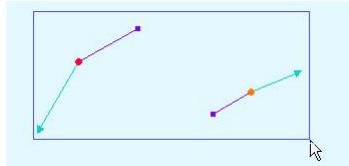
To exchange the body points of two subjects within one sample (or range of), after checking that these were assigned incorrectly by EthoVision XT.

- If a large number of samples contain body points assigned to the wrong subject, it may mean that the detection settings defined to discriminate between subjects are not optimal, or that you need to improve the lighting or the color marking of your setup. See Chapter 8.
- You can set a Restore point so that if you are editing many swaps and make a mistake, you do not lose your edits. See page 373.

Procedure

- 1 Play the track until you find samples with wrongly-assigned subjects.
- 2 Select the sample (or range of) in which you want to swap the subjects. To do so, select the corresponding rows in the Samples List, or drag the mouse around the subject's body points.

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Result – The selected samples are highlighted.

- 3 Click the **Swap subjects** button  on the component tool bar, or press **D**.

Result – The subjects in the selection are swapped. In the **Samples List**, the coordinates are swapped between subjects for those samples.

Notes



- You can also check the swap from the change in the colors of the body points. Compare the colors of the body points with those selected in the Show/Hide tab in the **Track Plot Settings** pane.
- If you select several samples and choose to have one sample every n-th visualized (see page 348), you also swap the body points in the selected range that are not visualized.

11.5 Swapping nose-point and tail-base

Aim of Swapping points

- To exchange the nose-point and the tail-base within a sample (or range of), after checking that these were assigned incorrectly by EthoVision XT.

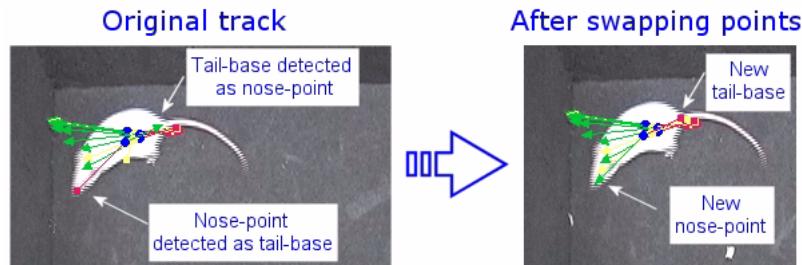


Figure 11.2. An example of swapping nose-point and tail-base. Left: The tip of the animal's nose of the current sample is detected as tail-base, while in the previous samples it was correctly detected as nose-point. Swapping results in the nose-point becoming tail-base and vice versa.



- By selecting the **Advanced model-based** nose-tail detection method, you can minimize the occurrence of nose-tail swaps (see page 258). However, this method requires much processor load, so make sure you do not miss samples.
- If a large number of samples contain wrongly-assigned nose-point and tail-base, it may mean that the detection settings for nose-point and tail-base detection are not optimal, or that you need to improve the lighting or other settings of your setup. See page 258.
- You can set a Restore point so that if you are editing many swaps and make a mistake, you do not lose your edits. See page 373.



For the samples that are swapped, the Head direction value is removed and cannot be recovered.

How do I check that nose-point and tail-base were mistakenly exchanged?

- **In all cases** – From sudden and large turns of nose-point and tail-base. For example, the subject makes a turn of almost 180 degrees.
- **If you track from media files or you have created an MPEG-4 file whilst live tracking** – Have your track displayed with the video on the background (see page 349). Check that the nose-point and tail-base do correspond to the actual orientation of the subject.

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Manual vs. Automatic swapping

There are two main methods for swapping body points:

- **Manual swapping** (see below) – You look for the samples with exchanged nose-point and tail-base, and give the Swap command for those samples.
- **Automatic swapping** (see page 358) – You define a criterion for automatic detection of changes in orientation of the nose-point and tail-base. EthoVision XT searches your tracks for the samples where a sudden change in orientation has occurred. It is up to you to swap nose-point and tail-base for those samples.

Manual swapping

- 1 Play the track until you find samples with wrongly-assigned nose-point and tail-base (see the left picture in Figure 11.2.).
- 2 Select the sample (or range of) in which you want to swap the nose-point and tail-base.

Result – The selected samples are highlighted.

- 3 Click the **Swap nose/tail** button  on the component tool bar, or press the **W** key.

Result – The nose-point and tail-base within each sample in the selection are swapped. In the **Samples List**, the coordinates are swapped between **Nose-point** and **Tail-base** for those samples.

Automatic swapping

With automatic swapping, EthoVision looks for the samples where the subject made a turn exceeding a user-specified threshold relative to the previous sample. These turns are interpreted as nose-point and tail-base being incorrectly assigned. You can then select those samples and let EthoVision exchange the corresponding nose-point and tail-base.

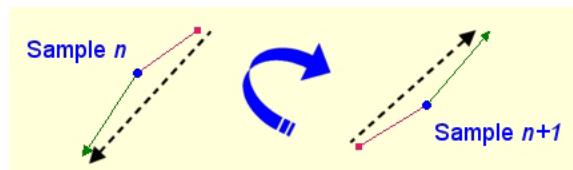


Use this method when a large number of consecutive samples have body points wrongly assigned.

Editing Data

What does 'turn' mean here?

Turn is referred to the change in orientation of the nose-tail base vector. The nose-tail base vector is the vector connecting the nose-point and the tail-base point within a sample (see the dotted arrow in the picture below). With automatic swapping, if the change in the vector's direction from sample n to $n+1$, is larger than the angle you specify, the sample $n+1$ is considered for swapping. Then, it is up to you to decide whether to swap the points.



If the nose-point or the tail-base is missing, the nose-tail base vector is the vector connecting the center with the other point. If both nose- and tail-base points are missing, the sample is not considered for swapping anyway.

If samples following $n+1$ have a vector's turn lower than the specified threshold, they too are assumed to have nose-point and tail-base detected wrongly. Therefore, they are included in the swapping interval (see samples under B in Figure 11.3.).

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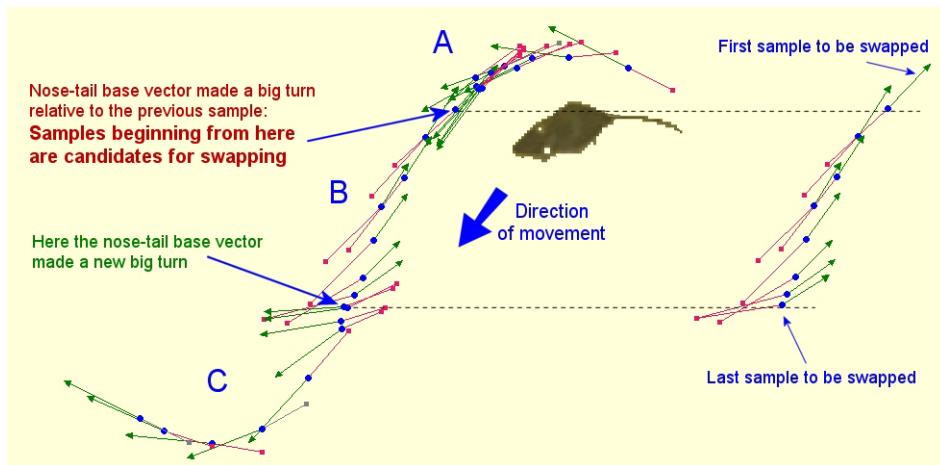


Figure 11.3. Example of how samples are considered for swapping. The subject is moving in the direction of the arrow. In the interval marked with A, the subject turns to an extent lower than a specified threshold (for example, 150°). Starting from the first sample of A, EthoVision scans the track and finds the first sample with turn greater than the threshold. This is the Start of the swapping interval. When it finds a new significant turn, it assumes that all samples in the middle are considered to be swapped (B). The swapping interval is copied to the right-hand side for clarity. The dotted lines highlight the Start and the End samples of the swapping interval. Samples of the interval marked with C are considered correct, until a new significant turn is found.

Procedure



- 1 Position the track at the beginning and check whether the first sample in your track is orientated correctly.

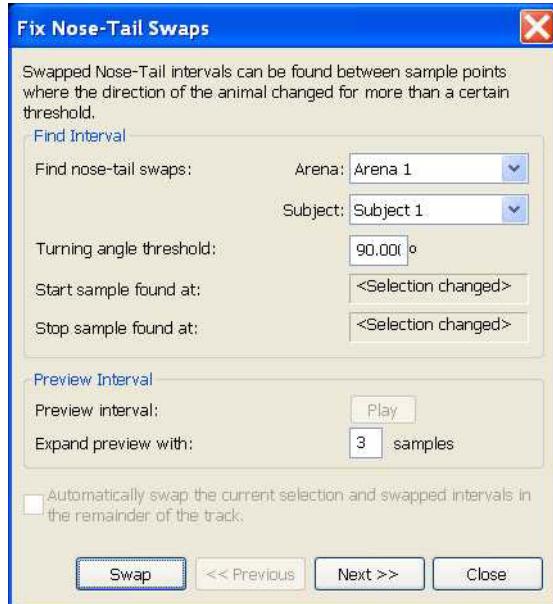


How do I check this? – If you have tracked data from a video file, make sure that the video file is on the background (see page 349). If you have tracked live, make sure that the nose-tail base orientation matches the direction of movement.

- 2 Click the **Auto Swap** button on the component tool bar.

Result – The **Fix Nose-Tail Swaps** window appears on top.

Editing Data



3 Under **Find Interval**, do the following:

- i** From the **Find nose-tail swaps** in the track list, select the arena and the subject you want to search. For single-area setups and single-subject setups, only one arena/subject is available, so go to step **ii**.
- ii** In the **Turning angle threshold** field, enter the threshold angle. The first sample with a change in orientation of the nose-tail base vector larger than this threshold is considered for swapping.

4 Click the **Next >>** button.

Result – A number of samples are highlighted (selected). Under **Find Interval**, the following times are displayed:

- **Start sample found at:** – The time of the sample in the track with turning angle greater than the threshold relative to the previous sample. It is the start of the interval of samples considered for swapping (see **B** in Figure 11.3.).
- **Stop sample found at:** – The time of the sample immediately preceding the sample with turning angle greater than the threshold. It is the end of the interval of samples considered for swapping (see **B** in Figure 11.3.).

5 Do one of the following:

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- If the first sample of the selected interval in your track is oriented correctly, click the **Next >>** button until the first sample of the new interval is **not** oriented correctly.
- If the first sample of the selected interval is **not** oriented correctly, this is the start of the interval of samples to be swapped. Proceed to step **6**.

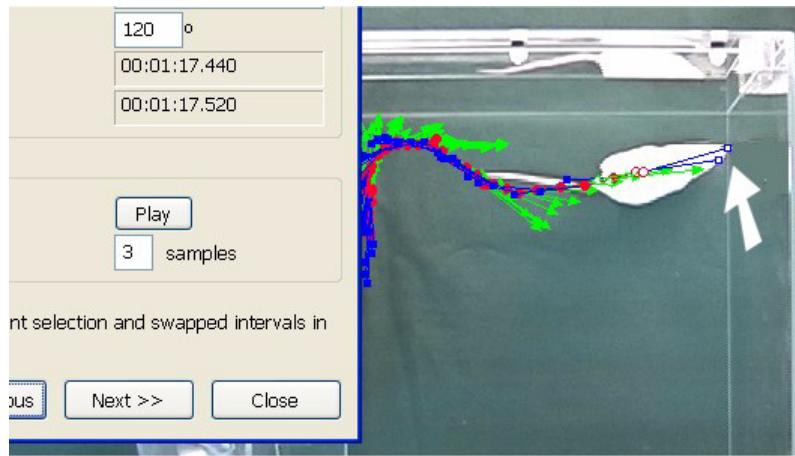


Figure 11.4. Click the **Next >>** button until the first sample of the interval highlighted in the track plot does not match the orientation of the animal. In this example, an interval of two samples to be swapped has been found.

- 6 Once you have selected an interval of samples that does not match the orientation of the animal, do one of the following:
 - If you want to let EthoVision XT swap the intervals of samples throughout the track based on the above criteria, select the **Automatically swap the current selection and swapped intervals in the remainder of the track** option, and click the **Swap** button in the **Fix Nose-Tail Swaps** window.
 - If you only want to swap the selected interval, make sure that the option above is cleared and click the **Swap** button in the **Fix Nose-Tail Swaps** window.
- Result – The nose-point and tail-base in the selected intervals are swapped.
- 7 Do one of the following:
 - If you want to search for the next interval to be swapped, go back to step 5.

Editing Data

- If the track contains only samples oriented correctly, click the **Close** button.
- To swap nose-point and tail-base in another arena, select that arena from the appropriate list (step 3-I).

Notes



- When you click **Next>>** for the first time, the interval from the start of the track (0:00:00.000) to the first change in the nose-tail vector orientation greater than the threshold is displayed. If you click the **Next>>** button more times, other intervals are displayed depending on where in the track other significant turns are found.
- Select the **Automatic swap option** above only if you are confident that the angle threshold specified in step B results in identifying the correct intervals to be swapped. This usually happens when you have checked the intervals yourself a few times.
- If you select points outside the **Fix Nose-Tail Swap** window, the **Start sample found at** and **Stop sample found at** fields show **<Selection changed>**. In this case the Automatic swap option is not available. Click the **<<Previous** button to return to one of the sample intervals found by the program.
- If you want to view the movement of the subject around the selected sample, click the **Play** button in the Preview Interval group. If you want to view additional samples immediately before the start and after the end of the selected interval, enter the number of such samples in the **Expand preview with** box.
- To go back in the track and select the previous intervals that start and stop at samples where the turn angle exceeds the threshold, click the **<<Previous** button.
- If you want to undo the Swap action, click the **Swap** button again.



11.6 Deleting points

Aim

To remove a sample (or some of the points of your sample) from your data set. Deleting points means they are set to missing.

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If you delete a point, you cannot recover the original coordinates, you can only interpolate it. If you delete an entire sample, the surface area is also lost. If you are not sure about the consequences of setting points to missing, make a Restore point first (see page 373). You can always return to your original track data.

Procedure

- 1 Select the sample (or range) that contains the points you want to set to missing (see page 349).
- 2 Do one or more of the following:

If your track contain only center-points:



- To set the center-points to missing, click the **Set to missing** button on the component tool bar or press **Ctrl+Del**.

If your data also contains nose-point and tail-base:



- To set all points to missing, click the **Set to missing** button or press **Ctrl+Del**.



- To set only nose-points to missing, click the **Set nosepoint to missing** button or press **Ctrl+Shift+M**.

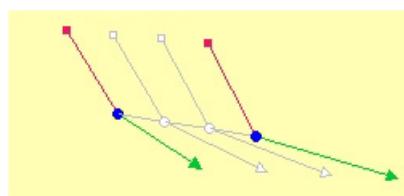


- To set only tail-base points to missing, click the **Set tailbase to missing** button or press **Ctrl+M**.

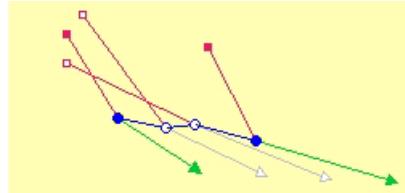
Result – The selected samples or points are set to missing.

What Setting to missing means

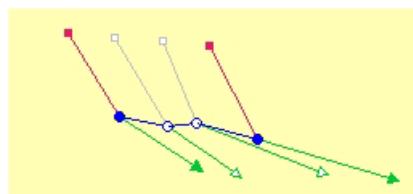
- **In the track plot** – Points set to missing are displayed in the color you have chosen (see page 348). Compare the pictures below with the one in the previous page.
 - If you have set the whole selection to missing:



- If you have set the nose-points to missing:



- If you have set the tail-bases to missing:



Missing points are shown as linear interpolations between the non-missing points of their own type. However, their coordinates are not in the track data, and are not subject to analysis. For example, if sample n is missing, the distance moved between the two non-missing points $n-1$ and $n+1$ is given by the straight line connecting the two points of the same type (nose-point to nose-point, etc.).

Notes



- **In the Samples List** – The points' X,Y coordinates are removed from the Samples List. If you set the sample to missing, the surface area is also removed. Missing values are indicated by "-".
- **For Head direction data** – If you set a nose-point to missing, the Head direction value for that sample is removed.

11.7 Moving and interpolating points

Moving points

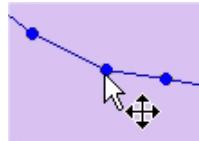
Aim

To change the X,Y coordinates of a point on the track plot.

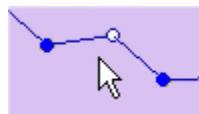
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Procedure

- 1 Hover the mouse on the point you want to move. The mouse cursor changes to a four-headed arrow.



- 2 Press and hold the left mouse button and drag to the position you require. Next, release the mouse button. The point is moved to the new position.



- 3 To de-select the point, press **Esc**.

The X,Y coordinates of the point are updated in the **Samples List**.

Notes



- If you move a missing sample, this becomes non-missing and therefore included in your data.
- If you drag a sample outside the Arena, the action is canceled, however the sample stays selected.
- If your experiment is set to **Center-point, nose-point and tail-base detection**, a sample is made of three points (no matter whether those are actual data points or missing). You cannot move a sample as a whole, you must move each point separately (center-, nose-, and tail-base points).
- If you move a nose-point, the **Head direction** value for that sample is invalidated, because of the conflict between the new nose-point position and the original contour which determines the Head direction.
- If you want to move a point by just one or two pixels, make sure you drag it a bit further away otherwise your action has no effect.

Interpolating points

Aim

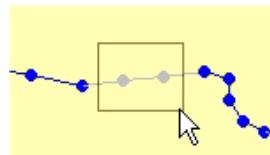
To replace one or more missing points (or samples) or points clearly misplaced with points calculated on the basis of the position of the first previous and next non-missing points.



If your experiment is set to **Center-point, nose-point and tail-base detection**, you can choose between two types of interpolation: interpolate all selected body points or the center-points only (see below).

Procedure

- 1 If you want to interpolate non-missing points clearly misplaced (for example, the one in the picture on page 342), set them first to missing (see page 363). In other cases go to step 2.
- 2 Select the missing sample (or the range of) you want to interpolate (see page 349 for details).



- 3 Click one of the following buttons on the component tool bar:



- **Interpolate selection** (or press **Ctrl+I**) – Click this button if you want to interpolate all the body points of the selected samples. See the notes below for how points are interpolated.



- **Interpolate center points** (or press **Ctrl+Shift+I**) – Click this button if you want to interpolate the Center points for the selected samples, and leave the nose-point and tail-base as they are currently (no matter whether missing or not).



If your experiment is set to **Only center-point detection**, only the first button is available. Click this button to interpolate the center-points.

A message appears after you have clicked the interpolate buttons, informing you that EthoVision XT has extended the range of samples to be interpolated, if needed (see the note below). Click **Yes** if you want to continue. If you are not sure about the result of the interpolation, click **Cancel**, set a new Restore point (see page 373) to save the current edit, and repeat the interpolation procedure. If you are not satisfied about how



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data are interpolated, click the **Restore Last Restore point** button in the **Recovery** tool bar to return to the situation prior to interpolation.

- 4** If you have clicked **Yes**, the samples (or center points within samples) are interpolated.

Result – The interpolated points are highlighted in the track plot and get the color of non-missing points. In the **Samples List**, the points obtain X,Y coordinates, not the Area value.



To cancel the selection (not the interpolation), press **Esc**. If you want to cancel the interpolation, set the samples to missing (see page 363).

Notes



- **Missing points** – When you visualize the data, missing points are shown as linear interpolation between the first previous and next valid points. However, their coordinates are not calculated (you can check this in the **Samples List**). This means that missing points are actually not in the data set. If you want to include them in your data set, you must first interpolate them.
- **Interpolated points are set as missing when outside the arena** – In some cases interpolation results in points being located outside the arena. In those cases, they are automatically set to **missing**. This is why you may still see missing points after you have carried out interpolation.

When is the interpolating range expanded?

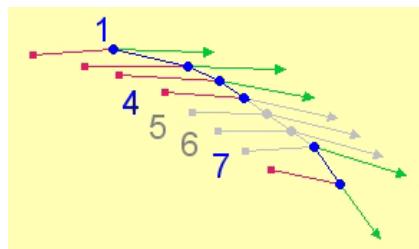
To interpolate points, EthoVision needs complete samples immediately before and after the sample to be interpolated. 'Complete samples' means that:

- For **Only center-point detection**, the Center point of the adjacent samples must not be missing.
- For **Center-point, nose-point and tail base detection**:
 - If you want to interpolate the entire samples (that is, you click **Interpolate Selection** on the component tool bar), all the three points of the adjacent samples must not be missing.
 - If you want to interpolate the center-point only (that is, you click **Interpolate center points** on the component tool bar), the Center-point of the adjacent samples must not be missing.

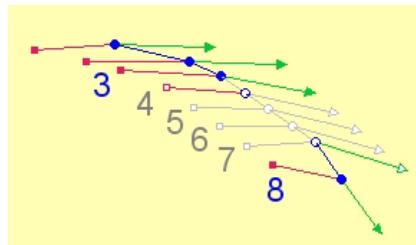
Editing Data

If the samples adjacent to the selected range are not complete, EthoVision XT searches for the first complete sample before and after that sample (or range). Therefore, all (incomplete) samples in the middle are included in the range to interpolate.

Example – The missing samples 5 and 6 in the picture below have been selected for interpolation. Samples 4 and 7 are adjacent to the range, but not complete: sample 4 lacks the nose-point, while sample 7 lacks the tail-base point (missing points and connectors look faded here).

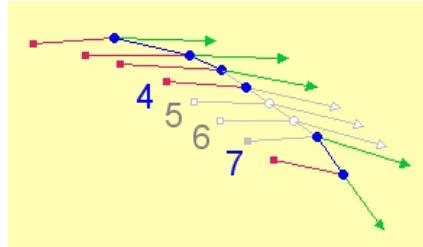


- If you choose to interpolate the entire selection, EthoVision XT needs the coordinates of the three points for both adjacent samples in order to calculate the coordinates of the three points of samples 5 and 6. Therefore, samples 4 and 7 are not used. Instead, the complete samples 3 and 8 are used as adjacent samples, so the interpolating range is expanded to samples 4 and 7 (see the open dots below). The coordinates of the missing points of samples 4 to 7 are calculated.



- If you interpolate only the center points, EthoVision needs the coordinates of the center points for the adjacent samples in order to calculate the coordinates of the center-points 5 and 6. Sample 4 and 7 are used because they are the first previous and next samples with valid center-points, respectively. The interpolating range is not expanded (see the open dots below). The coordinates of the missing center-points, not nose-point and tail-base, of samples 5 and 6 are calculated.

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How body points are interpolated

Center-point

The center-points of the selected samples are a linear interpolation between the first previous non-missing center-point and the first next non-missing center-point found around the interpolating (expanded) range.

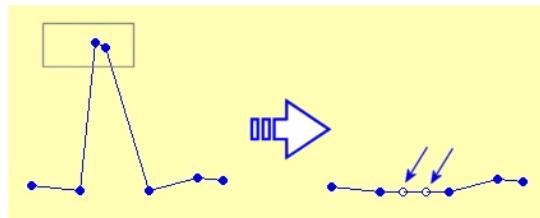


Figure 11.5. Linear interpolation of two Center points. Left: the two points are first set to Missing (see page 363). Right: After interpolation, samples are still highlighted (see the arrows).



If your track contains nose-point and tail-base, interpolation of center-points is independent of whether you choose to also interpolate nose-point and tail-base. In any case, center-points are interpolated first.

Nose-point and tail-base point

Nose-points and tail-base points are always interpolated after the center-points have been found (see above), and their interpolation is dependent on the

Editing Data

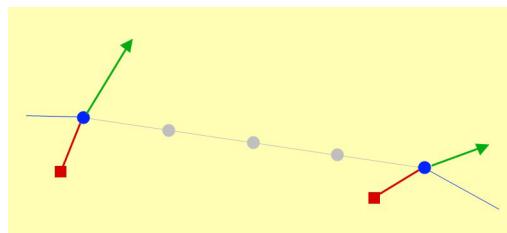
orientation of the non-missing nose-points and non-missing tail-base points immediately preceding and following those missing samples, respectively.



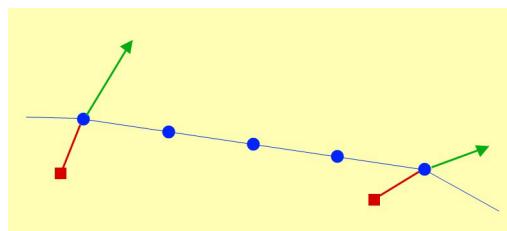
Before interpolation, the missing points are indicated as a linear interpolation between the first previous and the first next non-missing points of their type.

The direction of movement does not play any role in interpolation.

Consider the following example with three missing samples (missing nose- and tail-base points are not shown for clarity):



First, center points are linearly interpolated:

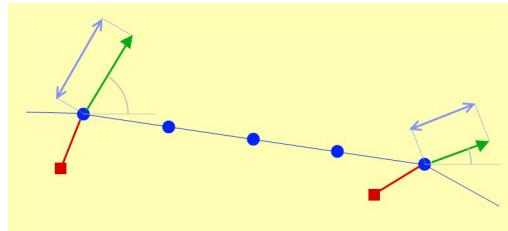


Second, for the two adjacent non-missing samples, the program determines:

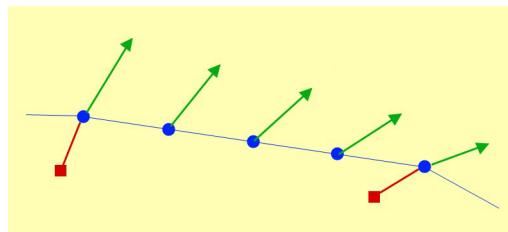
- The length of the segment joining the center and the nose- (or tail-base) points.
- The angle formed by this segment and the horizontal x-axis generating from the center point (this angle can be either positive or negative depending on whether the segment lies above or below the axis).

The picture below shows this step for the nose point only:

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Third, the program calculates the interpolated values of segment length and angle for the missing points, using linear interpolation of the values obtained in the previous step.



So for example:

- If the length of the center-nose point segment for the two adjacent samples is 50 and 10 mm, respectively, then the length of the three segments joining the interpolated nose-points and their center points will be 40, 30 and 20 mm respectively.
- If the orientation of the center-nose point segment for the two adjacent samples is 90 and -10 degrees, respectively, then the orientation of the three segments joining the interpolated nose-points and their center points will be 65, 40 and 15 degrees respectively.

The second and third step is repeated to interpolate the tail-base points.

Interpolating points at the beginning or end of the track

If the range of missing points to interpolate is at the beginning or the end of the track, the missing points are not interpolated. This is also the case when you want to interpolate nose- or tail-base points at the beginning/end of the track and the corresponding center points are non-missing.



Notice that this differs from how EthoVision 5 and 6 deal with interpolation at the beginning/end of the track. For more information, see the EthoVision XT 5/6 Reference Manual.

11.8 Managing your edited tracks

The Restore point

A **Restore point** is a copy of the track that includes the edits done up to that time. If you make more changes after setting the Restore point, you can always recover that point if you are not satisfied with those changes.

Example – You want to interpolate points, but are not sure whether the result is satisfactory. Save the current track by setting a Restore point, then do the interpolation:

- If the result of editing is good, set a new Restore point. This replaces the previous Restore point.
- If the result is not good, you can go back to the last saved data by reverting to the Restore point.



Please note that setting a Restore point is not the same as saving the data.

What is the difference between setting a Restore point and the normal Saving?



To save your edited tracks at any time, from the File menu, select **Save Experiment** or press **Ctrl+S**. However, once you have saved the data, you cannot revert to the first edit. In other words, you cannot **Undo** any Save action. However, you can always return to your original track data (see page 375).

Unlike with the Save action, with the Restore points you can always revert to the previous set of changes (see **Setting and reverting to the Restore point** below). Therefore, using the Restore point is handy when you want to make sure you can recover the data after you carry out some editing.

A Restore point persists until you close the experiment. If you set a Restore point, then make further changes to the data and close the experiment, all changes are saved but when you re-open the experiment you cannot revert to the Restore point (see page 374).

How many Restore points can I set?

You can set one Restore point per trial. Every time you set a new Restore point, that replaces the previous one. This means that you can always keep only one copy of edited data.

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What about my original data?

The original (unedited) data are always stored in your experiment, no matter of whether you edit the tracks and set Restore points. You can revert to the original data at any time (see page 375).

Setting and reverting to the Restore point

Setting the Restore point

- 1 Edit the track (see this chapter).
- 2 When you want to save the changes, click the **Set restore point** button on the component tool bar.

Result – The edits are saved.



- If you have already set a Restore point and you set a new one (step 2 above), a message appears asking you whether you want to replace the existing Restore point. Click **OK** if you want to do so. Click **Cancel** to return to the main screen.
- After you have saved edits, the tracks that are visualized (see Chapter 12) and subject to analysis (see Chapter 13) always include the changes. If you want to visualize or analyze the original data, you must first restore the original data (see below).
- **Auto save** in EthoVision XT works like a normal Save command. Therefore, if an auto save action occurs, all changes made so far to the currently edited trial are applied. However, if you make further changes and an auto save action occurs, you can always revert to the Restore point that was made before the auto save action. To alter your auto save settings, from the **File** menu, select **Preferences** and click **Auto Save** in the menu on the left.

Reverting to the Restore point



Use this function to revert to previously saved data after you have changed the data further and you do not want to keep the last edits.

- 1 Click the **Revert to restore point** button on the component tool bar.

Result – A message appears, asking you whether you want to recover the Restore point and lose all changes made since that.

- 2 Click **Yes**. The track plot is updated.

Editing Data



If you click **Cancel** at step 2, you return to the main screen without reverting to the Restore point.

Saving data when switching between trials

If you edit data in one trial and then select another trial in the trial selection list on the component tool bar (see page 346), the changes you have made to that trial are saved automatically, including those you made after setting the Restore point.

Saving data when exiting the Track-editing screen

If you open another EthoVision component (for example, to calculate statistics) or close the experiment after making changes to the track, those changes are saved, and retained when you switch back to the Track editing screen.

However, if you close the experiment you cannot revert to the Restore point you set. This is because the Restore point is always deleted when closing the experiment.

Consider the following sequence of actions:

- 1 Edit data, then set a Restore point.
- 2 Make a second run of changes, then close the experiment.
- 3 Re-open the experiment and from the **Acquisition** menu select **Edit tracks**.

Result – The screen shows the last changes made, but you cannot revert to the Restore point.

Restoring the original data



If you want to revert to the original tracks, that is, data as they were acquired, and discard all changes, click the **Revert to original** button on the component tool bar.

12

Selecting Data for Analysis

This chapter is about:

- **Track Smoothing** – Two track smoothing methods are available to filter out system noise and outliers: Smoothing (Lowess) and Minimal distance moved.
→ See the next page
- **Why select data?** – Explains why it is handy to analyze or visualize part of the data you have acquired, and shows a few examples. See this section for the important distinction between **Filtering** and **Nesting over data**.
→ See page 382
- **The Data Selection screen** – Describes the software interface and how to work with selection boxes.
→ See page 384
- **Selecting your Data** – Explains how to select tracks (**Filtering**), specific portions of tracks (**Nesting**), regular time intervals (**Results per time bin**), or different Zones (**Results per Zone**). It also contains examples of data selection.
→ See page 393 (Filtering), page 400 (Nesting), page 430 (Results per Time bin), page 430 (Results per zone)

12.1 Track Smoothing

In any tracking system, there are three sources of noise that potentially affect the values of dependent variables such as Distance moved or Velocity:

- **System noise** – The noisy output of tracking systems means that when you track an object that does not move, the position of the object irregularly oscillates between two or more locations. This way the track may show continuous movement of the object that corresponds to a real-world distance of, for example, 1 cm, while in fact the animal is sitting still. As a result, the data show that the immobile object traveled quite a distance.
- **Outliers** resulting from tracking noise – Occasionally the tracking system generates a single outlier which obviously affects a dependent variable such as Velocity.
- **Small movements of the animal** ('body wobble') – When you track a moving animal using a high sample rate, sideways movements from the wobbling of the animal's body are also tracked. This results in an overestimation of, for instance, the total distance moved.

EthoVision XT 8 has two track smoothing methods:

- **Smoothing (Lowess)** - You should use Smoothing when you want to eliminate small movements, such as body wobbling during locomotion, that might affect dependent variables such as total distance moved. On the other hand, do not use Smoothing when you are specifically interested in small, erratic movements of the animal, for instance, when it has been treated with a drug such as amphetamine.
- **Minimal Distance Moved** - Use Minimal Distance Moved when the animal sits still, yet the body points still move slightly due to system noise or breathing.



Please note that these two track smoothing methods affect the tracks after acquisition. In some cases you may already want to smooth the track during acquisition. This may especially be the case if you use Trial and Hardware Control. As an example, if the centre point of an animal is detected in a zone, you want the pellet dispenser to drop a pellet. If the detected centre point is moving rapidly because of noise, this may result in a number of consecutive pellets to be dropped, every time the centre point crosses the border of the zone. Track Smoothing does not solve this problem. Instead, use Track noise reduction in the detection settings (see page 229).

Smoothing

The Smoothing method in EthoVision XT uses the **Lowess** (Locally weighted scatter plot smoothing) method. The Lowess method fits a curve to the dataset, using least square regression that is modified as follows:

- The Lowess method uses a moving time window (or half size window) that contains a subset of sample points.
- The Lowess method uses a 2-degree (non-linear) polynomial fit. This fit is applied to the center-point. If you use center-point, nose-point and tail-base detection, the fit is also applied to the angle between center-point and nose-point and between the center-point and tail-base. (see Notes below).
- The Lowess method is weighted; sample points nearest to the point being fitted have a larger influence on the fit than sample points further away.

The Lowess method has been successfully applied on track data from EthoVision.

To apply Smoothing to your acquired track data:

- 1 Open a **Track Smoothing Profile** (see also "Managing settings and profiles" on page 581).
- 2 Select the **Smoothing (Lowess)** check box and click the **Edit** button.
- 3 Change the number of sample points in the half window size list.
 - The default value is '10' which is the recommended value. Selecting a larger half window size results in a smoother path, selecting a smaller half window size results in a path that more resembles the original path. With a lower sample rate, you should use a larger half window size to get the same smoothing as with a higher sample rate.

Notes

- For more information on the Lowess method, see the web page <http://en.wikipedia.org/wiki/Lowess>. and the White Paper about smoothing on the EthoVision XT download page on www.noldus.com.
- For more information on the application of the Lowess method on EthoVision track data, see **Drai and Golani**, 2001, SEE: a tool for the visualization and analysis of rodent exploratory behavior, *Neurosci. & Biobehav. Rev.* 25(5): 409-426 and **Kafkafi et al.**, 2005, Genotype-environment interactions in mouse behavior: a way out of the problem, *Proc. Natl. Acad. Sci. USA*, 102(12): 4619-4624.
- Smoothing is applied **after** data acquisition but **before** the Minimal Distance Moved method.

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- Smoothing is applied to the center-point, and subsequently to the angle between the center-point and the nose-point (if you also track nose-point and tail-base).
- Applying Smoothing does not change the track file. The acquired data remain unchanged.
- When you have edited your data, Smoothing is applied to the edited data.
- When you have applied Smoothing, the smoothed data are used for analysis and for export.

Acknowledgment

We gratefully acknowledge the ongoing collaboration with Prof. Ilan Golani, Prof. Yoav Benjamini and their colleagues at Tel Aviv University. Their pioneering work on the detailed analysis of animal movement has been a source of inspiration for the developers of EthoVision and for many of its users around the world.

Distance moved

With the Minimal Distance Moved method, you can pick out the data when the subject moved a minimal distance from one sample to the next. For example, when the distance moved between the current and the previous sample is above (or equal to) the threshold for Minimal Distance Moved, the current sample is used for analysis. If the distance moved between the current and the previous sample does not exceed the threshold, the previous sample is used for analysis.

In contrast to the Minimal Distance Moved method used in EthoVision XT 7, in EthoVision XT 8, no samples are excluded for analysis.



Minimal Distance Moved is only applied to the center-point.

Under some circumstances, using the Minimal Distance Moved filter might affect dependent variables other than Distance Moved and Velocity. To apply Minimal Distance Moved to some dependent variables, not others, create two Track Smoothing profiles: one with Minimal Distance Moved selected, the other without Minimal Distance Moved selected. Run analysis twice, using first one Track Smoothing profile, then the other one.

Selecting Data for Analysis

Procedure

- 1 Open a **Track Smoothing Profile** (see also “Managing settings and profiles” on page 581).
- 2 Select the **Minimal Distance Moved** check box and click the **Edit** button.
- 3 Under **Select**, enter the **Minimal Distance Moved** threshold (in centimeters).
- 4 Next, choose one of the two options:
 - **Direct (A > MDM)** – To select samples on the basis of the shortest distance (beeline distance) between samples. Select **Direct** to exclude movements such as breathing, when the animal is sitting still.
 - **Along the path (B + C + D > MDM)** – To select samples on the basis of the actual path between samples. Select **Along the path** whenever you want to filter samples according to the distance moved along the path.



See Figure 12.1. for an example.

- 5 Click **OK**.

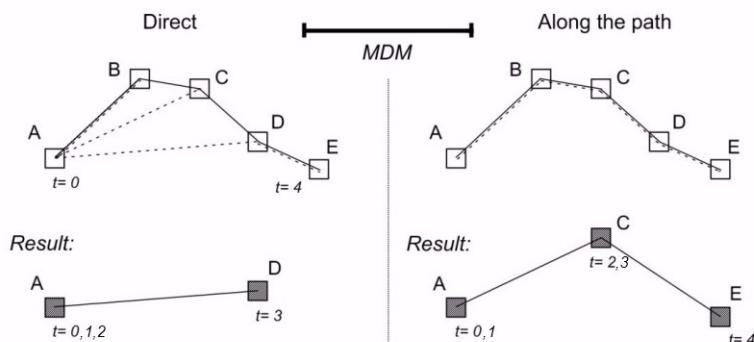


Figure 12.1. Difference between the two options for the Minimal Distance Moved method: **Direct** (left) and **Along the path** (right). Top: A, B, ... E are the center-points of a subject along a track. The horizontal bar at the top (**MDM**) represents the threshold distance set in the procedure. The dotted segments are the distances calculated to find a sample with distance greater than the threshold. **Result:** the samples selected for analysis are shown as hatched squares. See also the text below this figure.

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Direct vs. Along the path Minimal Distance Moved

- **Direct** (left-hand side in Figure 12.1.) – The program calculates the shortest distance between a sample and the next (A-B). If this distance is shorter than the threshold distance, sample B is set to sample A. Then the program calculates the distance to the second next sample (A-C), etc. until it finds a segment (A-D) longer than the threshold distance. In this example, samples B and C are both set to sample A. This procedure is then repeated from sample D up to the end of the track.
- **Along the path** (right in Figure 12.1.) – The program calculates the distance between a sample and the next (A-B). If this distance is shorter than the distance threshold, sample B is set to sample A. Then the program calculates the cumulative distance to the second next sample (A-B + B-C, etc.) In this example, sample B is set to sample A, and sample D is set to sample C. The procedure is repeated from sample C up to the end of the track.



Notes

- Calculation of the dependent variables is not affected by what Minimal Distance Moved option you choose.

12.2 Selecting data

There are several good reasons why you may want to select data before carrying out analysis (that is, creating track plots or calculating statistics):

Analyze some tracks, not others.

Filter tracks manually or according to values of independent variables, then run analysis.

Example 1 – Remove Track 12 from analysis, because the test was disturbed and the data were declared invalid.

Example 2 – Calculate distance moved by the animal for all tracks of female subjects, treated with dose higher than 0.01 mg/l.

Example 3 – In an experiment with multiple cages, you want to calculate locomotor variables for the tracks obtained in the cages with enriched environment.

➔ See page 393

Selecting Data for Analysis

Analyze segments of tracks corresponding to a time interval or the behavior of the subjects

Nest data over Time, Zones or behavioral states, then run analysis. See page 400.

Example 1 – Visualize the first 5 minutes of the track.

→ See **Nesting over Time**, page 404

Example 2 – Calculate distance moved and visualize heart rate data when the subject was in each of the arms of a plus maze.

→ See **Nesting over zones**, page 406

Example 3 – Visualize all samples when the animal was moving.

→ See **Nesting over behavioral states**, page 410



- If a track is divided in more segments, these are still considered as one unit (therefore, one result per track is shown). If you want to analyze separate intervals within a track, see the next option.
- If you track multiple animals per arena, you can also analyze the track segments of one subject, defined by another subject.

Example – Analyze Subject 1 when Subject 2 was in Zone A.

To do so, choose one of the options under **Nesting over Subjects**, and specify the subjects (named **Actors**) that should be in a zone, or in a particular state.

In the example above, choose **In zone** under **Nesting over Subjects**, and specify that the Actor **Subject 2** must be in **Zone A**.

→ See also **Selecting subjects in nesting intervals**, page 425

Analyze segments of tracks corresponding to Trial Control states

Nest data over Trial Control states. ATC states are time intervals defined by events of trial control.

Example – Calculate the time from when the cue light switched on to when the food item was dropped.

→ See **Nesting over Trial Control states**, page 424

Analyze segments of tracks corresponding to regular time intervals

Define **Results per time bin**, then run analysis.

Example 1 – Visualize the tracks split in 10-minutes intervals.

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Example 2 – Calculate statistics of distance moved in each of the 30-minute intervals.

→ See **Results per time bin**, page 430

Analyze segments of tracks corresponding to different zones

Define **Results per zone**, then run analysis.

Example – Calculate statistics of distance moved in each of the zones.

→ See **Results per zone**, page 430

General notes



- If you select data, the data filtered out are not deleted from your PC.
- Data selection is optional. If you do not select data, the default data selection profile is used, which contains all data in your experiment.
- You can always create a combination of Filtering and Nesting criteria in your Data Selection. For example, **Filter** the tracks with female subjects, and **Nest over** the zone "Center of Open field".
- You cannot group tracks in the current version of EthoVision XT.

12.3 The Data Selection screen

To select your data, you first have to create a **Data Profile**. A Data profile is a collection of settings that refer to how you select your data.

1 Do one of the following:

- In the Experiment Explorer, in the Analysis folder, right-click **Data Profiles**, select **New**, type in a name and press <Enter>.
- From the **Analysis** menu, choose **Data Profile**, select **New**, type in a name and click **OK**.

2 The Data Selection screen appears.

Selecting Data for Analysis

The Data Selection screen contains two main objects:

- The **Components** pane, listing a number of variables and selection criteria, each provided with a button.
- The **Data Selection** window, showing the current content of the Data profile. It contains a sequence of boxes connected by arrows.

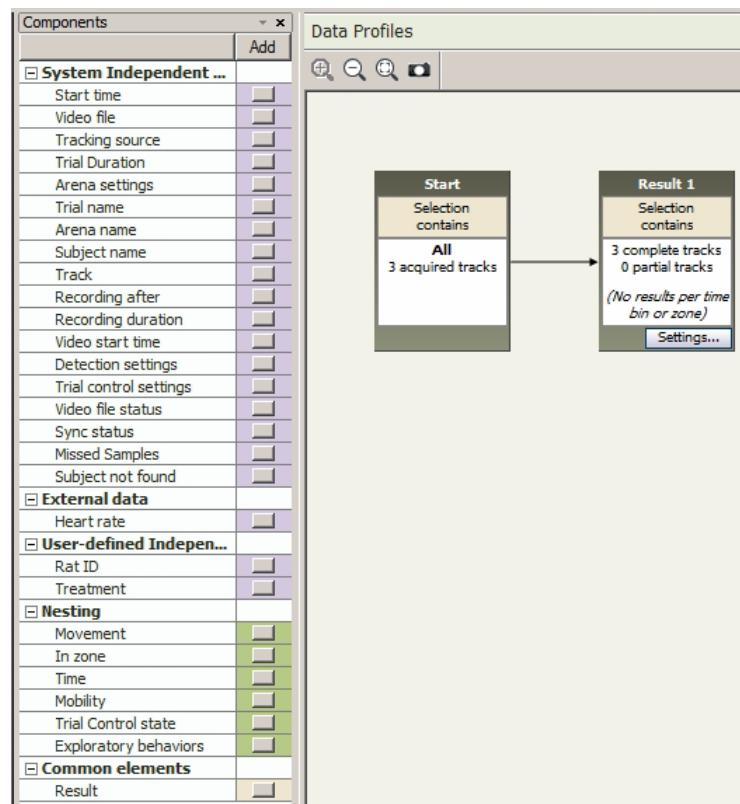


Figure 12.2. The Data Selection screen, with the **Components** pane (left) and the **Data Selection** window (right).

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Notes



- You can create as many Data profiles as you want, containing different criteria for data selection.
- You can also open an existing Data profile: click **Open** in the **Data Profiles** window, and choose the Data profile from the list (for more information on managing Data profiles, see page 581).

The Components pane

The **Components** pane lists the following:

- **System independent variables** – To filter tracks according to the values of system variables. See page 394.
- **User-defined independent variables** – To filter tracks according to the values of the variables you have defined in the experiment. See page 394.
- **External data** (only if external data have been imported to the experiment) – To filter the tracks according to the physiological data files associated with them. See page 395.
- **Nesting** – To select segments of tracks of each subject corresponding to time intervals or states of that subject. See page 403.
- **Nesting over Subjects** (available only when two or more animals are tracked simultaneously in the arena) – To select segments of tracks of each subject corresponding to time intervals or states of another subject or combination of subjects. See page 402 and page 425.
- **Common elements** – Contains the **Result** button to create multiple data selections in the same screen. See page 438.

Notes



- The independent variable list includes all system and user-defined variables no matter whether they are shown in the **Trial List**.
- If you do not see the Components pane, click the **Show/Hide** button in the component tool bar and select **Components**.

Selecting Data for Analysis

How to use the Components pane

To select data, do one of the following:

- Double-click the criterion of data selection.
- Click the button next to it.
- Drag the name from the **Components** pane to the **Data Selection** window.

A new selection box appears in the top-left corner of the **Data Selection** window. Insert the new selection box in the sequence (see below).

The Data Selection window

Default selection

When you create a new Data profile (see page 581), the **Data Selection** window contains two selection boxes (see Figure 12.3.):

- The **Start** box (left) containing all tracks currently stored in the experiment, and
- The **Result 1** box (right), containing the data used for analysis. The two boxes are connected through an arrow. This means that all data in the **Start** box are used for analysis.

This is the basic **selection sequence**. It is now up to you to add selection boxes to refine your selection.

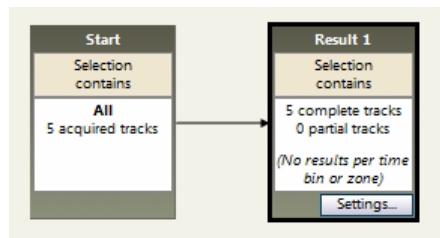


Figure 12.3. The **Start** box (left) and the **Result 1** box (right). In this example, the **Start** box contains 5 tracks.

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Creating your selection

By default, data from all trials are selected. To refine your selection and focus on a smaller data set, insert a sequence of selection boxes between the **Start** box and the **Result 1** box.

- 1 In the **Components** pane, click the button next to the variable you want to use (see 1 in Figure 12.4.).
- 2 A new box appears in the top-left corner of the Data selection window. top (see 2 in Figure 12.4.). A new window appears on top, listing all possible values or characteristics of the chosen variable. For example, which zone you want to consider for analysis.
- 3 Choose the values that specify your selection (see 3 in Figure 12.4.) and click **OK**. Drag the box over the arrow that connects the two pre-existing boxes. When the arrow turns white, release the mouse button.

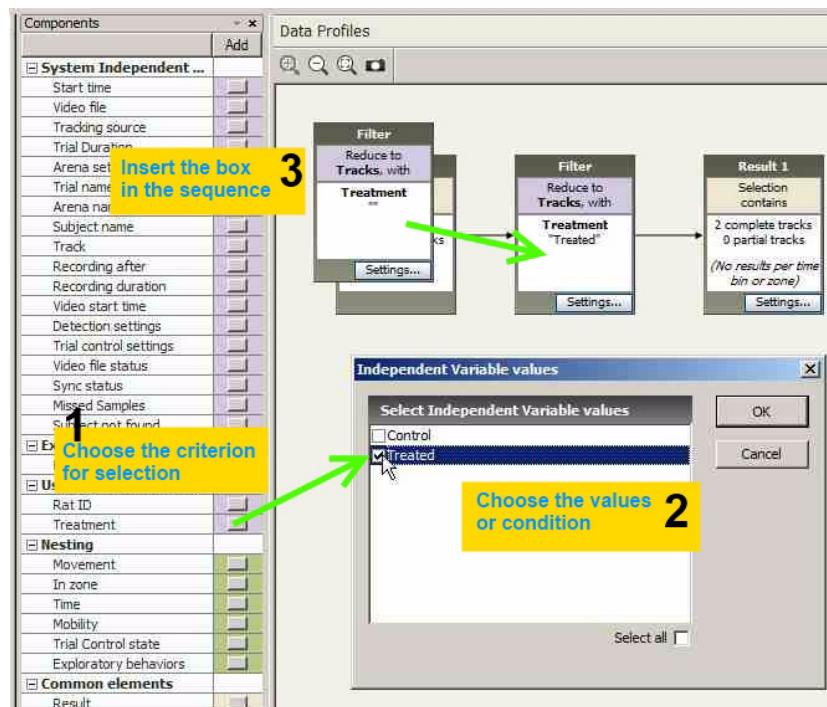


Figure 12.4. Visualization of the basic steps you must follow to select data. See the text for explanations.

Selecting Data for Analysis

Creating complex selections

You can define complex data selections by inserting two or more selection boxes in the sequence.

- *Example 1* – You want to analyze data for the subjects which received 0.01 mg/l of drug, (where **0.01** is one of the values of the User defined variable **Dose**), and tested 3 days after treatment (where **3** is one of the values of the variable **Days after treatment**).
Solution – Insert two **Filter** boxes: one for the variable **Dose**, one for the variable **Days after treatment**. Select the appropriate values in each box.
- *Example 2* – You want to analyze data for the subjects with ID number A21088, A21089 and A21093 (provided that **ID number** was entered as user-defined variable), when they entered the **closed arms** of the plus maze (provided that the closed arms were defined as zones).
Solution – Insert the following: one **Nest** box for the **In zone** criterion, and one **Filter** box for the variable **ID number**.



The order in which you place Filter boxes does matter. See the note on page 439.

Creating different selections in the same Data Selection window

By clicking the button next to **Result** (last button in the **Components** pane), you get a new **Result** box.

Additional **Result** boxes allow you to create different data selections in the same Data profile. This way, you can visualize and compare the effect of different data selections without the need to run analysis two or more times. For more information, see page 438.

Grid

The data selection boxes automatically snap to a grid. You can change this by clicking the **Show/Hide** button on the component tool bar and selecting/deselecting the two Grid options (**Snap to Grid** and **Show Grid**).

Zoom

The component tool bar of the Data Selection window shows three zoom icons:

- **Zoom in** – You can keep zooming in until all trial control boxes fit in the window.

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- **Zoom out** 

- **Zoom to fit**  – Clicking this button fits all trial control boxes into the window.



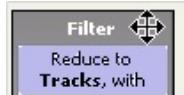
The Data Selection window is ‘dynamic’: this means that it expands when you move trial control boxes to the right. In this case, you can navigate ‘from left to right’ in the Data Selection window by using the scrollbar at the bottom. use the **Zoom to fit** button in the component tool bar to make all trial control boxes visible.

Working with selection boxes

This section contains instructions for the basic operations with selection boxes. For the actual selection procedure, see page 393 and page 400.

Moving a selection box

- 1 Click the margin of the box. The mouse cursor changes to a four-headed arrow.



- 2 Drag the box to the position you require.

Moving a group of selection boxes

- 1 Draw a box around the boxes you want to move or click on the boxes you want to select while holding the **Ctrl** key.

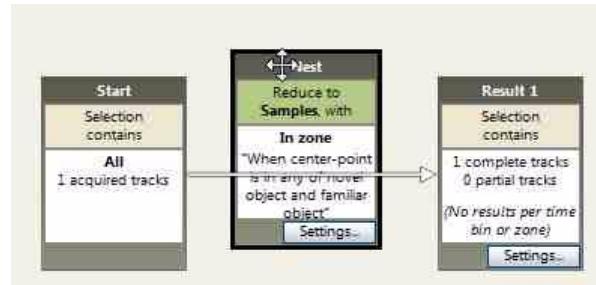
As a result, the border of the selected boxes becomes darker.

- 2 Hover the mouse on the margin or the colored area of one of the selected boxes. The mouse cursor changes to a four-headed arrow.
- 3 Drag the group of boxes to the position you require.

Selecting Data for Analysis

Inserting a box in a data selection sequence

- 1 Drag the selection box between two boxes, until the connecting arrow turns white.



- 2 Release the mouse button. The new box is inserted.

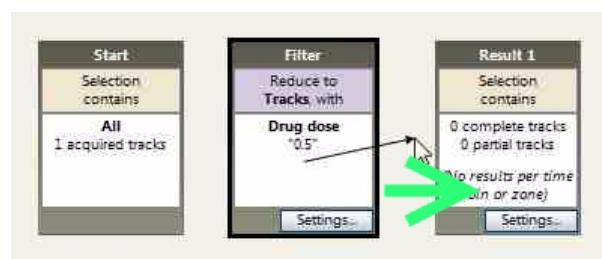
Connecting two selection boxes

- 1 Point the mouse to the center of the first box, press and hold the left mouse button and drag toward the center of the other box.
- 2 Release the mouse button when the pointer has reached the center of the other box. The two boxes are connected.



You cannot create connections:

- From the **Result** box to any other box.
- From any box to the **Start** box.



Changing the selection criteria in a selection box

Follow the instructions below when you have inserted a selection box, and you

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want to adjust the settings to restrict/widen your selection. For example, when you have selected drug dose = 0.01 and 0.05 in the first instance, and you want to remove 0.05.

- 1 Locate the selection box that specifies the criterion you want to change.
- 2 Click the **Settings** button.
- 3 Select the appropriate values in the window that appears.

Deleting a selection box

- 1 Click the title of the box so the mouse pointer changes to a four-headed arrow.
- 2 Press **Delete**.

Deleting a group of selection boxes

- 1 Draw a box around the boxes you want to delete or click on the boxes you want to select while holding the **Ctrl** key.
- 2 Press **Delete**.



You cannot delete the **Start** box. You can delete a **Result** box only if another one has been inserted in the **Data Selection** window.

If you delete a box within a sequence, the arrows connecting the adjacent boxes are lost. Therefore, you must re-connect the adjacent boxes (see above).

Deleting a connecting arrow

- 1 Click the connecting arrow you want to delete. The arrows turns bold to show it is selected.
- 2 Press **Delete**. The arrow is deleted.

Creating a new Result box

When do I need to create a new Result box? – When you want to display analysis results from independent data selection criteria at the same time. For example, to calculate statistics for when the subject was in two areas of the open field, and you want to display results for the two areas separately. (See page 438).

Selecting Data for Analysis

-  1 In the **Components** pane, under Common elements, click the button next to **Result**.

The new **Result** box appears in the top-left corner of the **Data Selection** window. Connect the selection box to other boxes.

- 2 Drag the new **Result** box to the right-hand side of the **Data Selection** window, below the first **Result** box.
- 3 Complete the selection sequence by making a branching so that each data selection criterion ends up in its own **Result** box.

For more information on complex data selections, see page 433.

Customizing the Result box name

The **Result** boxes are named automatically as you create them: **Result 1**, **Result 2**, etc.

The **Result** box name is shown in the Analysis results (statistics and track plots), so you always know from which data selection the results come from. If you want to change that name, click the button in the bottom-right corner of the box, and enter the new name in the **Name** field.

12.4 Selecting your data - Filtering

What is Filtering?

Filtering is the process of picking out entire tracks (single or multiple) according to the value of variables (System or User-defined). Filtered tracks and the corresponding video and external (physiological) data are subject to analysis.

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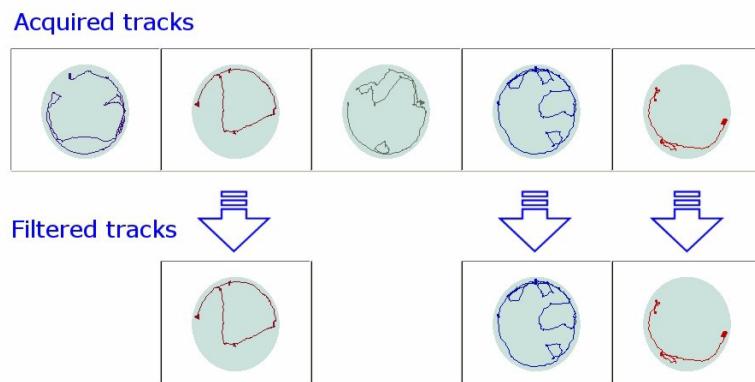


Figure 12.5. By filtering data, you choose a subset of tracks from the data set in your experiment.



In the current version of EthoVision XT you cannot group tracks. If you want to analyze segments of tracks, use the Nest function (see page 400). You can also combine Filter and Nest boxes to create complex selections (see page 433).

See the general procedure on page 395.

Filtering by System Variables

Aim – Analyze the tracks that are associated with specific values of system variables.

Example 1 – Analyze the set of Trials from Trial 1 to Trial 20. To do so, filter by the system variable Trial name.

Example 2 – Analyze data acquired on a certain date and time. To do so, filter by the system variable Start time.

Filtering by User-defined Variables

Aim – Analyze the tracks corresponding to specific values of user-defined variable.

Example – You want to analyze all subjects tested with apomorphine. To do so, apomorphine must be one of the values of a user-defined variable. Next, filter by this variable.

Selecting Data for Analysis

Filtering by External (physiological) data

Aim – Analyze the tracks linked to the specified physiological data files.

Example – You have acquired tracks for 10 subjects, but co-acquired EEG data for eight of them. You want to visualize those eight data sets. Filter the tracks by the EEG variable.

Filtering procedure in short

1 Make sure that the Data Profile in which you want to insert the filtering criterion is open. If this is not the case, create a new data profile or open an existing one (see page 581).

2 Locate the variable in the **Components** pane that you want to use for filtering data, and click the corresponding button.



If you do not see the single components, click the + sign next to System Variables and User defined Variables.

See the next section for details on the independent variables available for filtering.

A **Filter** box appears in the top-left corner of the **Data Selection** window. The **Independent Variable values** window opens on top.

3 Select the values of the variable for the tracks you want to analyze, and click **OK**.

4 Drag the **Filter** box to the desired position in the selection sequence between the **Start** and the **Result** box (see page 391 for how to do so).

5 Repeat steps 2 to 5 to add more **Filter** boxes and refine your selection (see also page 433).

Notes



- **Scope of the variable** – Within a single trial, you can select different data sets depending on the **Scope** of the variable used (**Trial**, **Arena** or **Subject**; see page 113). Depending on your setup, a trial may consist of one or more arenas. On its turn, each arena can include one or more subjects.
 - By filtering by a variable with **Trial** scope, you analyze all data for the trials that you specify, no matter how many subjects and arenas those trials contain.

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- By filtering by a variable with **Arena** scope, you analyze all subjects recorded in the arenas that you specify, and you ignore the data recorded in other arenas in the same trial.
- By filtering by a variable with **Subject** Scope, you analyze the tracks referring to the subjects that you specify, and ignore the tracks of other subjects recorded in the same arena and trial.
- If the **Independent variable values** window does not list any value available for selection, it means that no values have been assigned to any trial in the Trial List.
- You can combine different filtering criteria to create complex data selections. To do so, insert multiple **Filter** boxes and combine them as described on page 433.
- You cannot filter tracks according specific values of external (physiological) data. For example, filter all tracks for which average heart rate was higher than 600 bpm.
- If you want to edit an existing Filter box, click the **Settings** button of that box.

Variables available for filtering

We assume at this point that you have followed steps 1 to 3 in the procedure above. As a result, a **Filter Variables** window appears on top, listing the values of the variable chosen available for filtering (see below). Select the values you want to use as filter.

System Variables

- **Start time** – Filters the tracks belonging to the trials started at the times you select from the list.



Tracks acquired from video files have the time of creation of the video files as their start date/time.

Tracks that belong to the same trial have equal **Start Time** (that is, the time you started the Trial). If you have applied Trial Control with **Start** conditions, actual data recording may have started at different times in each track (see Chapter 7). Therefore, if you want to select individual tracks according to when actual data recording started, filter tracks by the variable **Recording after** (see below).

- **Video file** – Filters the tracks recorded from the video files you select from the list.

Selecting Data for Analysis

- **Tracking source** – Shows the camera source if you track live. Since EthoVision allows one camera source per experiment, this variable will show only one camera.
- **Trial Duration** – Filters the tracks belonging to the trials of the duration you select from the list.



Tracks that belong to the same trial have equal **Trial Duration** (that is, the difference between the Stop time and the Start time of the trial). If you have applied Trial Control with **Start** or **Stop** conditions, the duration of actual data recording may be different for each track (see Chapter 7). Therefore, if you want to select individual tracks according to the duration of actual data recording, filter tracks by the variable **Recording Duration** (see below).

- **Arena settings** – Filters the tracks recorded using the Arena Settings profile you select from the list.
- **Trial name** – Filters the tracks belonging to the trials you select from the list.



Tracks recorded in the same trial have the same **Trial name**. If you want to select individual tracks, filter by the variable **Arena name** or **Track** (see below).

- **Arena name** – Filters the tracks recorded in the arenas you select from the list.



This option is handy if you have a multiple arena setup, and you want to analyze data from some arenas, not others.

- **Track** – Filters the tracks you select from the list.



Each track name corresponds to data of one individual subject recorded in one arena and in one session (trial). If you want to select data according to the recording sessions (which, in the case of multiple arena setups, may contain multiple tracks), filter by **Trial name** (see above).

- **Recording after** – Filters the tracks that started a specific time after the start of the Trial. Select this time from the list.



This option is handy if you apply Trial Control with **Start** conditions (see Chapter 7). If this is the case, actual data recording for each track may have started later than the trial's start time. You can filter tracks started a certain time after the start of the Trial they belong to. For example, in a multiple arena setup, Trial 1 started at 10:23:00, but actual data recording started after 5 seconds in Arena 1 and after 30 seconds in Arena 2. You can select those time lags from the list to filter specific tracks.

If you do not use Trial Control with **Start** conditions, data recording starts at the start of the Trial. In this case, the **Filter Variables** window for **Recording after** shows only the **0** value.

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- **Recording duration** – Filters the tracks recorded for the time you select from the list.



This option is handy if you apply Trial Control with **Start** and **Stop** conditions (see Chapter 7). If this is the case, actual data recording may be shorter than the trial duration. You can filter tracks that have a certain duration, independent of the trial's duration. For example, in a two-area setup, a trial lasted 15 minutes. In Arena 1, actual data recording lasted 15 minutes while in Arena 2 12 minutes because a **Stop** condition was met for that arena before the trial stopped. You can select those duration values from the list to filter specific tracks.

If you do not use Trial Control, actual data recording lasts as long as the Trial, so **Recording duration** is the same as trial **Duration** (see above). In this case, the **Filter Variables** window for **Recording Duration** shows the same values as that for **Duration**.

- **Video start time** – Filters the tracks recorded from the video file created (or last saved) on the date and time you select from the list.
- **Detection settings** – Filters the tracks recorded using the Detection Settings profiles you select from the list.



The **Detection profile** variable has **Trial Scope**; this means that within a trial, all tracks have the same **Detection Profile**. If you want to know which **Detection Settings** were used for a specific trial, see page 581.

- **Trial control settings** – Filters the tracks recorded using the Trial Control profile you select from the list.



If you want to know which Trial Control profile was used in a certain trial, make sure the System Variable **Trial control settings** is selected for display in the Trial List (page 113).

- **Video file status** – Filters the tracks corresponding to the status of the video file you select from the list.

External – Filters the tracks recorded from media files obtained with systems other than the Picolo Diligent MPEG-4 or the Picolo U4 H.264 encoding board.

Skipped – Filters the tracks for which no video file is available. It is the trials acquired live with the **Save video** option not selected.

Generated – Filters the tracks for which an MPEG-4 or H.264 video file is available. It is the trials acquired live with the **Save video** option selected.



The last two options are available if you acquire tracks live with a Picolo Diligent MPEG-4 or a Picolo U4 H.264 encoding board.

Selecting Data for Analysis

- **Sync status** - Filters the tracks corresponding to the status of the synchronization between track and co-acquired external data.
 - Planned** - Filters the tracks for which no external data co-acquisition has been carried out.
 - Acquired** - Filters the tracks for which co-acquisition was carried out.
- **Missed Samples** - Filters the tracks corresponding to the proportion of missed samples caused by, for example, a too high processor load.
- **Subject not found** - Filters the tracks corresponding to the proportion of samples which were processed by EthoVision but in which no subject was detected.

User-defined Variables

This list depends on the variables that have been defined in your experiment (see page 103). Click the button next to the variable you want to use as a criterion for filtering data.

<input type="checkbox"/> User-defined independent variables
<input type="checkbox"/> Treatment level
<input type="checkbox"/> Drug dose
<input type="checkbox"/> Seizure occurred



See also the note **Scope of the variable** on page 395.

External data

If you import external data into one or more trials (see Chapter 9), the **Components** pane shows each type of physiological variable under **Stream Variables**. Click the button next to the variable you want to use to filter data.

<input type="checkbox"/> External data
<input type="checkbox"/> Temperature
<input type="checkbox"/> EEG



In the window that appears on top, select the imported data files you want analyze. Tracks linked with those data files are selected for analysis.

If you filter by External data, the data being subject to analysis also includes the tracks and the video files associated.



What track data are selected with external data files depends on how you linked the external data files to the track data during import (whether at the **Trial**, **Arena** or **Subject** level; see page 319). For example, if ECG data files are linked at the Trial level, entire trials are filtered. If ECG data files

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are linked to Subjects, only the tracks for the subjects linked to those ECG files are filtered.

12.5 Selecting your data - Nesting



If you open an EthoVision XT 7 experiment in EthoVision XT 8 and the EthoVision XT 7 experiment contains a Nesting by Minimum Distance Moved box, this box is automatically removed from the data selection. Make sure you add Minimal Distance Moved to a Track Smoothing profile (see page 380 how to do this).

What is Nesting?

Nesting is the process of picking out portions of tracks specified by time intervals or states of the subject. Track portions and the corresponding video and external (physiological) data are subject to analysis (see Figure 12.6.).

See the general procedure on page 403.

Nesting over time

Aim – Analyze a time interval defined by a Start time and a Stop time.

Example – Analyze the first 15 minutes or each trial.

→ See page 404

Nesting over zones

Aim – Analyze the track segments when one or more subjects is within a zone.

Example 1 – Analyze the samples when the animals were in the closed arms of the plus maze.

Example 2 – Analyze the samples when the animals were in each of the quadrants of the water maze.

→ See page 406

Selecting Data for Analysis

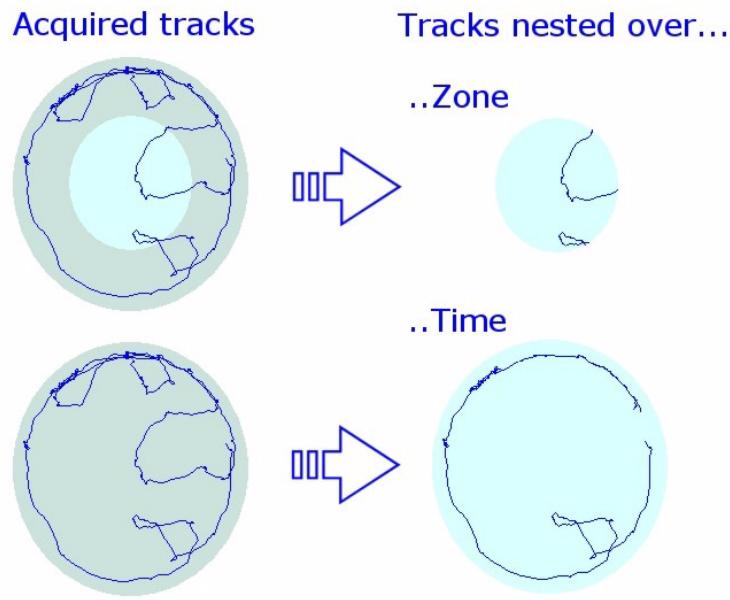


Figure 12.6. Nesting over data means choosing to analyze segments of tracks. Top: A track is nested over a zone – only the samples collected in the center of the open field are analyzed. Bottom: the same track is nested over time – only the samples collected in the first 60 seconds are analyzed. You can also nest over behavioral states of the subject, for example to analyze the samples collected when the animal was moving.

Nesting over behavioral states

Aim – Analyze the track segments corresponding to state of one or more subjects (for example, **Movement**).

Example 1 – Analyze all the samples collected when the animal was moving faster than a certain speed (use the Movement dependent variable).

Example 2 – Analyze all the samples collected when the animal's body was stretched above a certain threshold (use the Elongation dependent variable).

➔ See page 410

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Nesting over Trial Control states

Aim – Analyze the track segments corresponding to the time between two events of Trial Control.

Example – Analyze the time from when the cue light switched on to when the mouse consumed the food item delivered.

→ See page 424

Nesting over subjects

If your arena contains multiple subjects, like in a social interaction test, you can choose to select track segments that correspond to the behavior of that subject (Nesting), or of another subject, or all/any subjects in the arena (Nesting over Subjects).

Example of Nesting – Each trial consists of 3 tracks, one for each interacting animal. The researcher wants to analyze the track segments of each subject when it was Moving.

Example of Nesting over Subjects – In the setup described above, the researcher wants to analyze the track segments of Subject 1 when Subject 2 was In proximity.

You can do this by selecting the option under **Nesting over Subjects**.

→ See page 425

If you want to analyze entire tracks, use the Filter function (see page 393).



You can also combine Filter and Nest boxes to create complex selections (see page 433).

Nesting over Subjects is not possible when each subject is in a separate arena.

Nesting over manually scored data

If you have manually scored behaviors, you can choose to select track segments when a behavior was active.

Example - You manually scored the behavior 'sniffing' of an animal in an open field. You want to analyze the velocity of the animal while it was sniffing.

→ See page 429

Nesting procedure in short

- 1 Make sure that the Data Profile in which you want to insert the nesting criterion is open. If this is not the case, create a new data profile or open an existing one (see page 581).
- 2 If you have tracked one subject per arena, locate the **Nesting** component and go to step 3.

If you have tracked two or more subjects per arena, you have two categories of nesting options:

- **Nesting** – Choose this category if you want to analyze the track segments that correspond to the behavior of the subject of that track. This corresponds to Nesting in EthoVision XT 4 and 5.
 - **Nesting over Subjects** – Choose this category if you want to analyze the track segments that correspond to the behavior of subjects other than the subject of that track, or a combination of subjects.
- 3 Locate the variables under **Nesting** or **Nesting over Subjects** in the **Components** pane that you want to use to select track segments, and click the corresponding button.
 - **Time** – To analyze a specific time interval (see page 404).
 - **In Zone** – To analyze the time a subject was in a specific zone or combination of zones (see page 406).
 - Behavioral states (**Movement**, **Elongation**, **Mobility**, **Head directed to zone**, **Proximity**, and **Relative Movement**) – To analyze the time the subject was in a certain state. For example, the state "Moving" for the variable **Movement**. See page 410).
 - **Trial Control state** – To analyze the interval defined by two Trial Control events.
 - **Manually scored behavior** – To analyze the samples in which the subject performed a manually scored behavior.
-  If you have chosen an option under **Nesting over Subjects**, the subjects that define the time interval are other than the subject considered in each track.
- A **Nest** box appears in the top-left corner of the **Data Selection** window. A window opens on top.
- 4 Select the values of the dependent variable that define the track segments you want to analyze, and click **OK**. See the next sections for details.

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- 5 Drag the **Nest** box to the desired position between the **Start** and the **Result** box (see page 391 for how to do so).
- 6 Repeat steps 2 to 5 to add more **Nest** boxes and refine your selection (see also page 433).



Selecting **Time** under **Nesting** or **Nesting over subjects** makes no difference, because within one arena tracks of multiple subjects start at the same time.

If you want to edit an existing Nest box, click the **Settings** button on that box.

Nesting over time

Aim

Pick out the data collected in a specific time interval.

Procedure

We assume at this point that you have followed steps 1 to 3 in the procedure above, and you have clicked the button next to **Time** in step 2. As a result, the **Time** window appears on top.

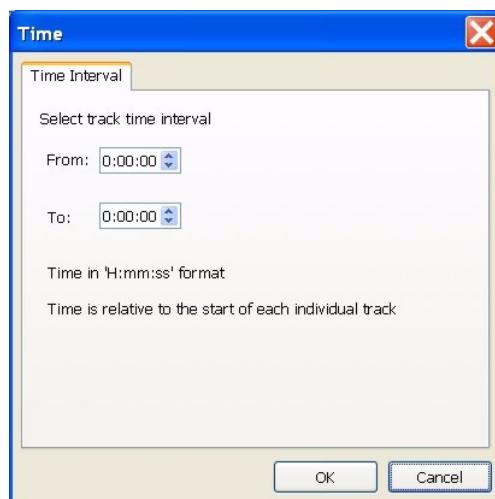


Figure 12.7. The **Time** window.

Selecting Data for Analysis

- 1 In the **From** field, enter the start time of the interval to analyze.
- 2 In the **To** field, enter the end time of the interval to analyze.
- 3 Click **OK**. Insert the **Nest** box in the appropriate position between the **Start** and the **Result** box.



For information on how to connect selection boxes, see page 391. To create multiple selections, see page 433.

Notes



- **From** is applied in each track independently (see the notes below). By default, 0:00:00.0 (h:mm:ss.d) is selected. The 0 time is the time that recording started for that specific subject, no matter if it started later in the trial (this can occur when you apply Trial Control with **Start** conditions).
Example – When selecting 0:00:10.0, EthoVision XT analyzes all samples collected from 10 seconds on in each track file.
- **To** is applied in each track independently (see the notes below). It always refers to the time that recording stopped, no matter of whether it stopped before the end of the trial (this can occur when you apply Trial Control with **Stop** conditions).

Example – By selecting 0:02:00.0, EthoVision XT analyzes all samples collected up to 2 minutes in each track file.

- If you want to analyze data in multiple time intervals (for example, analyze a one-hour recording in six 10-minutes intervals), do not nest over time; rather, define **Results per time interval** (see page 430).
- **Multiple arena setups** – if you apply Trial Control with **Start** and **Stop** conditions for each arena separately, actual data recording may start and stop at different times in each arena. Since the time stamp in each track file correspond to the time since the start of the actual recording in that arena, a sample collected at 10.0 in Arena 1 and a sample collected at 10.0 in Track 2 do not necessarily correspond to the same 'real' time. Therefore, when you consider tracks recorded in a multiple arena setup, analysis is likely to be done on different 'real time' intervals for each arena (see Figure 12.8.).

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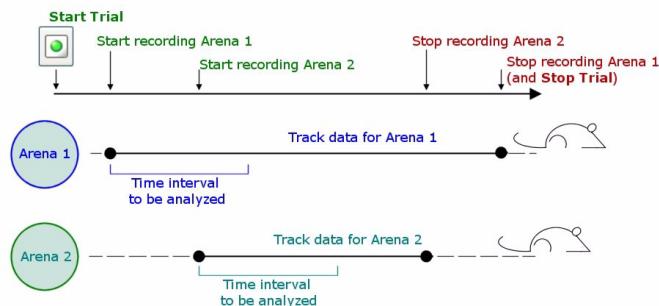


Figure 12.8. An example of analyzing time intervals in a multiple arena setup. If a Start condition is defined in Trial Control, actual data recording can start independently in each arena. This results in tracks having different duration (see the thick time lines next to the arenas). Nesting is used to analyze the first minute of each track (From 0:00:00.0 to 0:01:00.0). The From and To times are applied to each arena independently (see the horizontal bars).

- **Multiple subject setups** – Within an arena, tracking starts at the same time for all subjects. This means that a sample collected at 10.0 for Subject 1 and Subject 2 corresponds to the same 'real' time. Therefore, choosing **Time** under **Nesting** and **Nesting over subjects** results in the same interval being selected for all the subjects.

Nesting over zones

Aim

Pick out the data collected when the subject was in a specific zone (or in any of a group of zones).

Procedure

We assume at this point that you have followed steps 1 to 3 in the procedure on page 403, and you have clicked the button next to **In Zone** in step 2. As a result, the In Zone window appears (see Figure 12.9. on page 408):

Selecting Data for Analysis

- 1 Under **For the following body points**, select the body points of the animal that determine whether or not the animal is considered to be within a zone ('In zone' vs. 'Not in zone').



If your experiment is set to **Only center-point detection**, the **From following body points** options are not available. Calculations are based on the center-point of the body.

- 2 If you specify two or three body points, a list becomes available. Choose one of the following:

- **Any selected point** – To select the samples when at least one of the chosen body points was within the zone.
- **All selected points** – To select the samples when all the chosen body points were simultaneously within the zones.



Select the three points and **All selected points** to analyze the samples when the whole body was in a zone.

- 3 Under **In the following zones**, select the zones in which the animal's body points must be.

- 4 From the drop-down list, choose one of the following:

- **When in any of the selected zones** – To select the samples when the body points were in any of the chosen zones.
- **When in all selected zones** – To select the samples when the body points were in all the chosen zones simultaneously.
- **When not in any of the zones** – To select the samples when the animal was anywhere but the chosen zones.



Select **When in all selected zones** when the zones are overlapping at least partially (for example, North Quadrant and Center of an open field). If the zones are not overlapping, and you have chosen When all points are in zone in step 2, the second option results in no data being selected as a body point can never be in two different locations at the same time.

- 5 If you have chosen In zone under **Nesting over Subjects**, the **Actors** tab is also available. Select the subjects that, when in the zones selected above, define the nesting intervals. If you select two or more subjects, select (see also page 425):

- **Any selected subject** – To analyze the time that at least one subject was (or was not) in the zones.
- **All selected subjects** – To analyze the time that all subjects were (or were not) in the zones simultaneously.

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- 6 Click **OK**. Insert the Nest box in the appropriate position between the **Start** and the **Result** box.

For information on how to connect selection boxes, see page 391. To create multiple selections, see page 433.

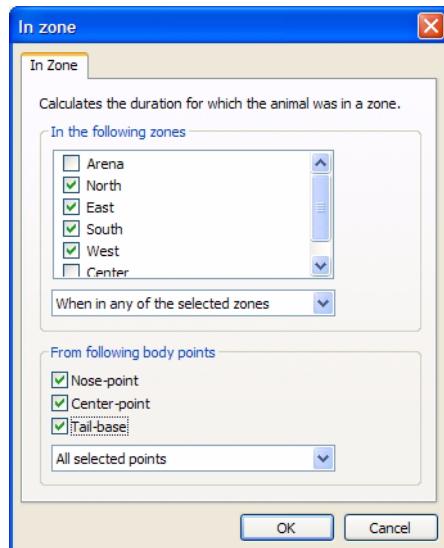


Figure 12.9. The **In Zone** window.



Notes

- Nesting over zones is not the same as selecting the **In zone** depending variable for calculating statistics (see Chapter 14).
 - **Nesting over zones** means that you analyze segments of tracks when the subject is in specific zones.
 - Selecting **In zone** in the Analysis profile means that you calculate the time or the number of times the subject has entered specific zones.
- If you want to calculate distance moved for each zone separately, create a separate **Result** box for each Nest box that specifies a zone.
- The **In the following zones** group of options lists the zones defined in all your Arena Settings.

Selecting Data for Analysis

- Notice the difference:
 - If you select In zone under **Nesting**, data are selected based on the presence of the subject of the current track in the zone. Therefore, different subjects are analyzed in different time intervals (see **B** in Figure 12.18.).
 - If you select In zone under **Nesting over Subjects**, data are selected on the basis of the presence of the subjects specified in the **Actors** tab in the zone (see also **C, D** in Figure 12.18.).

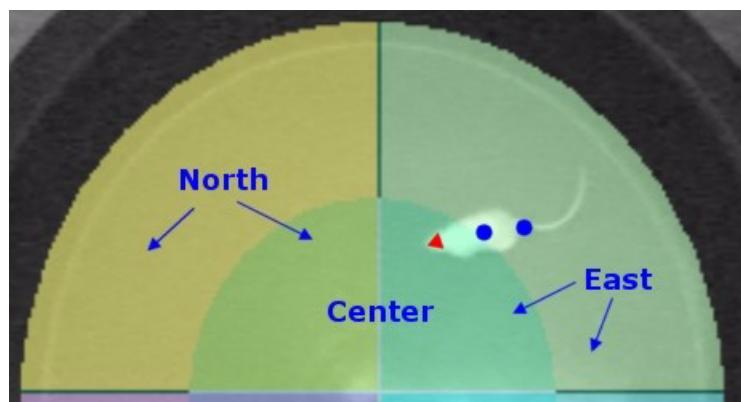
Example 1

An open field has been divided into two zones. If you select Nose-point and Center-point and If any point is in zone, and then you choose **Zone 1** under **In the following zones**, the sample shown in the picture below is selected for analysis. However, if you select When all the points are in zone, the sample is not analyzed, since only the nose point is within Zone 1.



Example 2

An open field has been divided into four quadrants (North, South, East, West) and an overlapping zone named Center.



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If you select:

- Under **From following body points**, **Nose-point** and
- Under **In the following zones**, **North** and **Center**, and from the list you select **When in all the selected zones**;

it means that you consider the samples when the animal was in the area shared by Center and North. Then the sample shown in the picture above is not considered for analysis, because the animal's nose is outside the area where North and Center overlap. However, if you select **When in any of the selected zones** under **In the following zones**, the sample is selected for analysis.

Nesting over behavioral states

Aim

Pick out the data collected when the subject was in one of the behavioral states:

- For **Movement** – States **Moving** or **Not Moving**.
- For **Elongation** – **Stretched**, **Normal** or **Contracted**.
- For **Mobility** – **Mobile**, **Highly mobile** or **Immobile**.
- For **Head directed to zone** – **True** or **False**.
- For **Proximity** – **In proximity** or **Not in proximity**.
- For **Relative movement** – **Moving to**, **Moving from**, **No relative movement** or **No interaction**.



Nesting over behavioral states is not the same as selecting those states for calculating statistics (see Chapter 14).

- **Nesting over dependent variables** in your Data profile means that you analyze segments of tracks when the subject is in a specific state (moving, stretched, etc). However, you can calculate any dependent variable on those segments. For example, calculate the distance moved when the subject was moving.
- **Calculating the dependent variables** in your Analysis profile means that you calculate statistics for those variables. For example, calculate the time the subject was moving.

Selecting Data for Analysis

Procedure

We assume at this point that you have followed steps 1 to 3 in the procedure on page 403, and you have clicked the button next to one of the behavioral states in step 2. As a result, the window for the chosen state appears.

Nesting over Movement

Movement is the state variable that determines whether a subject is moving or not moving by comparing the subject's current velocity with two thresholds you specify (see page 526).

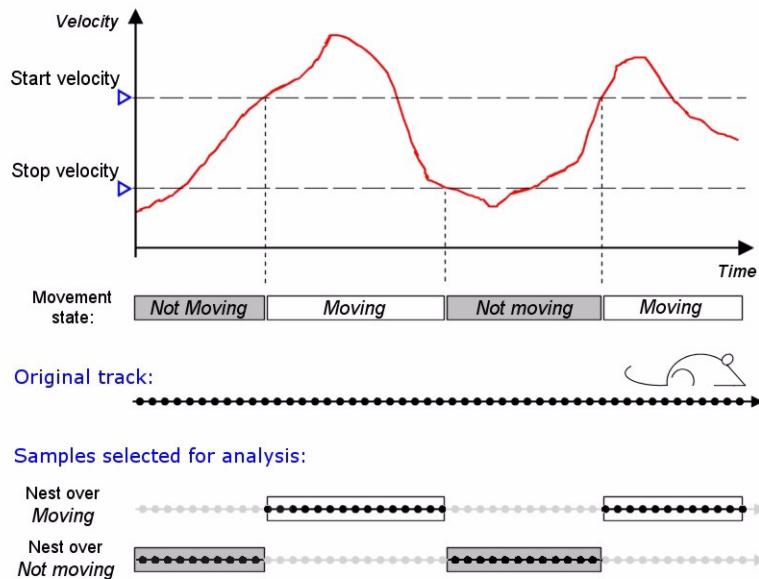


Figure 12.10. An example of nesting over Movement. The Movement dependent variable, with the two possible discrete states Moving and Not Moving, is calculated for each sample according to the running average speed. Samples are considered for analysis which are assigned the Moving or the Not moving state. Depending on your choice, different segments of the track are selected.

1 On the **Movement** tab, select the following:

- Under **Filter**, enter the **Averaging Interval** (Range 1 - 1000) – This is the number of samples across which changes in speed are calculated to

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determine whether the subject is moving or not. In order to reduce the sensitivity of the **Movement** variable to brief changes in velocity, the velocity data can be smoothed by taking the running average of the last n samples. Enter the averaging interval n or leave 1 if you do not want to smooth velocity data.

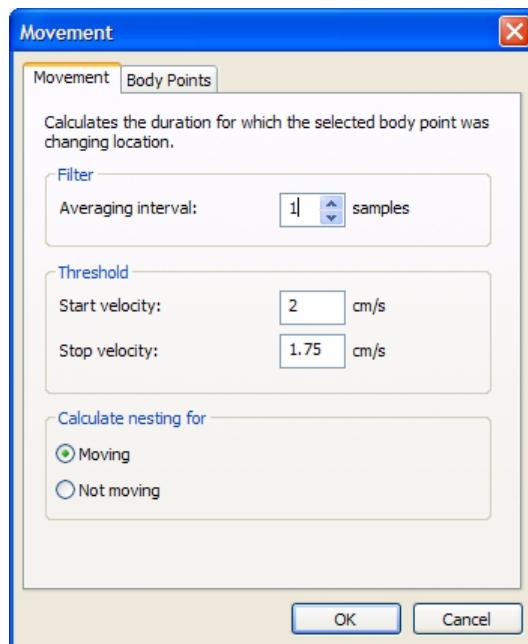


Figure 12.11. The **Movement** window.

- Under **Threshold**:

- **Start velocity** – Enter the velocity above which the subject is considered to be **Moving**.
- **Stop velocity** – Enter the velocity below which displacements of the subject's body point are no longer attributed to locomotion but to system noise, body wobble or pivoting on the spot. The subject is considered to be **Not moving**.

- Under **Calculate nesting for**:

Selecting Data for Analysis

- Moving** (default) – Select this option if you want to analyze the samples assigned to **Moving**.
 - Not moving** – Select this option if you want to analyze the samples assigned to **Not moving** (see Figure 12.11.).
- 2 On the **Body Points** tab, select the body points movement refers to. For example, select **Nose-point** to select the samples when the nose-point was moving (or not moving). Default is **Center-point**. If you select two or more body points, select:
- **Any selected point** – To consider the samples where any body point is moving (or not moving)
 - **All selected points** – To consider the samples where all body selected points are moving (or not moving) simultaneously.
- 3 If you have chosen **Movement** under **Nesting over Subjects**, the **Actors** tab is also available. Select the subjects which, when in the state selected above, define the nesting intervals. See page 425 for more information.
- 4 Click **OK**. Insert the **Nest** box in the appropriate position between the **Start** and the **Result** box.

For information on how to connect selection boxes, see page 391. To create multiple selections, see page 433.

Notes



- In some cases the number of samples available for smoothing can be less than the averaging interval entered. For example, in tracks with missing samples or at the beginning of the track. In such cases EthoVision XT uses the samples available in the specified interval.
- When the velocity is between the two thresholds, the current state of the subject does not change relative to the previous sample.
- For Movement, it is possible to select different thresholds for nesting (see above) and for analysis (see page 526). You should either use the same thresholds or make the thresholds in your analysis profile more restrictive, so that the variable specified in the Analysis profile is in effect a fine-tuning of your nesting criteria.
- Notice the difference:
 - If you select Movement under **Nesting**, the Actor is the subject of the current track. Therefore, different subjects are analyzed in different time intervals (see **B** in Figure 12.18.).
 - If you select Movement under **Nesting over Subjects**, the Actors are the subjects specified in the **Actors** tab (see also **C, D** in Figure 12.18.).

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Nesting over Elongation

Body elongation is the state variable that determines whether the subject is Stretched, Normal or Contracted by comparing the subject's current Body elongation index (expressed as percentage) with two thresholds you specify (see page 528).

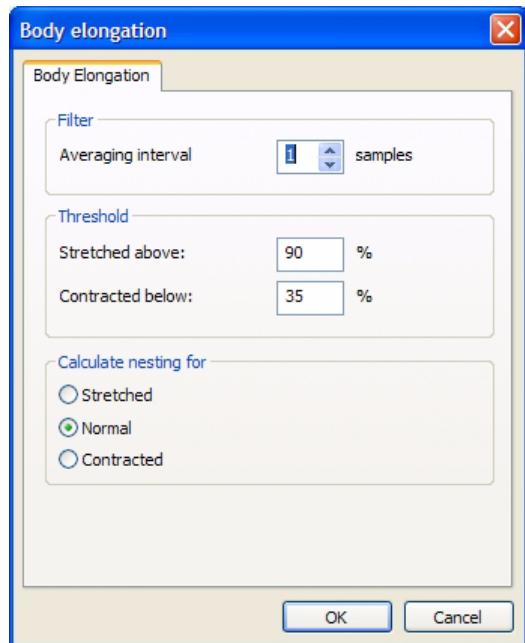


Figure 12.12. The **Body elongation** window.

1 Select the following:

- Under **Filter**, enter the **Averaging Interval** (Range 1 - 1000) – This is the number of samples across which changes in the Elongation index are calculated to determine whether the subject is Stretched, Normal or Contracted. In order to reduce the sensitivity of the **Elongation** variable to brief changes in Elongation index, the elongation data can be smoothed by taking the running average of the last n samples. Enter the averaging interval n or leave 1 if you do not want to smooth elongation data.
- Under **Threshold**, enter the following values:

Selecting Data for Analysis

- **Stretched above** – The value of Elongation index above which the subject is considered **Stretched**.
- **Contracted below** – The value of Elongation index below which the subject is considered **Contracted**.

Percentage values range from **0** to **100%**. When the subject's Elongation index is between the two thresholds, the subject is considered **Normal**.

- Under **Calculate nesting for**:
 - Stretched** – Select this option if you want to analyze the samples assigned to **Stretched**.
 - Normal** (default) – Select this option if you want to analyze the samples assigned to **Normal**.
 - Contracted** – Select this option if you want to analyze the samples assigned to **Contracted** (see Figure 12.12.).
- 2 If you have chosen **Body elongation** under **Nesting over Subjects**, the **Actors** tab is also available. Select the subjects that, when in the state selected above, define the nesting intervals. If you select two or more subjects, select
 - **Any selected subject** – To analyze the time that at least one subject was in that state.
 - **All selected subjects** – To analyze the time that all subjects were in that state simultaneously.For more information see page 425.
- 3 Click **OK**. Insert the **Nest** box in the appropriate position between the **Start** and the **Result** box.

For information on how to connect selection boxes, see page 391. To create multiple selections, see page 433.



Notes

- In some cases the number of samples available for smoothing can be less than the value n entered. For example, when there are missing samples or at the beginning of the track. In such cases EthoVision XT uses the samples available in the specified interval.
- Notice the difference:
 - If you select Body elongation under **Nesting**, the Actor is the subject of the current track. Therefore, different subjects are analyzed in different time intervals (see **B** in Figure 12.18.).
 - If you select Body elongation under **Nesting over Subjects**, the Actors are the subjects specified in the **Actors** tab (see also **C**, **D** in Figure 12.18.).

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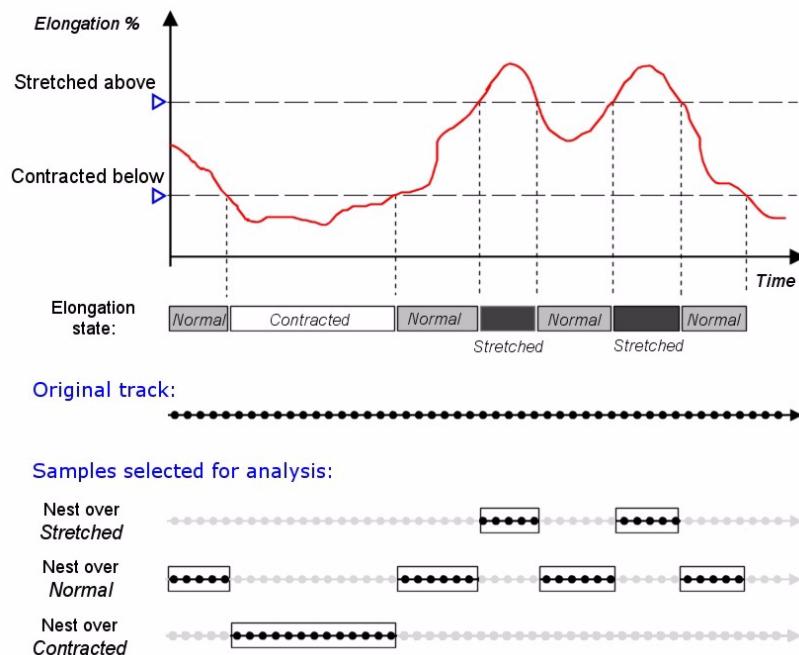


Figure 12.13. An example of nesting over Body elongation. The Body elongation variable with the three possible discrete states Stretched, Normal and Contracted is calculated so each sample is given one of the three states according to the subject's elongation percentage. Samples are considered for analysis which are assigned to one of the three states. Depending on your choice, different segments of the track are selected.

Nesting over Mobility

Mobility is the state variable that determines whether a subject is Immobile, Mobile or Highly mobile by comparing the changes in the subject's area from one sample to the next with two thresholds you specify (see page 530).

Selecting Data for Analysis

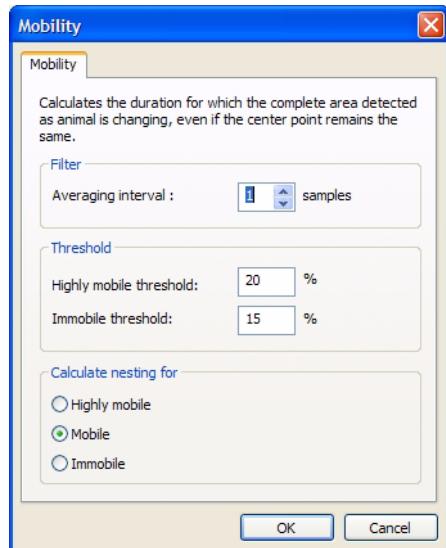


Figure 12.14. The **Mobility** window.

1 Select the following:

- Under **Filter**, enter the **Averaging Interval** (Range 1 - 1000) – This is the number of samples across which changes in the subject's area are calculated to determine whether the subject is **Mobile**, **Immobile** or **Highly mobile**. In order to reduce the sensitivity of the Mobility variable to brief changes in area, the area data can be smoothed by taking the average change in area in the last n samples. Enter the averaging interval **n** or leave **1** if you do not want to smooth area changes.
- Under **Threshold**, enter the following:
 - **Highly mobile threshold** – Enter the percentage change in area above which the subject is considered to be **Highly mobile**.
 - **Immobile threshold** – Enter the percentage change in area below which the subject is considered to be **Immobile**.



When the change in the subject's area is between the two thresholds, the subject is considered to be **Mobile**.

- Under **Calculate nesting for**:

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- Highly mobile** – Select this option if you want to analyze the samples assigned to **Highly mobile**.
 - Mobile** (default) – Select this option if you want to analyze the samples assigned to **Mobile**.
 - Immobile** – Select this option if you want to analyze the samples assigned to **Immobile**.
- 2 If you have chosen **Mobility** under **Nesting over Subjects**, the **Actors** tab is also available. Select the subjects that, when in the state selected above, define the nesting intervals.
If you select two or more subjects, select
- **Any selected subject** – To analyze the time that at least one subject was in that state.
 - **All selected subjects** – To analyze the time that all subjects were in that state simultaneously.
- 3 Click **OK**. Insert the **Nest** box in the appropriate position between the **Start** and the **Result** box.

For information on how to connect selection boxes, see page 391. To create multiple selections, see page 433.



Notes

- For Mobility, it is possible to select different thresholds for nesting (see above) and for analysis (see page 530). You should either use the same thresholds or set the thresholds in your analysis profile more restrictive, so that the variable specified in the Analysis profile is in effect a fine-tuning of your nesting criteria.
- Notice the difference:
 - If you select Mobility under **Nesting**, the Actor is the subject of the current track. Therefore, different subjects are analyzed in different time intervals (see **B** in Figure 12.18.).
 - If you select Mobility under **Nesting over Subjects**, the Actors are the subjects specified in the **Actors** tab (see also **C, D** in Figure 12.18.).
- In some cases the number of samples available for smoothing can be less than the averaging interval entered. For example, in tracks with missing samples or at the beginning of the track. In such cases EthoVision XT uses the samples available in the specified interval.

Selecting Data for Analysis

Nesting over Head directed to zone

Head directed to zone is the state variable that determines whether the head of the subject (or more subjects, named Actors) is pointing to a zone (or a circular area around a point) defined in the Arena Settings.

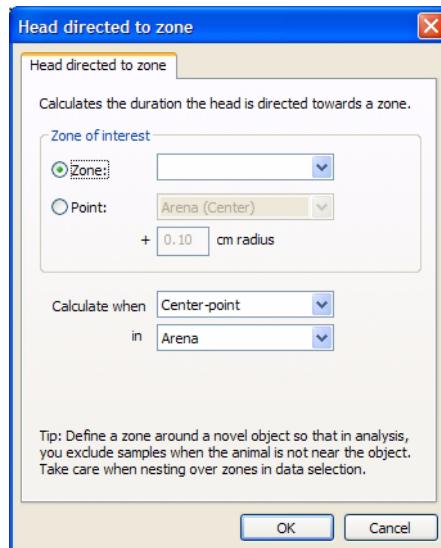


Figure 12.15. The **Head directed to zone** window.

1 Select the following:

- Under **Zone of interest**, select one of the following:
 - **Zone** – From the list, select the zone the subject should point at.
 - **Point** – From the list, select the point or the center of a zone the subject should point at.

Because a point has an infinitely small surface area, you need to define a circular zone around the point (default= 0.1 cm). Make sure you select a radius large enough. The smaller the radius around a point, the less likely it is that the animal's head is exactly directed at this point.

- Under **Calculate when**, define the position of the animal when the **Head directed to zone** state should be calculated. This allows you to exclude instances when the animal is very far from the zone or point. In such instances, pointing to a zone or point is likely to have no biological meaning.

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From the first list, select the body points of the subject that should be in the zone selected in the second list, when it is pointing to the zone (or point) of interest.

Example – An arena for a novel object test is divided in two zones: Proximal (which on its turn contains the Novel object zone) and Distal. To nest over the instances that the subject is pointing to the Novel object when all its body points are in the Proximal zone, under **Zone of interest** select **Novel object**, and under **Calculate when** select **All detected body points in Proximal**. This way, you exclude all instances when the animal points to the novel object as it walks in the Distal zone.

- 2 If you have chosen **Head directed to zone** under **Nesting over Subjects**, the **Actors** tab is also available. Select the subjects that, when pointing to the zone/point of interest, define the nesting intervals. If you select two or more subjects, select
 - **Any selected subject** – To analyze the time that at least one subject was in that state;
 - **All selected subjects** – To analyze the time that all subjects were in that state simultaneously.
- 3 Click **OK**. Insert the **Nest** box in the appropriate position between the **Start** and the **Result** box.

For information on how to connect selection boxes, see page 391. To create multiple selections, see page 433.



Notice the difference:

- If you select Head directed to zone under **Nesting**, the Actor is the subject of the current track. Therefore, different subjects are analyzed in different time intervals (see **B** in Figure 12.18.).
- If you select Head directed to zone under **Nesting over Subjects**, the Actors are the subjects specified in the **Actors** tab (see also **C, D** in Figure 12.18.).

Nesting over Proximity

Proximity is the state variable that determines whether one or more subjects (Actors) are within a user-defined distance from one or more other subjects (Receivers).

Selecting Data for Analysis

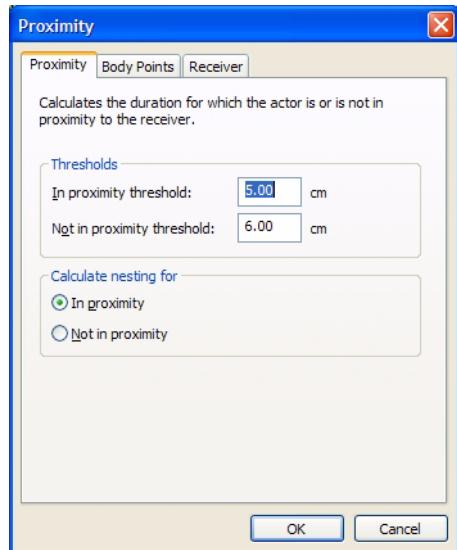


Figure 12.16. The **Proximity** window.

1 Under **Thresholds**, enter the following:

- **In proximity threshold** – The distance below which the Actor is considered to be In proximity of the Receiver (default 5 cm).
- **Not in proximity threshold** – The distance above which the Actor is considered to be In proximity of the Receiver (default 6 cm).

When the distance is between the two thresholds, the state does not change from the previous sample.

2 Under **Calculate nesting for**, select one of the states

- In proximity** – To analyze the samples assigned to "In proximity".
- Not in proximity** – To analyze the samples assigned to "Not in proximity".

3 Click the **Body Points** tab. Select the body point of the subject defined as Actor. For example, select **Nose-point** to select the samples when the nose-point of each subject was in proximity of the other subjects (Receivers). Default is **Center-point**. If you select two or three body points, select:

- **All selected points** – To consider the samples when all selected points were in proximity (or not in proximity) of the Receivers simultaneously.

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- **Any selected point** – To consider the samples when at least one selected point was in proximity (or not in proximity) of the Receivers.
- 4 Click the **Receivers** tab. Select the subjects that you want to define as Receivers, and their relevant body points. Analysis is done on the those samples when the Actor's body points are in the state selected above relative to the Receivers' body points. For more information, see page 425.
- 5 If you have chosen **Proximity** under **Nesting over Subjects**, the **Actors** tab is also available. Select the subjects that, when in proximity (or not in proximity) of the Receivers, define the nesting intervals. If you select two or more subjects, select
 - **Any selected subject** –To analyze the time that at least one subject was in state selected;
 - **All selected subjects** – To analyze the time that all subjects were in the state selected simultaneously.

See the note below about Actors.

- 6 Click **OK**. Insert the **Nest** box in the appropriate position between the **Start** and the **Result** box.

For information on how to connect selection boxes, see page 391. To create multiple selections, see page 433.



Notice the difference:

- If you select **Proximity** under **Nesting**, the Actor is the subject of the current track. Therefore, different subjects are analyzed in different time intervals (see **B** in Figure 12.18.).
- If you select **Proximity** under **Nesting over Subjects**, the Actors are the subjects specified in the **Actors** tab (see also **C, D** in Figure 12.18.).

Nesting over Relative movement

Relative movement is the state variable that determines whether one or more subjects (Actors) are moving to or away from one or more other subjects (Receivers).

- 1 In the **Maximum interaction distance** field, enter the value of distance between subject above which the subject are not considered as interacting.
- 2 Under **Calculate nesting for**, select the state that defines the nesting interval.

Selecting Data for Analysis

- **Moving to** – To analyze the samples when the Actor is moving towards the Receivers.
- **Moving from** – To analyze the samples when the Actor is moving away from the Receivers.
- **No relative movement** – To analyze the samples when the Actor does not change its distance from the Receivers.
- **No interaction** – To analyze the samples when the distance between Actor and Receivers is greater than the Maximum interaction distance.

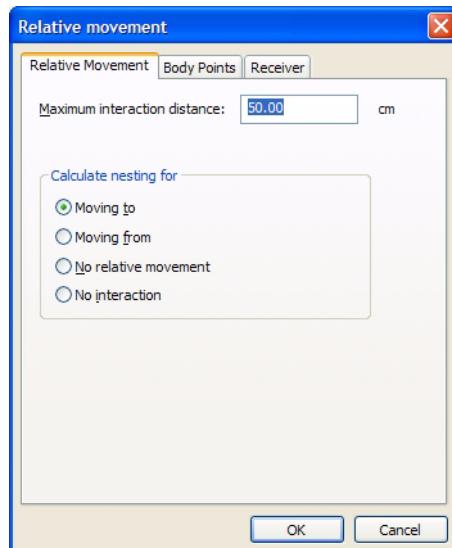


Figure 12.17. The **Relative movement** window.

- 3 Click the **Body points** tab. Select the body point of the Actor. For example, select **Nose-point** to select the samples when the nose-point of each subject was moving to other subjects (Receivers). Default is **Center-point**. If you select two or three body points, select:
 - **All selected points** – To consider the samples when all selected points were in the state selected simultaneously relative to the Receivers.
 - **Any selected point** – To consider the samples when at least one selected point was in the state selected relative to the Receivers.

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- 4 Click the **Receivers** tab. Select the subjects that you want to define as Receivers, and their relevant body points. Analysis is done on those samples when the Actor's body points are in the state selected above relative to the Receivers' body points. For more information, see page 425.
- 5 If you have chosen **Relative movement** under **Nesting over Subjects**, the **Actors** tab is also available. Select the subjects that, when in the state selected above, define the nesting intervals. If you select two or more subjects, select
 - **Any selected subject** – To analyze the time that at least one of the Actors was in that state relative to the Receivers.
 - **All selected subjects** – To analyze the time that all the Actors were in that state simultaneously relative to the Receivers.See page 425 for more information.
- 6 Click **OK**. Insert the **Nest** box in the appropriate position between the **Start** and the **Result** box.

For information on how to connect selection boxes, see page 391. To create multiple selections, see page 433.



Notice the difference:

- If you select Relative movement under **Nesting**, the Actor is the subject of the current track. Therefore, different subjects are analyzed in different time intervals (see **B** in Figure 12.18.).
- If you select Relative movement under **Nesting over Subjects**, the Actors are the subjects specified in the **Actors** tab (see also **C**, **D** in Figure 12.18.).

Nesting over Trial Control states

Aim

Pick out the track segments corresponding to a Trial Control state. A Trial Control state is an interval defined by two events occurred during the trial (a condition met, a sub-rule started, an action taken etc.).

To learn more about Trial Control states, see page 556.

Procedure

We assume at this point that you have followed steps 1 to 3 in the procedure on page 403, and you have clicked the button next to **Trial Control state** in step 2. As

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a result, the **Trial Control state** window appears.

- 1 Next to **From**, from the **Element** list, select the Trial Control element that makes the criterion for the start of the interval. From the **Event** list, select the event for that element that makes the start of the interval.
- 2 Next to **To**, from the **Element** list, select the Trial Control element that makes the criterion for the end of the interval. From the **Event** list, select the event for that element that makes the end of the interval.



Since there may be multiple occurrences of the event marking the interval end, choose which **occurrence** (from **1th** to **9th**) should be used. **1th** is the first occurrence of the event selected under **To** available after each occurrence of the event selected under **From**.

The **State** options available depend on what you have chosen as **Element** (see **Analysis of Trial Control data** in the Trial and Hardware Control Manual).

- 3 Click **OK**. Insert the **Nest** box in the appropriate position between the **Start** and the **Result** box.



You can create Trial Control elements specifically for data selection or analysis, as well as directly for controlling the trial.

The **Actors** tab in the **Trial Control state** window is of no use, because Trial Control states are independent of subjects.

Selecting subjects in Nesting intervals

With Nesting, analysis is done on segments of tracks (intervals) based on one of the criteria available under **Nesting** and **Nesting over Subjects**.

Intervals are applied to each track.

- To analyze the intervals based on the behavior of the animal that refers to that track, choose an option under **Nesting**, and follow one of the procedures on page 404, page 406, page 410 or page 424.

Result – Each subject will be analyzed in a different interval, according to its own behavior (see **B** in Figure 12.18.).

- To analyze the intervals based on the behavior of a specific subject (or combination of subject, called Actors), choose an option under **Nesting over Subjects** and see the procedure below.

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Result – Within the same arena, subjects will be analyzed in equal intervals (see **C** and **D** in Figure 12.18.).

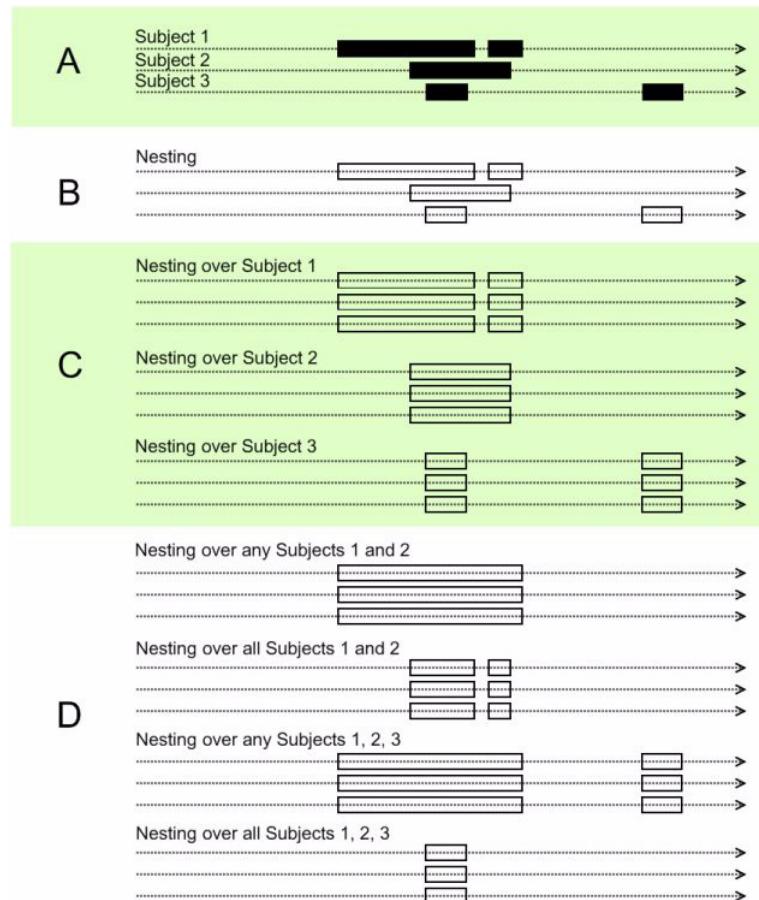


Figure 12.18. Effect of Nesting in one arena with three animals. Dotted lines: tracks. Black bars: time intervals when a behavioral state or ATC state is active (for example: **In zone** for the variable **In zone**, or "Highly mobile" for **Mobility**). White bars: data selected for analysis. **A** - Original data. **B** - Results of **Nesting**. Track segments are selected according to the state of the corresponding subject. **C** - Results of **Nesting over Subjects**, with one subject selected in the **Actors** tab (page 425). **D** - Results of **Nesting over Subjects**, with two or more subjects selected in the **Actors** tab. For reasons of space, the combinations "Any/All of Subjects 1 and 3" and "Any/All of Subjects 2 and 3" are not displayed.

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Arenas are independent replicates. Therefore, nesting intervals are applied to each arena separately depending on the state of their subjects.

Nesting is equivalent to Nesting in EthoVision XT 4/5.

Nesting over Subjects

This category is only visible if your experiment includes multiple subjects per arena.

We assume that you have clicked the **Actors** tab in the nesting window.

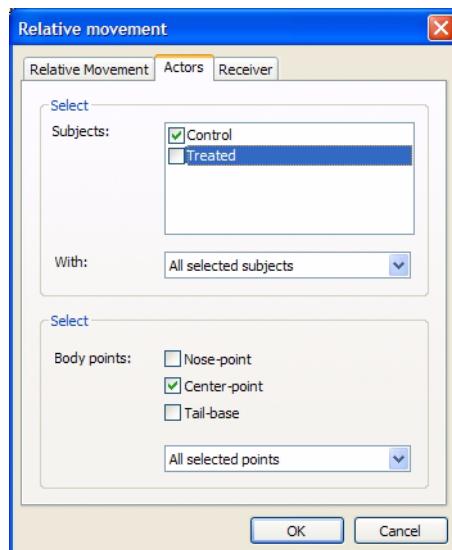


Figure 12.19. Nesting over Relative movement of the Control subject towards the Treated subject 2.

- 1 In the **Actors** tab, you select the actors of the behavior that define the nesting interval. Under **Select**, select the subject that should show the behavior. If you select two or more subjects in the **Actors** tab, select one of the two options from the list immediately below the **Select** box:
 - **All selected subjects** – To analyze the samples when all the actors show that behavior simultaneously.
 - **Any selected subject** – To analyze the samples when at least one actor shows that behavior.

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- 2 If the **Actors** tab also contains the **Body points** options, select one or more body points of the subjects selected above (for details, see page 410).
 - 3 For the dependent variables of social interaction, a **Receivers** tab is also available. Receivers are the subjects towards which the behavior of the Actor is directed to. For example:
 - To analyze when Subject 2 is moving to Subject 1, Subject 2 is the Actor and Subject 1 is the Receiver.
 - To analyze when Subject 1 is in proximity of Subject 2, Subject 1 is the Actor and Subject 2 is the Receiver.
- Click this tab and select the subjects that you want to define Receivers (see an example below).
- 4 Click **OK** and insert the **Nest** box in the appropriate position between the **Start** and the **Result** box.



Be careful when selecting the same subject under **Actors** and **Receivers**. In such case you may also get unwanted selections, for example **Subject 1**'s nose point (Actor) In proximity of **Subject 1**'s tail base (Receiver). This nesting criterion is usually of no use.

Examples

- **Example of Social interactions** – You want to analyze the time that the nose-point of Subject 1 was in proximity of the tail-base point of Subject 2 (ano-genital sniffing).

Solution – Subject 1 is Actor, Subject 2 is Receiver.

Under **Nesting over Subjects**, click the button next to **Proximity**.

- In the **Actors** tab select **Subject 1** and de-select **Subject 2**. Under **Body points**, select **Nose-point** only.
- In the **Receivers** tab select **Subject 2** and de-select **Subject 1**. Under **Body points**, select **Tail-base** point only.

If you want to select the time that the nose point of Subject 1 was in proximity of the tail-base point of Subject 2, or vice versa, create two nesting boxes, one that specifies Subject 1's nose in proximity of Subject 2's tail base, and the other that specifies Subject 2's nose in proximity of Subject 1's tail base. Next, combine the two boxes with OR logic (see page 433).

- **Example with one Actor, no Receivers** – The researcher wants to analyze the time that Subject 2 was moving. In particular, he wants to quantify the distance moved by other subjects in that period of time.

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Solution – Subject 2 is the Actor. Under **Nesting over Subjects**, click the button next to **Movement**.

- In the **Actors** tab select **Subject 2** and de-select the other subjects.

In the Analysis profile, select **Distance moved**. The statistics results refer to each subject in the specified interval.

Nesting over manually scored behaviors

With nesting over manually scored behaviors you can pick out the track segments in which a manually scored behavior was active.



To learn more about manually scoring behaviors, see “Defining behaviors” on page 121 and “Scoring behaviors manually” on page 299.

We assume at this point that you have followed steps 1 to 3 in the procedure on page 403.

Nesting start-stop behaviors

- 1 Click the button next to the start-stop behavior.
- 2 In the <behavior> window, make sure <behavior> is selected and click **OK**.



To analyze the track segments in which the behavior was not active, you use an Analysis Profile (see page 555).

Nesting mutually-exclusive behaviors

- 1 Click the button next to the mutually-exclusive group.
- 2 In the <behavior group> window, select a <behavior> and click **OK**.



If you select a behavior from a mutually-exclusive group, the other behaviors of the same mutually-exclusive group cannot be used for Visualization and Analysis.

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12.6 Selecting your data - Results per time bin or zone

What are Results per time bin?

If you select Results per time bin, your data are divided into time intervals of equal duration. This way you analyze data as if your tracks were split in two or more segments.

What is the difference between Nesting over Time and Results per time bin?

- Nesting over Time (see page 404) allows you to analyze a single time interval within each track.
- Results per time interval allows you to analyze a series of time intervals within each track. **Each interval is analyzed separately.**

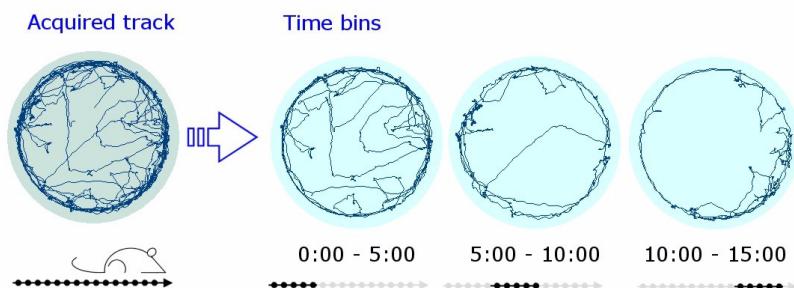


Figure 12.20. Applying **Results per time bin** results in each track being split in a number of time intervals of equal length. Analysis results are shown for each interval separately. Compare this with Nesting over Time in the picture on page 401.

Results per time interval is applied for each track separately.

- In a **multiple arena setup**, tracking may start at different times for each arena, depending on when the Trial Control's **Start** conditions are met in a specific arena. Therefore, the same time interval may refer to different 'real' times for different arenas.
- In a setup with **multiple subjects per arena**, tracks start simultaneously for all the subjects in each arena, because the Trial Control's **Start** condition is

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applied at the arena level. Therefore, the same time interval refers to the same 'real' time for all the subject in that arena.

What are Results per zone?

If you select Results per zone, you analyze your data for different zones separately.

What is the difference between Nesting over Zone and Results per zone?

- There is no difference in the results between Nesting over Zone (see page 406) and Results per zone. In both cases, you analyze data for each zone separately (for example, you calculate the mean velocity in each of four quadrant zones of an open field).
- However, when you want to analyze data for multiple two zones, it is much easier to use Results per zone, because Nesting over Zone requires you to create multiple Results boxes in a Data Profile (see the figure below for an example).

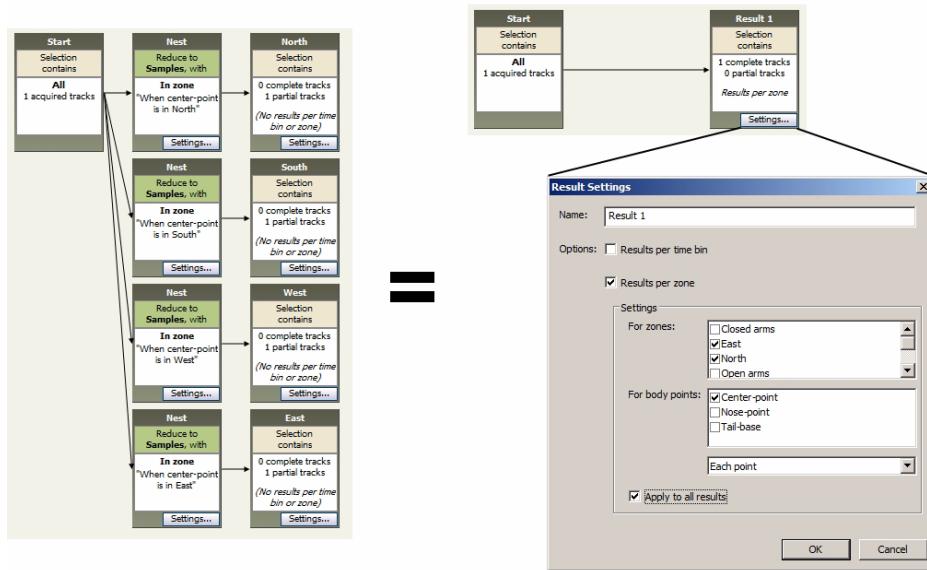


Figure 12.21. This figure shows an example of Nesting over zones (left) and Result per zone (right) which both lead to the same result in the Analysis Output. In this case, in which the data is analyzed for the four arms of an elevated plus maze separately, it is much faster and easier to use Results per zone.

How to specify Results per time bin or zone

- 1 Make sure that the Data profile in which you want to define the Results per time interval is open. If this is not the case, create a new data profile or open an existing one (see page 581).
- 2 Click the **Settings** button on the **Result** box. In the case your data profile contains more **Result** boxes, make sure you open the one corresponding to the data you want to be split in time interval.

Result – The **Result Settings** window appears.

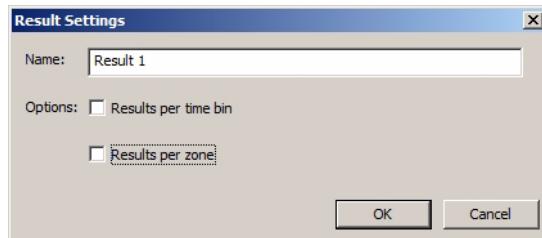


Figure 12.22. The **Result Settings** window.

- 3 Select **Results per time bin** if you want to analyze your data in time intervals of equal duration.
- 4 Under **Settings**, select:
 - The **Length** of the single interval (range: 1 second to 24 hours).
 - Ignore last time bin if incomplete** – Select this option if you want to exclude the last interval in the case this is of a shorter duration than the **Length** you have set.
 - Apply to all Results** – Select this option if your data profile contains more than one **Result** box (see an example on page 438) and you want to apply **Results per time bin** to all of them.
- 5 Select **Results per zone** if you want to analyze your data for multiple zones separately.
- 6 Under **Settings**, select:
 - For zones** – Select the zones you want to analyze your data for.

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- For body points** – Select which body points should be in the selected zones. This option is only available if you have the multiple body points module.
 - From the list, select:
 - **Each point** – The data is analyzed for each body point in each zone separately.
 - **Any selected point** – The data is analyzed when at least one of the selected body points was in a selected zone.
 - **All selected points** – The data is analyzed when all the selected body points were simultaneously in a selected zone.

7 Click **OK**.

12.7 Multiple selections

Selection boxes can be combined in a variety of ways to create complex data selections.

Below you find a few basic rules to combine selection boxes. On page 439, you find a note on the correct order of selection boxes in a sequence.

The basic rules for combining selection boxes

A very general rule – AND, OR and Multiple-results logic

- **AND Logic** – When you line up boxes in a single sequence, the data in the **Result** box (and thus selected for analysis) satisfy all conditions set by the boxes. Selection criteria are connected by **AND** logic.

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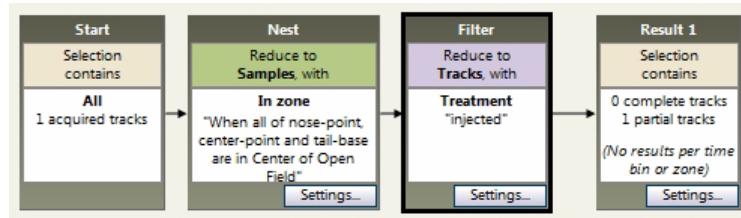


Figure 12.23. The data selected in this example are first nested over the center of the open field (second box), then filtered according to the value “injected” of the variable Treatment (third box).

- **OR Logic** – When you split a sequence in two or more branches which end in the same **Result** box, the data in the **Result** box (and thus selected for analysis) satisfy either one condition or another set in those branches. The analysis result combines the selection criteria with **OR** logic. For example, the total time the animal was either Moving or Mobile.
- **Multiple Results Logic** – When you split a sequence in two or more branches and those end in different **Result** boxes, the data selected for analysis satisfy the conditions set in those branches independently. Unlike in the previous example, selection criteria are not combined and there will be as many analysis results as **Result** boxes, each referring to its own selection criterion. See also page 438.

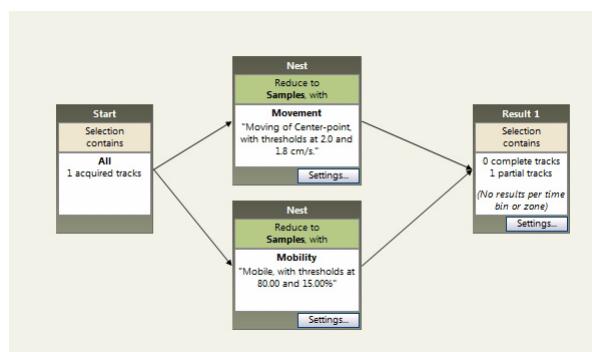


Figure 12.24. The data selected in this example is the portion of the track when the animal was either Moving (top, middle box) or Mobile (bottom).

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Rule 1 – Filtering by two or more values of a variable

To create multiple filters using values of the same independent variable, specify those values in one Filter box. Do not insert multiple Filter boxes.

Example – Your experiment contains a number of tracks recorded on different days from the treatment (1, 2 or 3 days). We assume here that Day after treatment has been entered as User-defined variable.

You want to analyze the tracks for days 1, 2 and 3 after treatment.

- 1 Create a **Filter** box corresponding to the filtering variable (see page 395).
- 2 Specify your selection.
- 3 Insert the **Filter** box in the sequence (See Figure 12.25.).

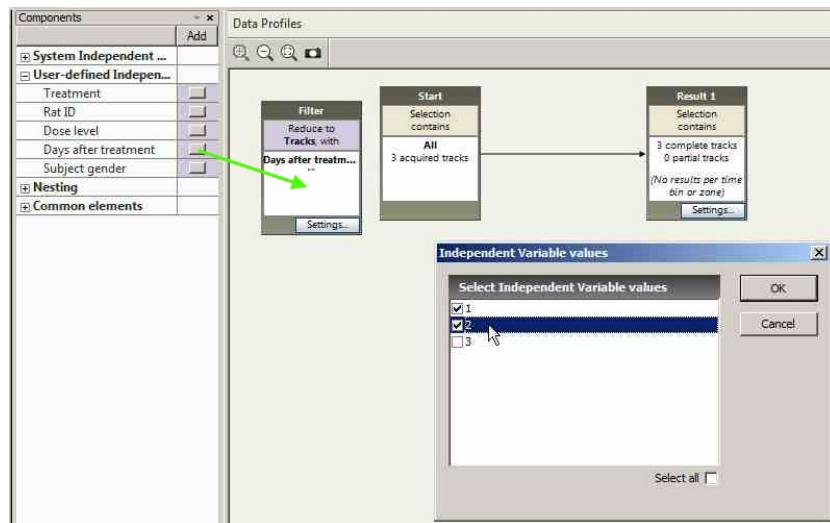


Figure 12.25. Edit a Filter box to refine filtering based on one variable. The thick arrow indicates which independent variable was used to define the selection. **Result** - when visualizing the plots or calculating the statistics, the data are shown for 1 and 2 days after treatment.

Rule 2 – Filtering by two or more variables

To create multiple filters using different independent variables, create one box per variable.

Filtering allows you to select entire tracks from your data set. To refine filtering

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according to two or more independent variables, create the corresponding **Filter** boxes (see page 395) and connect them between the **Start** box and the **Result** box using the logic you require (AND, OR or Multiple Results; see page 433).

Example – Your experiment contains a number of tracks recorded for male and female subjects, treated with two dose levels of a drug. We assume here that Dose level and Subject gender have been entered as User-defined variables. Since the selection criteria depend on different variables, two Filter boxes must be inserted in the selection sequence.

You want to calculate statistics for the female subjects, and for the dose level 0.5 mg/kg.

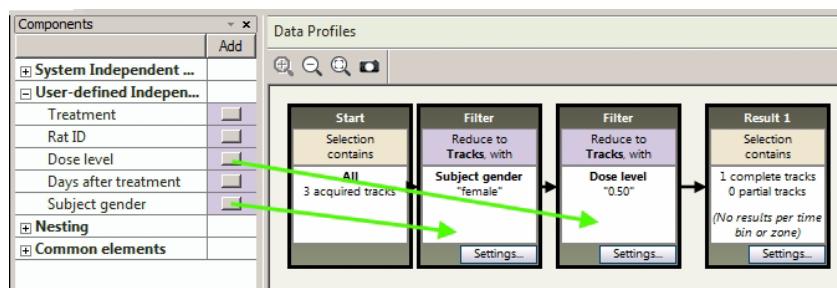


Figure 12.26. Connect **Filter** boxes to create a complex filter. The thick arrows indicate which independent variables were used to define the selection. **Result** - When visualizing the plots or calculating the statistics, the results are shown for female subjects treated with drugs dose= 0.5 mg/kg.

Rule 3 – Nesting over multiple values of one criterion

To select track segments based on two or more values of the same nesting criterion, specify those values in one Nest box. Do not insert multiple Nest boxes.

Example – You want to visualize the track segments when the subject was entirely in one of the open arms of the elevated plus maze. We assume that the open arms 1 and 2 have been defined as a zones.

- 1 Create a **Nest** box corresponding to the nesting criterion (see page 403).
- 2 Specify your selection.
- 3 Insert the **Nest** box in the sequence (see Figure 12.27.).

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Notes

- **In zone** – For the **In zone** criterion, you can apply the AND and OR logic by choosing the appropriate option in the **In zone** property window. For example, to specify that the subject must be either in Open arm 1 or in Open arm 2, select **When in any of the selected zones**.
- **Movement, Elongation and Mobility** – For those nesting criteria it is not possible to specify multiple values in the same **Nest** box (for example, nest over Highly mobile and Mobile). To specify multiple values, see the next rule.

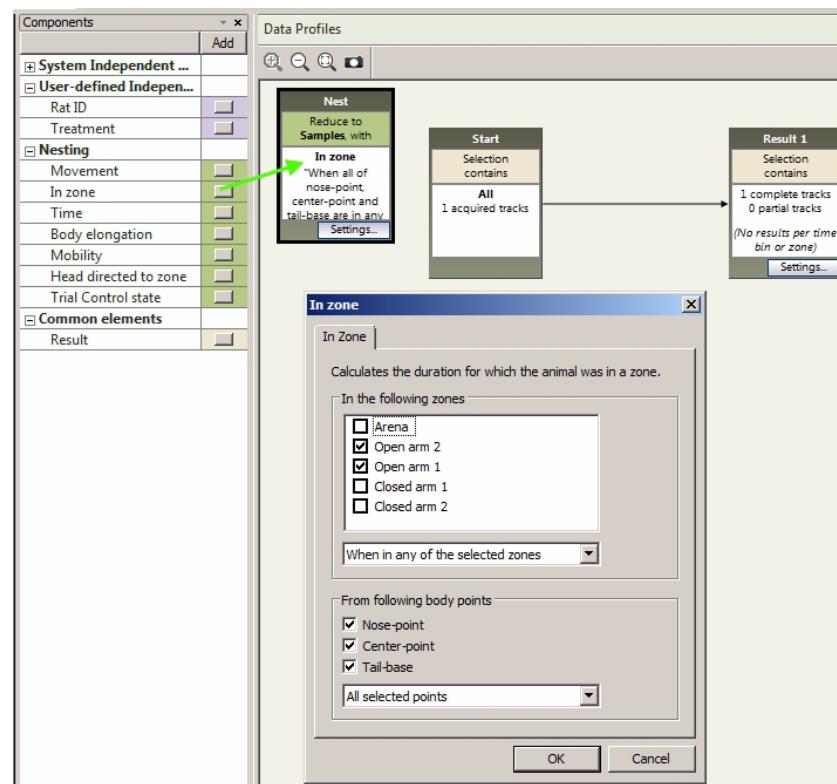


Figure 12.27. Edit a **Nest** box to refine nesting based on one variable. The thick arrow indicates which dependent variable was used to define the selection. **Result** - Analysis is done on the samples collected when the center point, nose-point and tail-base of the subject were all in any of the open arms of the plus maze.

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Rule 4 – Nesting over two or more criteria

To select track segments using different nesting criteria, create one Nest box per criterion.

To refine nesting according to two or more nesting criteria, create the corresponding **Nest** boxes (see page 403) and connect them between the **Start** box and the **Result** box using the logic you require (AND, OR or Multiple Results; see page 433).

Example – You want to visualize the track segments when the animal was completely in any of the arms of the elevated plus maze and its body was stretched. We assume that the open arms 1 and 2 have been defined as a zones.

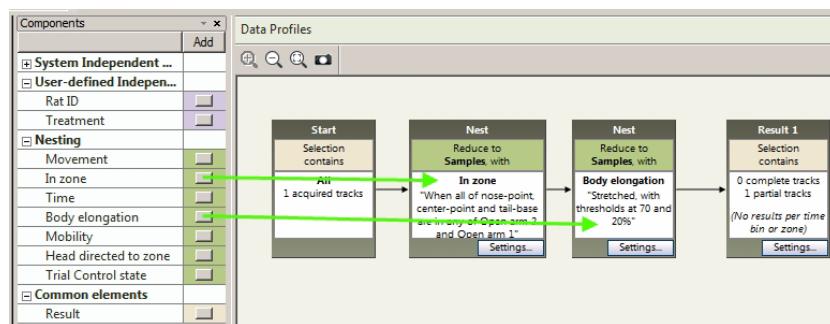


Figure 12.28. Connect **Nest** boxes to select track segments on the basis of different dependent variables. The thick arrows indicate which variables were used to define the selection. **Result** - The result shows the samples collected when the animal was stretched and its body was completely detected in any of the open arms.



If you want to nest over multiple values of the same variable (for example, nest over **Highly mobile AND Mobile**), create one **Nest** box per value and connect them using the logic you require (AND, OR or Multiple Results).

Rule 5 – Creating independent data selections

To show results for different data selections, connect each sequence to a separate **Result box.**

You can create different data selections in your Data profile in the form of

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separate box sequences. Each sequence must end in a separate **Result** box. When you analyze the data using that Data profile, the results (whether statistics figures or track plots) are displayed for each selection separately.

Example – You want to calculate statistics of locomotor behavior of rats in the two open arms of a plus maze named Open arm 1 and Open arm 2. We assume that the two open arms have been defined as zones. You want to show the statistics for the two arms separately.

To add a Result box, click the button next to Result in the Components pane.

In the analysis results, you can see which data selection generated a specific result from the name of the Result box under the Selection Result heading (see page 455 for track plots and for statistics results).

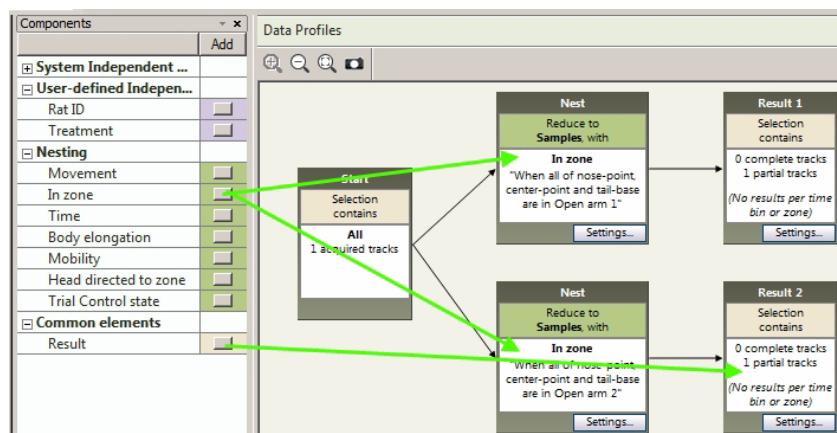


Figure 12.29. Create multiple Result boxes to show a separate analysis result for each data selection criterion. The two **Result** boxes receive the data nested over the zones Open arm 1 and Open arm 2, respectively. The thick arrows indicate which buttons in the **Components** pane were used to create the selection boxes. **Result** - The statistics are calculated separately for when the animal was in Open arm 1 and when it was in Open arm 2.

Order of selection boxes

In some instances the order in which you place selection boxes in the Data selection sequence may be important

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Filter boxes

- If two or more **Filter** boxes refer to different variables (for example, one to filter trials, another one to filter drug doses), then the order you place the **Filter** boxes in does not matter.
- If two or more **Filter** boxes refer to the same variable, then selection will only work if the elements selected in the second box are also selected in the first box.

Example – If you filter male subjects in one Filter box and female subjects in the second Filter box, the selection contains no data.

Nest boxes

The order you place the **Nest** boxes relative to **Filter** boxes does not matter.

12.8 Changes in Data Selection relative to EthoVision 2/3

Please read carefully this section if you have used previous EthoVision versions.

Applying data selection to analysis

In EthoVision 2/3, you select data after you have started the **Analysis** module. In EthoVision XT, you first create your Data profile (see page 384) or activate an existing one (see page 581), then carry out your analysis (see Chapter 14).

Selecting tracks

In EthoVision 2/3 you select tracks for analysis by choosing **Select Tracks** from the **Data** menu. This corresponds to the **Filtering** function in EthoVision XT (see page 393).

Input filters

Minimal Distance Moved is now implemented in a Track Smoothing profile not in a Data Profile. Also, in EthoVision XT 8, no samples are excluded from analysis.

Nesting over Time, Zone, behavioral states

In EthoVision 2/3, you select track segments by choosing **Nesting** from the **Data**

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menu. This corresponds to the **Nesting** function in EthoVision XT (see page 400). If you want to define regular time intervals like you would do in EthoVision 2/3, choose **Results per time interval** (page 430).

Defining Receivers

In EthoVision 3, the Receivers defined were applied to all the parameters chosen for analysis. In EthoVision XT, you can define Receivers per dependent variable. Therefore, each dependent variable can have its own set of receivers.

Complex selections

In EthoVision 2/3 you can create complex data selections by operating different commands from the **Data** menu. In EthoVision XT, this corresponds to combining different **Filter** and **Nest** boxes (see page 433).

Wider choice of data selections

In EthoVision XT you can create a wider array of data selections.

- **AND vs. OR logic to combine selection criteria** – For example, when two or more zones overlap, you can nest over those zones to analyze the data in the overlapping area (select **When in all selected zones** in the **In Zone** window; see page 406). This was not possible in EthoVision 2/3, where you can only select data when the subject is in any of the selected zones.
- **Apply different selections to the same analysis result** – You can create separate selection sequences in a Data profile, so you can immediately compare results (track plots or statistics tables) from different selection criteria. For example, you can compare statistics of locomotor behavior according two nesting criteria. To do so, create two or more selection sequences that end in different **Result** boxes (see page 438 for how to do so).

Grouping tracks

In EthoVision 2/3, you group tracks by choosing **Group Tracks** from the **Data** menu. In the current version of EthoVision XT, you cannot group tracks.

To obtain overall results, export the Analysis output and then carry out the calculations in your statistics software.

 Example – You have an experiment with the user-defined independent variable **Treatment**. Export the Analysis output to Microsoft Excel and remove the Units row and any blank rows in the header. Then create a Pivot Table with your independent variable **Treatment** as a Row Label and the Average Value of the EthoVision dependant variable calculation as the Pivot table Value. You will then

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see your track results grouped by Treatment.

12.9 What next?

Make sure that the Data profile containing your data to analyze is highlighted in blue. Next:

- To visualize track plots, see Chapter 13.
- To calculate statistics, see Chapter 14.
- To export data, see Chapter 15.

13

Visualizing Data

This chapter is about:

- **The Visualization screen** – Windows and objects of the software interface.
→ See the next page
- **Plotting tracks** – To visualize track data in form of plots.
Choose this option if you want to visualize data from multiple trials.
→ See page 454
- **Plotting integrated data** – Visualize tracks together with video (provided that tracking was carried out from video files) and external (physiological) data. You can also view Time Event plots of your dependent variables.
With this option, you can only visualize data from one trial at a time.
→ See page 465



You cannot print track plots directly in this version of EthoVision XT.
You can export track plots as image files (JPG, PNG, etc.; see page 463).

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13.1 The Visualization screen

To start visualization, do one of the following:

- From the **Analysis** menu, select **Results** and then **Plot Tracks** or, in the Experiment Explorer, click **Track Visualization** in the Analysis folder under **Results**.
- From the **Analysis** menu, select **Results** and then **Plot Integrated Data** or, in the Experiment Explorer, click **Integrated Visualization** in the Analysis folder under **Results**.



If you selected **Plot Integrated Data** from the **Analysis** menu, you can visualize one trial at a time. Select the trial name from the list in the tool bar (see page 465 for details).

The Visualization screen appears (see Figure 13.1.).

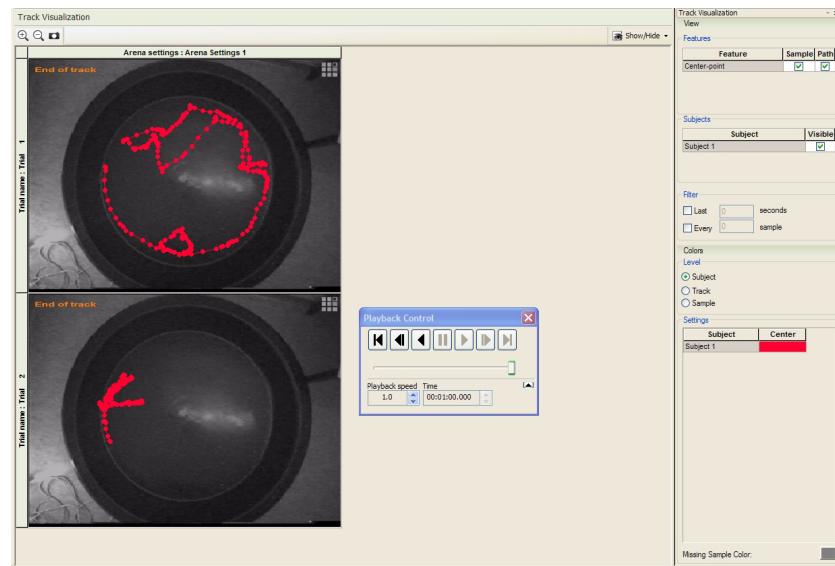


Figure 13.1. The Track Visualization screen. The content of the screen may vary depending on your data selected and what layout you have chosen.

The Visualization screen contains:

- The plotting area with track plots and video – See page 445.

Visualizing Data

If you choose **Plot Integrated data**, the plotting area will also include plots of your external data and one or more **Time Event plots** of dependent variables.

- The **Playback Control** window – See page 446.
- The **Track Plot Settings** pane (on the right hand side) – See page 447.
- The **Trial Control Events** pane (only if you select **Plot Integrated Data**) – See page 475.

Plotting area

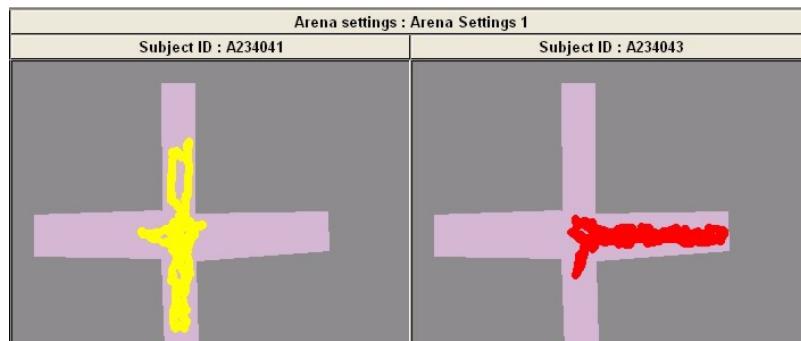
Track data

- If you choose **Track Visualization** in the Experiment Explorer, the plotting area shows the tracks selected in the currently active data profile. The static background is the picture grabbed as background when you defined your arenas.
- If you choose **Integrated Visualization** in the Experiment Explorer, the **Trial** window appears showing the tracks for the trial you have chosen from the list in the component tool bar. The trial name is displayed in the video window title bar.
 - If you have tracked data live, the window shows the tracks on the background picture.
 - If you have tracked data from video files, the window shows the content of the video file associated with the trial.

Plot labels

If you choose **Track Visualization** in the Experiment Explorer, each plot is identified by one or more labels like the headings of a table. Such labels show the values of independent variables associated with the tracks. You can sort the plots according to the values of one or more independent variables (see page 455).

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External data plots

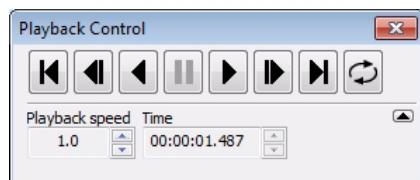
If a trial contains imported external data, the data plots are shown when you choose **Plot integrated data** from the **Visualize** menu (see page 465).

Time Event plots

If you choose **Integrated Visualization** in the Experiment Explorer, the plotting area includes one or more **Time event plots**. These plots show the change in the **Dependent Variables** selected in the **Analysis Profile** along the time line (see page 465).

Playback Control window

Use the **Playback Control** window to play back the tracks, the video, the external data (when applicable) and dependent variable values.



See page 461 for an overview of the functions of the buttons in the **Playback Control** window.

Visualizing Data



If you choose **Plot Integrated Data**, the slider and the three buttons on the bottom row are not available.



If you do not see the **Playback Control** window, make sure that **Playback Control** is selected under the **Show/Hide** button on the components tool bar.

You can hide/show the bottom-part of the **Playback Control** window by clicking the **Expand/Collapse** button on at the right.

Track Plot Settings

The **Track Plot Settings** pane (Figure 13.2.) allows you to control the appearance of the tracks, for example which subjects and body points are shown, how many and at which rate, and the tracks' color.

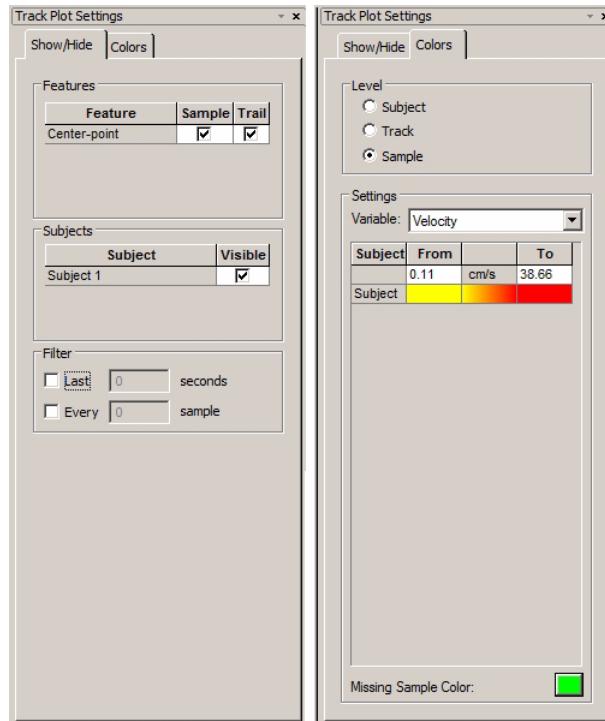


Figure 13.2. The two tabs of the **Track Plot Settings** pane.

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If you do not see the **Track Plot Settings** pane, click the **Show/Hide** button on the component tool bar and select **Track Visualization**.

Customizing the Track Plot Settings pane

By default, the **Track Plot Settings** pane is shown in the upper right corner of the visualization window. Choose one of the following options to change the visualization of the pane:

- **Move the pane** – Click its title bar and drag it to the desired position. You can either dock the pane or let it float on the screen.
- **Resize the pane** – To resize the pane, point with your mouse to the pane's left margin so the mouse pointer turns to a double arrow. Drag the margin to the desired position to have a larger pane.

The Track Plot Settings pane has two tabs:

The Show/Hide tab

In the **Show/Hide** tab you can choose which body points and which subjects to display, and how many samples you want to visualize.

Displaying the samples

Under **Features**, you find the following elements:

- **Nose-point** (only for Nose-tail tracking).
- **Center-point**.
- **Tail-base** (only for nose-tail tracking).
- **Connectors** (only for nose-tail tracking).

Features		
Feature	Sample	Path
Nose-point	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Center-point	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Tail-base	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Connectors	<input checked="" type="checkbox"/>	

If your experiment has been set to **Only center-point detection** (see page 102), you only see **Center-point**.

- To view the samples produced by a body point, select the box under **Sample** next to that body point.
- To view the line connecting samples for a body point, select the box under **Path** next to that body point.

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- ☐ **Connectors** refers to the line connecting the nose-point and the tail-base to the center-point.



In all tracks, the nose-point is always represented by a filled triangle, the center-point by a filled circle and the tail-base by a filled square. You cannot change the shape of body points in your tracks.

Displaying the subjects

In the **Subjects** section you can specify which subjects to visualize. This is handy if you track two or more animals per arena and you want to highlight the track of one or some of them.

Subjects	
Subject	Visible
Subject 1 - Control	<input checked="" type="checkbox"/>
Subject 2 - Treated	<input checked="" type="checkbox"/>

Under **Visible**, select which subject you want to have displayed, and clear the selection for those you want to hide.

Filter (displaying a partial track)

The **Filter** options allow you to choose how many samples to display and at which rate.

- ☐ **Last ... seconds** – If your track is long, you can choose to visualize only part of it, for instance only the last 5 seconds.

A dialog box titled "Filter". It contains two options: "Last" with a value of "5" and "seconds", and "Every" with a value of "0" and "sample". The "Last" option has a checked checkbox.

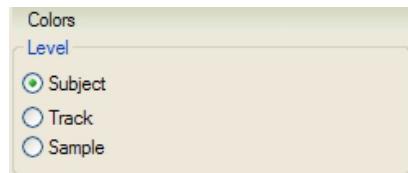
- ☐ **Every ... sample** – If your animal moves slowly, the samples are displayed accumulating at one spot. To prevent this you can choose to visualize every nth sample.

A dialog box titled "Filter". It contains two options: "Last" with a value of "5" and "seconds", and "Every" with a value of "2" and "sample". The "Every" option has a checked checkbox.

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The colors tab

In the **Colors** tab you can specify how colors should vary, for example between arenas, or within tracks.



You have three options:

- **Subject** – To have the tracks of multiple animals within an arena displayed in different colors. See page 451.
- **Track** – To have the tracks displayed in a color depending on the value of an Independent Variable. The samples *within a track* are shown in the same color. See page 451.
Example 1 – Show tracks obtained from each drug dose in a different color.
Example 2 – In a multi-arena setup, show tracks from each arena in a different color.
- **Sample** – To have the samples *within tracks* displayed in a color depending on the value of a dependent variable. Therefore, the color changes within tracks. See page 452.
Example 1 – Show samples with different values of velocity in different colors.
Example 2 – Show samples collected in different zones of the open field in different colors.



If you select **Plot Tracks** you can sort tracks according to one or more independent variables (see page 455). The way you select colors for tracks is independent of the way you sort tracks.

Missing Sample Color

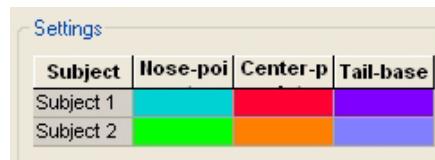
Missing sample points are always visualized in the track plot. You can change the color of missing samples to distinguish them more easily from the actual data. The current color for missing samples is indicated next to **Missing Sample Color**. To change the color for missing samples, click the button and select a new color in the **Color** window. For more information about missing samples, see page 227.

Visualizing Data

Specifying color variation at Subject level

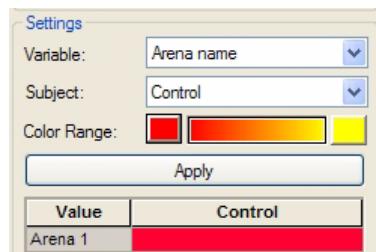
- **If you tracked one animal per arena in multiple arenas** – The tracks for all the animals are displayed in the same color. You can choose different colors for different body points.
- **If you tracked more than one animal per arena** – You can specify colors for each animal in the arena. The track of animal 1 in arena 1 is displayed in the same color as that of animal 1 in arena 2, arena 3, etc.

Under **Settings**, click the color you want to change and choose a new color.



Specifying color variation at Track level

When selecting **Track** as the level of **Color Variation**, you can choose an independent variable from the **Variable** drop-down list and specify what colors should be assigned to the tracks with specific values of that variable.



- 1 Select an independent variable from the **Variable** list.

Result – A list of variable **Values** appears. For example, if you have chosen 'Arena name', the list shows the treatments associated with the tracks available for visualization.

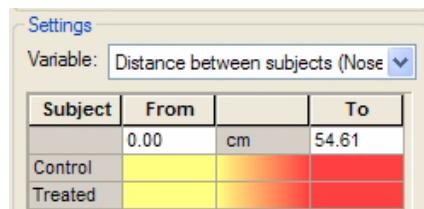
- 2 Choose one of the following two options:

- **Choose a color range** – Click the buttons next to **Color Range** to select the colors at the ends of the range and select two colors.

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- **Choose individual colors** – Each value of the selected variable is listed under **Value**. Click the **Color** button next to a value, and select the color you want to assign to the tracks with that value.
- 3 **If you tracked more than one animal per arena** – Select Subject 2 from the **Subject** drop-down list and repeat step 2. Repeat this for each animal in the arena.
 -  Choosing a color range is handy when you have a large number of values of the independent variable.
 -  If you select to display Nose-point and Tail-base (see page 448), the colors for the two corresponding tracks are derived from the color of the Center-point. The Nose-point's color has less intensity and the Tail base color has lower saturation than the center color.
 - Variable values are shown in alphabetic order (for Text and Boolean variables), or in ascending order (for Numerical, Time and Duration variables).

Specifying color variation at Sample level



When selecting **Sample** as the levels of **Color Variation**, you can choose a dependent variable from the **Variable** drop-down list and specify what colors should be assigned to the samples according to the values of the variable:

- 1 Select a dependent variable from the **Variable** drop-down list.



The list shows the dependent variables selected in the currently active Analysis profile, which is highlighted in blue under **Analysis Profiles** in the Experiment Explorer.

Result – Values are shown in the **From** and **To** field:

- For State variables – **0.00** and **1.00**.
- For continuous variables – The minimum and maximum value measured for that variable. For example, **-180°** and **+180°** for **Head direction**.

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If the **Variable** drop-down list does not include the dependent variable you would like to use, double-click it in the Experiment Explorer, add the dependent variable and edit its settings.

- 2 Choose the two colors for the 0.00/1.00 or minimum/maximum values of the dependent variable. To do so, click the cells below the values and select the colors. EthoVision calculates the spectrum of colors in between and shows it in the middle cell. See Figure 13.3. for an example of a plot with color variation at sample level.

If you tracked more than one animal per arena, carry out this step for each animal in the arena.

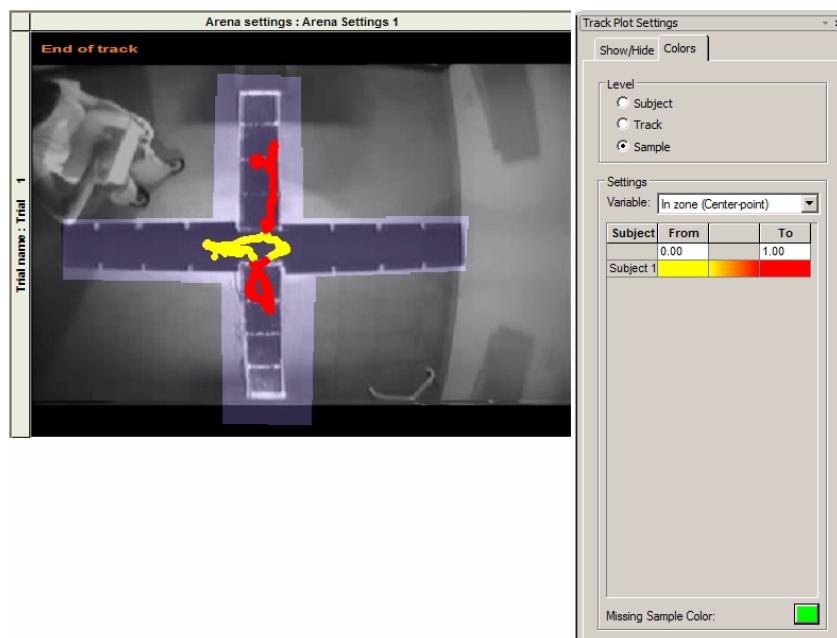


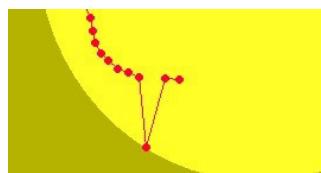
Figure 13.3. Example of a Sample level plot, where samples are displayed in different colors depending on whether the subject is in any of the open arms or in the rest of the plus maze. In the Analysis profile, the dependent variable **In zone** has been defined for the center-point, and the **When in any of the selected zones** option has been selected from the list. When plotting the tracks, samples in any of the open arms are displayed in the color selected for the True value of the **In zone** variable. Other samples are displayed in the color under **False**.

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You can change the range of dependent variable values to be visualized. Click the cell just below **From** or **To** and enter a new value.

Example – This way you get rid of outliers. Outliers may occur when EthoVision loses track of the animal like in the figure below.



The track shows a sharp change in direction and there is a peak in, e.g., 'velocity' and 'distance moved'. You can look up the values of these peaks in the variables' Time Event plot (see page 469) and omit them from the range of values to be visualized.

13.2 Plotting tracks

Aim

To visualize a set of tracks obtained from one or more trials.



This function is particularly useful when you want to have an overview of your data acquired so far. If you want to visualize data from **one** specific trial together with video or external data, see page 465.

Procedure

To plot tracks, do the following:

- 1 Make sure that the Data profile specifying the data you want to plot is active (that is, highlighted in blue in the Experiment Explorer), and, if you want to visualize tracks in colors depending on the values of a dependent variable, make sure that that variable is specified in the currently active Analysis profile.



To edit a profile, double-click it in the Experiment Explorer and make the necessary changes.

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- 2 In the Experiment Explorer, in the Analysis folder, under Results, click **Track Visualization**.
- 3 Choose the subjects and body points you want to display (see page 448 and page 449).
- 4 If you want to show only part of the tracks, select the appropriate options under **Filter** (see page 449).
- 5 Under **Colors**, in the Track Plot Settings pane, select one of the following:
 - Subject** – To have the tracks of multiple animals within an arena displayed in different colors. See page 451.
 - Track** – To have the tracks displayed in different colors depending on the values of an independent variable (for example, drug dose). Select an independent variable from the **Variable** drop-down list and choose either a color range or individual colors. See page 451.
 - Sample** – To have samples *within tracks* displayed in different colors depending on the values of a dependent variable (for example, velocity). Select a dependent variable from the **Variable** drop-down list and choose the two colors for the True/False or Minimum/Maximum values. See page 452.

Effect of data selection on track plots

If your data selection contains nesting criteria, all samples are plotted, however the samples excluded by nesting are displayed in a different color.

Sorting track plots

Track plots are displayed in the form of a matrix. You can sort rows and columns according to one or more independent variables.

Example – Subjects of both sexes were assigned to two different treatment levels (Treated or Control). You want to visualize tracks according to **Subject sex** (different sexes on different rows) and **Treatment** (different treatments on different columns). See the result in Figure 13.4.

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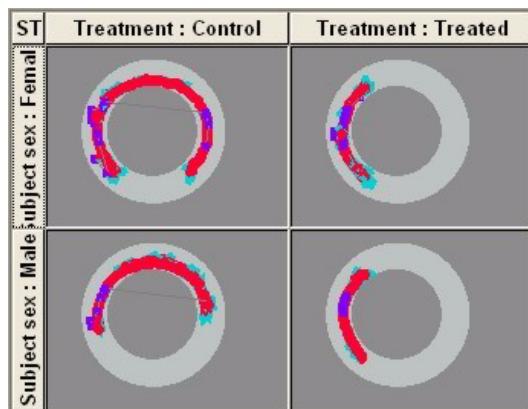


Figure 13.4. Track plots are sorted by two variables, Treatment (on the columns) and Subject sex (on the rows)

To sort track plots, click the **Show/Hide** button on the component tool bar and select **Layout** or right-click the headings of the plots (see below).



By default, plot headings include the name of an independent variable and the variable's value for that row/column. To remove the variables' name, right-click a heading and clear **Show Name**. The values of the variables and the resulting sorting order are not removed. To re-establish the variables' name, right-click the heading and select **Show Name**.

Layout window

In the component tool bar, click the **Show/Hide** button and select **Layout**. As a result, the **Layout** window appears (see Figure 13.5.):

- The **Available** box lists the independent variables available for sorting.
- The **On Columns** box lists the variables currently used to sort columns in the matrix.
- The **On Rows** box lists the variables currently used to sort rows in the matrix.

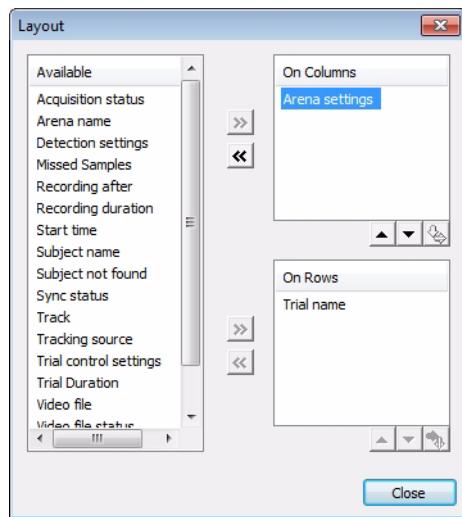


Figure 13.5. The **Layout** window.

Sorting by a new variable

Using the Layout window

- » Click the **Show/Hide** button on the component tool bar and select **Layout**. In the Layout window, click the variable name under **Available** and click one of the buttons next to **On Columns** or **On Rows**, depending on whether you want to sort columns or rows by that variable. Next, click **Close**.

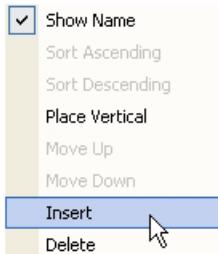
Directly on the plots

- i Do one of the following:

- If you would like to have tracks with a specific value of that variable displayed in their own column, right-click the heading above a plot.
- If you would like to have tracks with a specific value of that variable displayed in their own row, right-click the heading to the left of a plot.

- ii Select **Insert**.

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- iii The **Select Independent Variables to Insert** window appears. From the **Independent Variable Name** drop-down list, select the variable you would like to use as a sorting factor.

Result – The values of the variable that will be used to sort tracks appear under **Independent Variable Values**.



The variable list does not include the variables already present as headings in the plot matrix.

- iv Click **OK**.

Result – New columns or rows appear with their own headings. Track plots are sorted accordingly.



To reduce the range of values of the independent variables, filter the data (see page 393).

Removing a sorting variable

From the Layout window



Click the **Show/Hide** button on the component tool bar and select **Layout**. Click the variable name under **On Columns** or **On Rows**, and click the de-select button next to it. Next, click **Close**.

Directly on the plots

Right-click a heading for that variable and select **Delete**.



If you remove all variables from one side of the matrix, the heading will be empty. All tracks are plotted on one line or column.

If you obtained your data from more than one Arena Settings and you remove sorting variables, you may get an error message. See page 464.

Changing the sorting order

By default, track plots are sorted in ascending order of the corresponding variables (numeric or alphabetic).

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- To sort variable values in descending order, right-click a heading for that variable and select **Sort Descending**.
- To sort variable values in ascending order, right-click a heading for that variable and select **Sort Ascending**.

You cannot change the plot order from the **Layout** window.

Multiple sorting levels

When two or more variables are inserted or moved to the same side of the matrix, they form a multi-level structure. In the example below, plots are first sorted by **Treatment** (top level), and then by **Subject sex** (bottom level).

Treatment : Control		Treatment : Placebo	
Subject sex : Female	Subject sex : Male	Subject sex : Female	Subject sex : Male

You can re-arrange the sorting levels by moving each variable up and down across the headings:

- **Using the Layout window**



Click the **Show/Hide** button on the component tool bar and select **Layout**. Click the variable you want to move under **On Columns** or **On Rows**. To move the variable one level up, click the **Up** button. To move the variable one level down, click the **Down** button.

- **Directly on the plots**

Right-click a heading for that variable. To move a variable one level up, select **Move up**. To move a variable one level down, select **Move down**.

Result – The headings for that variable are moved one level up or down. Track plots are sorted accordingly.

In the example below, **Subject sex** has been moved above **Treatment**. As a result, plots are first sorted by Subject sex, then by Treatment (compare with the previous picture).

Subject sex : Female		
Treatment : Control	Treatment : Placebo	Treatment : Treated

Moving plots from columns to rows and vice versa

You can re-arrange your plots by moving the variables shown in the rows to columns and vice versa:

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- **Using the Layout window**

Click the variable name under **On Columns** or **On Rows**, and click the **Place Horizontal**  or **Place Vertical** button .

Result – The variable is moved from **On Columns** to **On Rows** or vice versa.

- **Directly on the plots**

- Right-click a row/column heading for that variable and select **Place Horizontal** or **Place Vertical** OR
- Click a row/column heading and drag it to a new position.

Displaying background, arenas and zones

Together with the track data, EthoVision can display the background image, the arena and the zones.

Customizing the background

- 1 On the component tool bar, click the **Show/Hide** button and select **Background**.
- 2 Select one of the following:
 - **Plain** – To have a uniform background. Click the button next to this option to select a color. To select colors other than the basic ones, click the **Other** button.
 - **Captured image** – If you want to have the captured image from the Arena Settings as background.
- 3 Click **OK**.



In the **Track Visualization** view, you cannot see the video as the background. This is only possible in the **Integrated Visualization** view (see page 468).

Showing/hiding arenas and zones

Arenas, zones and points can be displayed on top of the background as overlay objects. The color of each arena and zone depends on the color chosen in the Arena Settings.:.

- 1 Click the **Show/Hide** button on the component tool bar and select **Arena Features**.
- 2 Select the arena feature you want to have displayed.

Arenas and zones are shown in a semi-transparent color, so their appearance also depends on what you select as background.



If you want to change the color of an arena or zone group, open the appropriate **Arena Settings**, click the correct **Color** row and select the color of your choice.

For a multi-arena setup, you cannot select to view some arenas and not others.

Viewing and playing back tracks

Zooming in and out

The Zoom buttons are located on the component tool bar.

- To zoom in, click the **Zoom in** button .
- To zoom out, click the **Zoom out** button .

Customizing the length of tracks

- **Displaying the entire track**



→ Make sure that in the **Track Plot Settings** pane, under **Filter**, **Last ... seconds** and **Every ... sample** are not selected. Click the **Jump to end** button.

- **Displaying a partial track** – For example, always show samples for the latest 30 seconds.

→ Select **Last** under **Filter** in the **Track Plot Settings** pane and enter the time you want samples to be plotted.

- **Displaying one data point every n** – For example, display one sample every 5 to make the track plot more readable.

→ Select **Every** under **Filter** in the **Track Plot Settings** pane and enter *n* in the appropriate box.

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Playing back tracks

Play back your tracks if you want to see the movement of the tracked subjects.

The **Playback Control** window contains the following buttons:



- **Play forward (Ctrl+9)** – Plays the tracks.

You can set the speed of playback in the **Playback speed** box. Available speeds range from **1/25** to **16**. If the sample rate is lower than the maximum, samples are shown at a speed lower than the Playback speed.



- **Pause (Ctrl+0)** – Stops playing the tracks. If you click **Play forward**, it resumes playback from where it was when you clicked **Pause**.



- **Play backward (Ctrl+Shift+4)** – Plays the tracks backward. You can set the speed of playback in the **Playback speed** box.



- **Step frame forward (Ctrl+right arrow key)** – Plays the tracks one sample forward.



- **Step frame backward (Ctrl+left arrow key)** – Plays the tracks one sample backward.



One step forward/backward corresponds to the time interval for the sample rate used for detection (see page 223). For example, 0.2 seconds if the sample rate is 5/s.



- **Jump to begin (Ctrl+up arrow key)** – Jumps to the start of each trial (time= 0:00:00.000), and shows no samples.



- **Jump to end (Ctrl+down arrow key)** – Displays the last set of samples, depending on how many points you have chosen to display at a time.



If your trials have different durations, the end time is the end time of the longest trial. Therefore, it can happen that the longer tracks are played while the shorter ones have stopped because those have already reached the end.



- **Loop** - Loops the video, which is useful if you use the visualization for a demonstration.

Viewing tracks from multi-arena setups

If you plot tracks from a multi-arena setup, these are played back together starting from the first sample of each. If tracks were acquired at different times with Trial

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Control, the samples visualized at a given moment may refer to different 'real times'.

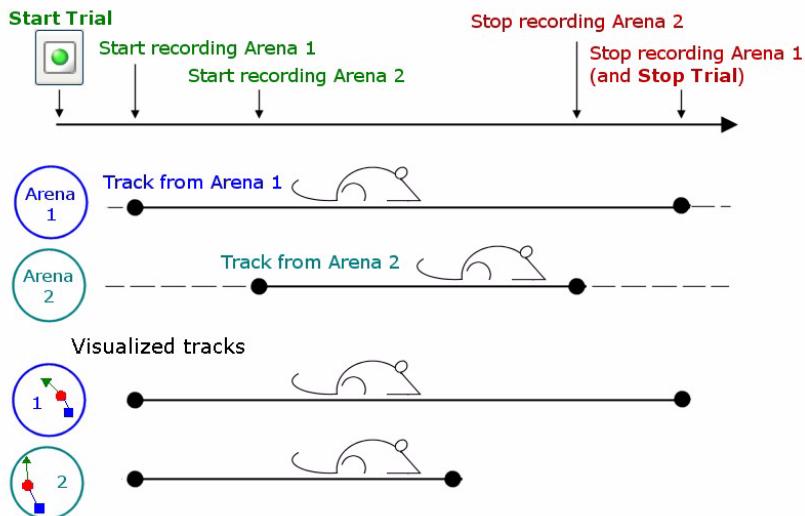


Figure 13.6. With the Plot Track function, the start times are lined up. Therefore, samples visualized in two arenas at the same time (see Visualized tracks) may refer to different times in the timeline (see the position of the mouse on the tracks).

Printing and exporting track plots

Printing track plots

You cannot print track plots from within EthoVision XT. To print plots, export them first (see below) and import them into other software, for example Windows Picture and Fax Viewer.

Exporting track plots

You can export your track plots by saving them to image files. To export track data (for example, the X,Y coordinates), see page 492.

- 1 Click the track plot you want to export.

Result – The border of the plot becomes highlighted in red.

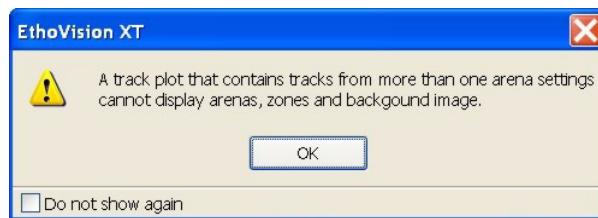
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- 2 On the component tool bar, click the **Export image** icon .
- 3 In the **Export Image** window, enter the name and location of your image file.
- 4 From the **Save as type** list, select one of the formats:
 - **Portable Network Graphic (*.png)** (default) – A picture format using lossless compression. It is especially useful for storing bitmap images containing line drawings.
 - **Enhanced Metafile (*.emf)** – A 32-bit version of the older Windows Metafile (*.wmf). Essentially, it stores a list of commands that have to be issued to the Windows' Graphic Device Interface to restore the image.
 - **JPEG file (*.jpg)** – A compression format for color images, particularly photographs. It can reduce file sizes to about 5% of their original size, but some detail might be lost in the compression.
 - **Windows Bitmap (*.bmp)** – A non-compressed format generally resulting in large files.
 - **Graphics Interchange Format (*.gif)** – A lossless format limited to 256 colors. This makes the GIF format suitable for storing graphics with relatively few colors such as simple diagrams.
- 5 Click **Save**.

Troubleshooting

I cannot display arenas, zones and background

You get the following message:



Problem – You have recorded data with more than one Arena Settings, and the layout of the plot matrix is such that tracks obtained with different Arena Settings are expected to be plotted together. EthoVision XT cannot display the background, the arena and the zones from two or more Arena Settings in the same plot.

Solution – Add the independent variable **Arena Settings** for sorting (see page 455). Alternatively, make sure that your Data Selection includes only data from one of the Arena Settings.

Some cells of the matrix are empty

This is caused by the fact that there are no track plots for some combinations of independent variable values. To create a more compact matrix, choose a different set of independent variables to sort track plots (see page 455).

13.3 Plotting integrated data

Aim

To visualize a set of tracks obtained from **one tracking session** (trial), together with video, external data (if applicable), and Time Event plots of your dependent variables (including manually scored behaviors).



This function is particularly useful when you want to compare track data with the video image from which those data were obtained. If you want instead to have an overview of your trials, see page 454.

Procedure

- 1 Make sure that the Data profile specifying the data you want to plot is active (that is, highlighted in blue).
- 2 Make sure that the appropriate dependent variables are specified in the active Analysis profile: (1) the variables which you want to use to visualize tracks (to plot tracks with color variation on sample level, see page 452); (2) the variables which you want to view in a Time Event plot (see page 469).



To edit a profile, double-click it in the Explorer and make the necessary changes.



If your data selection contains nesting criteria, all samples are plotted but the samples excluded by nesting are shown shaded.

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- 3 In the Experiment Explorer, in the Analysis folder, under Results, click **Integrated Visualization**.
- 4 From the trial list in the component tool bar, select the trial you want to visualize. By default, the first trial is selected.



To visualize the previous and next trials, click the buttons next to the list (**[** and **]**).



In some conditions depending on the amount of available memory, not all your trials are listed. To visualize the remaining trials, make a Data profile that includes only the trials you want to visualize.

- 5 If your current Data profile includes two or more Selection Results, a second list is available next to the trial drop-down list. Select the Result you want to visualize (see the note on the next page).

Result – The following windows and plots appear on the screen:

- The **Video** window showing the tracks, the arenas and zones and the content of the video file from that trial (only if tracking was done offline).
 - The **Playback Control** window – See page 446.
 - The **Track Plot Settings** pane – See page 447.
 - The **Trial Control Events** pane – see page 475.
 - **If you imported External Data** – One or more **External Data plots**, depending on how many external data files are associated with that trial. See page 468.
 - One or more **Time Event plots**, depending on how many dependent variables you have selected in the currently active Analysis Profile. See page 469.
- 6 In the **Track Plot Settings** pane choose the body points you want to display (see page 448).
 - 7 If you want to show only part of the tracks, select the appropriate options under **Filter** (see page 449).
 - 8 Under **Colors**, select one of the following (see also page 450):
 - **Subject** – To have the tracks of multiple animals within an arena displayed in different colors. See page 451.
 - **Track** – To have the tracks displayed in different colors depending on the values of an independent variable (for example, drug dose). Select an

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independent variable from the **Variable** drop-down list and choose either a color range or individual colors. See page 451.

- **Sample** – To have samples *within tracks* displayed in different colors depending on the values of a dependent variable (for example, 'In zone'). Select a dependent variable from the **Variable** drop-down list and choose the two colors for the True/False or Minimum/Maximum values. See page 452.
- 9 You can play back video, tracks, external data and Time Event plots by using the buttons in the **Playback Control** window (see page 461).



Notes

- **What are selection results?** – Selection Results correspond to the Result boxes you define in the Data Selection (see Chapter 12). They contain the data subject to analysis. If you have created two or more Result boxes in the same Data Profile, those Result boxes usually refer to different selection criteria (for example, different nesting conditions). Therefore, EthoVision XT must know what selection criterion you want to apply.
- Not all playback speeds might be available – Depending on the video format, you may not be able to play the video at a specific speed.
- The begin time in the **Video** window is the time of the first sample acquired in all arenas. If you have used Trial Control and the actual data recording started some time after the start of the trial, this time interval is not played back and the start time is greater than zero.



- The end time in the **Video** window is the time of the last sample from all arenas. Within each arena, the last sample is marked by the message **End of track** over the top-left corner of the corresponding arena.
- For multi-arena setups, the tracks are synchronized, that is, samples of different arenas visualized at a given moment were acquired at the same absolute time (but see page 462 when you choose **Plot Tracks** instead of **Plot Integrated Data**).

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Customizing the Video window

Showing/hiding the video image

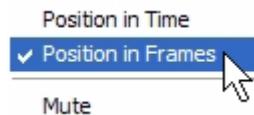
If you have tracked data from a video file, the video image is shown by default. To hide the video, click the **Show/Hide** button on the component tool bar and de-select **Video**.

Showing/hiding arenas and zones

Arenas and zones can be displayed in the **Video** window as overlay objects. See page 468.

Showing video position as time or frame number

You can specify how the current position of the video is displayed in the **Video** window. Right-click in the middle of the **Video** window and select the format you require, either **Position in Time** (elapsed time) or **Position in Frames** (frame number in the video).



Zooming in/out tracks

If you wish to zoom in/out tracks, resize the **Video** window by dragging the window's lower right corner. The zoom button on the component tool bar are for the plots.

External data plots

External data are shown in the form of X-Y plots (see Figure 13.7. for an example). The start and end time of the tracks does not always coincide with the start and end time of the co-acquired external data. When you visualize the integrated data, you can only view the external data acquired in the same time interval as the tracks associated with the external data file. Data acquired before the start and after the end of the tracks are not plotted.

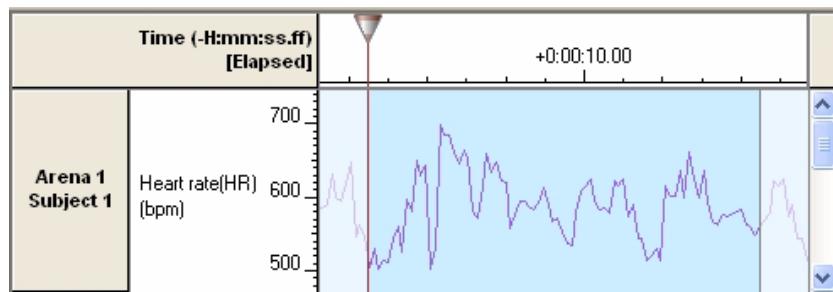


Figure 13.7. An example of an external data plot. The Plot margins are shown in semi-transparent color. These cover the first and last 10% of the plot length. The hairline is the plot cursor, it shows the current video/track position.

Time Event plots

Time Event plots show the values of the dependent variables selected in your active Analysis profile, on a time line (see Figure 13.8. for an example). By default, there are two dependent variables selected in your Analysis profile, **Distance moved** and **Velocity**. To plot other dependent variables:

- 1 Click the Analysis profile highlighted in blue under **Analysis Profiles** in the **Experiment Explorer** (or create a new Analysis profile).



- 2 Click the button next to the dependent variable you would like to add, and specify the variable properties. Next, click **Add**.
- 3 From the **Analysis** menu, select **Results** and then **Plot Integrated Data**.

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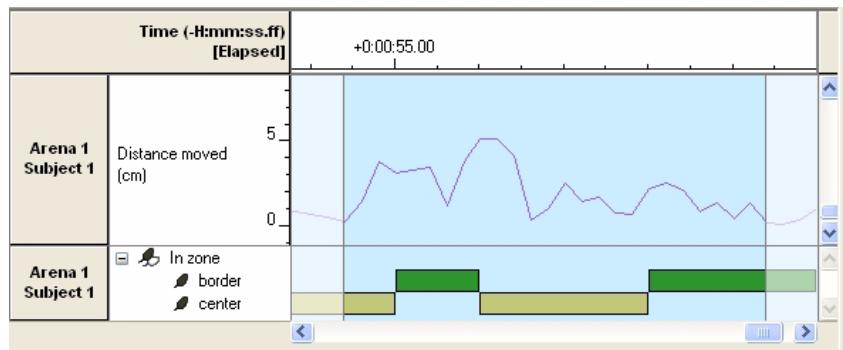


Figure 13.8. **Time Event plot** of the **Dependent Variables** ‘Distance moved’ and ‘In zone’.



If you have many Time Event plots, you can use your mouse wheel to scroll up and down.

If the dependent variable is already listed under **Select Dependent Variables** but has properties different from those you would like to set, return to the **Analysis Profile**, double-click the variable name and make the necessary changes. For more information about specifying dependent variable properties, see page 482.

Why are Time Event plots useful?

Time Event plots are especially useful:

- To check whether you set correct thresholds for state variables like body *Elongation*, *Movement* or *Mobility*.
 - i Open your analysis profile, double-click the variable's name and enter your best estimate for the thresholds.
 - ii From the **Visualize** menu, select **Plot Integrated Data**. Play back your data, watch the video and check whether the states shown in the **Time Event plot** correspond with the states of the animals in the video.
 - iii If necessary, repeat steps i and ii.



You can also set thresholds for state variables during acquisition and check whether the information about the subjects' state in the **Analysis Results**

Visualizing Data

pane corresponds with the state of the animals in the video or the live image. For more information see page 276.

- To check that you have scored behaviors manually at a good level of accuracy.
 - i Open your analysis profile and select the manually scored behaviors you want to check (see page 555).
 - ii From the **Analysis** menu, select **Results** and then **Plot Integrated Data**. Play back your data, watch the video and check whether the states shown in the **Time Event plot** correspond with the behavior of the animals in the video.
 - iii If necessary, correct manually scored data in The Observer XT. To export data, see page 495. To import data to The Observer XT, see page 575.
- To check whether your **Trial Control Settings** do what you want them to do. You can visualize **Trial Control events** and **Trial Control states**. For more information about **Trial Control**, see the Trial and Hardware Control Manual.

Effect of data selection on plots

If your data profile contains nesting criteria, the **External Data plots** and **Time Event plots** show all data points. The time segments included by the selection are shown in pale blue (Figure 13.9.).

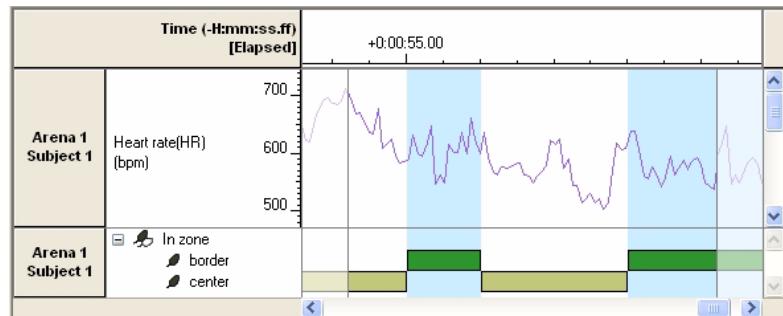


Figure 13.9. **Time Event plot** of the **Dependent Variables** ‘Heart rate’ and ‘In zone’ after nesting over the border zone of the open field.

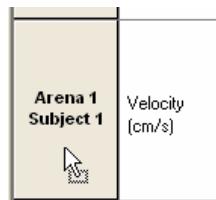
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Sorting plots

You can change the order in which **External Data plots** and **Time Event plots** are displayed by dragging them up and down on the screen.

- 1 Click the leftmost column next to the plot you want to move.

Result – The cursor changes (see figure below).



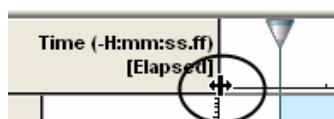
- 2 Drag the plot up or down to the position you require. When the mouse pointer is close to the line between plots, the line is highlighted in red. Release the mouse button to insert the plot.

Showing and hiding plots

You can choose which **External Data plots** and **Time Event plots** to display by clicking the **Show/Hide** button, selecting **Variables** and then making a selection in the **Variables** window. By default, all external data files are selected for that trial unless the associated tracks have been filtered out in the Data selection.

Resizing plots

- **Resizing the time window displayed** – Point to the Y-axis in the upper part of the plot, so the mouse pointer turns to a double arrow. Drag the Y-axis to the desired position to resize the time window.



- **Resizing the range of data values displayed** – Point to the lower margin of the grey cell on the far left of the plot so the mouse pointer turns to a double arrow. Drag the margin to the desired position to have a larger or narrower plot.



- **Zooming in/out the time scale (X-axis)**

With this function, you increase/reduce the time window displayed in your plot without changing the variable scale (Y-axis). If you have two or more External Data plots or Time Event plots, the time scale changes for all plots.

i Do one of the following:

- Click the **Zoom in**  or **Zoom out**  button on the component tool bar.
- Right-click the plot and select **Zoom in** or **Zoom out**.

Result – The mouse pointer turns to a magnifier symbol.

- ii Click the time bar one or more times to reach the zoom level you require.
- iii To stop zooming in/out and return to the normal mouse pointer, click the **No Zoom** button  on the component tool bar or right-click the plot and de-select **Zoom In** or **Zoom Out**.



Every time you zoom in/out, the plot scale is enlarged or reduced by a factor of 2.

- **Zooming in/out the Y-axis**

With this function, you increase/reduce the range of external data values or dependent variable values displayed in your plot without changing the time scale (X-axis). If you have two or more **External Data plots** or **Time Event plots**, the variable scale changes only for the plot you have clicked.

i Do one of the following:

- Click the **Zoom in**  or **Zoom out**  button on the component tool bar.
- Right-click the plot you want to zoom in/out and select **Zoom in** or **Zoom out**.

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Result – The mouse pointer turns to a magnifier symbol.

- ii Click the Y-axis scale of the plot one or more times to reach the zoom level you require.
- iii To stop zooming in/out and return to the normal mouse pointer, click the **No Zoom** button  in the tool bar or right-click the plot and again select **Zoom In** or **Zoom Out**.



Every time you zoom in/out, the plot scale is enlarged or reduced by a factor of 2.

Changing colors

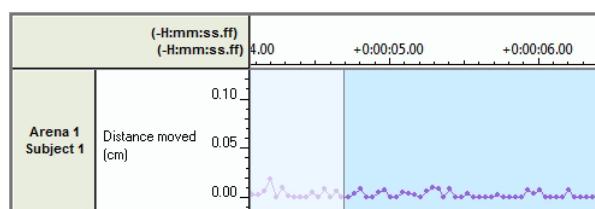
To change the color of a plot, double-click the plot with the line graph or the bar for a state variable and choose a new color from the window that appears. Then click **OK**.

Showing/Hiding data points

You can show or hide data points in the Time Event Plot with the Show/Hide button on the tool bar.



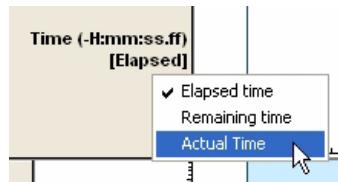
Select or deselect **Show Data Points**.



Specifying the time mode

You can choose to display time values on the X-axis as elapsed time, remaining time (that is, time to the end of the trial) or the actual time.

- 1 Right-click the top-left corner of the chart with the **Time (H:mm:ss.ff)** label.



- 2 Select one of the following:

- **Elapsed time** – The time elapsed since the start of the trial. The **0:00:00.00** time on the far left side corresponds to the start of the trial.
- **Remaining time** – The time to the end of the trial. The **0:00:00.00** time on the far right side corresponds to the end of the trial.
- **Actual time** – The actual date and time data were recorded (for example 10/22/2006 9:54:22.00).

Trial Control Events pane

The **Trial Control Events** pane gives you an overview of the events that occurred during the trial as a result of the **Trial Control Settings** you specified.

Trial Control Events	
Rule: 'Start-stop trial' - becomes active	
0:00:04.08	Action: 'Start track' - becomes active
0:00:04.08	Action: 'Start track' - becomes inactive
0:00:04.08	Condition: 'Time (2)' - becomes active
0:00:04.08	Condition: 'Time (2)' - becomes false
0:01:04.08	Condition: 'Time (2)' - becomes true
0:01:04.08	Condition: 'Time (2)' - becomes inactive
0:01:04.08	Action: 'Stop track' - becomes active
0:01:04.08	Action: 'Stop track' - becomes inactive

Figure 13.10. Example of a **Trial Control Events** pane.

In Figure 13.10. you can view an example of a **Trial Control Events** pane. The corresponding **Trial Control** profile is shown in Figure 13.11.. The first condition (the center-point of the mouse is in the arena for more than 1 second) is met after 2 seconds. At that moment the track is started. At t = 1 min, the track is stopped

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because the maximum trial duration has been reached. During playback, the hairline indicates the current position.

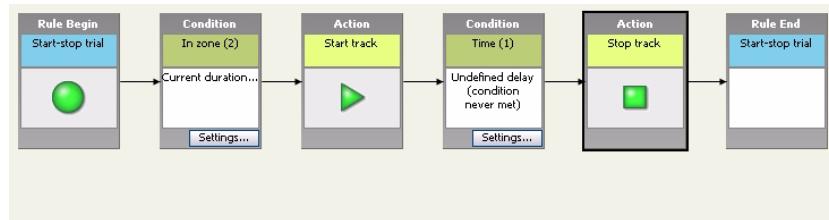


Figure 13.11. Trial Control profile with start and stop trial/track conditions.

Customizing the visualization of the Trial Control Events pane

By default, the **Trial Control Events** pane is shown in the lower right corner of the screen. Choose one of the following options to change the visualization of the pane:

- **Make the pane visible** – If you do not see the pane, click the **Show/Hide** button on the component tool bar and select Trial Control Events.
- **Move the pane** – Click its title bar and drag it to the desired position. You can either dock the pane or let it float on the screen.
- **Resize the pane** – To resize the pane do one of the following:
 - Point with your mouse to either the pane's upper margin or its left margin so the mouse pointer turns to a double arrow. Drag the margin to the desired position to have a larger pane.
 - Click the small black triangle in the upper right corner of the pane to have a full view of the pane and hide the **Track Plot Settings** pane.

Printing and exporting integrated visualization

Printing Integrated Visualization

You cannot print time event plots from within EthoVision XT. To print them, export them first (see below) and import them into other software, for example Windows Picture and Fax Viewer.

Exporting Integrated Visualization

You can export your track plots by saving them to image files. Please note that you will only obtain an image of the items that are visible on your screen.

To export track data (for example, the X,Y coordinates), see page 492.

- 1 On the component tool bar, click the **Export image** icon .
- 2 In the **Export Image** window, enter the name and location of your image file.
- 3 From the **Save as type** list, select one of the formats.
- 4 Click **Save**.

13.4 What next?

- To correct tracking errors, see Chapter 11.
- To select data for analysis, see Chapter 12.
- To calculate statistics, see Chapter 14.
- To export data, see Chapter 15.

14

Calculating Statistics

This chapter is about:

- **Calculating Statistics** – How to calculate statistics from the track data or for data acquired with hardware devices.
→ See the next page
- **Customizing your statistics result** – How to change the layout of your result table.
→ See page 484
- **Exporting dependent variables and statistics** –
→ See page 492
- **Dependent Variables and their Statistics** –
→ See page 498

See also:

- **Managing Settings and Profiles**
→ See page 581



The analysis results depend both on whether you applied Smoothing during acquisition, Track Smoothing, the variables and statistics you selected in your Analysis profile, and on the data you selected in your Data profile.

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You cannot print statistics results In EthoVision XT. To do so, export them to another program (see page 496).

14.1 Calculating Statistics

Before you perform calculations, you must choose the **Dependent variables** and their **Statistics**.

Dependent Variables

The *Dependent Variables* are the variables that quantify the behavior of your subjects. In EthoVision 3, dependent variables were called *Parameters*.

Example 1 – To quantify locomotor activity, choose ‘Distance moved’, or ‘Velocity’ as Dependent Variable.

Example 2 – To quantify spatial orientation behavior, choose ‘Heading’ as Dependent Variable.

Example 3 – To calculate how many food pellets were dropped by your pellet dispenser, choose ‘Hardware continuous variable’ as Dependent Variable.

Dependent variables are calculated for each sample of the track data. The table below shows the **Distance moved** variable calculated for a few samples. X and Y are the coordinates of the center of the subject's body. Sample rate = 12.5 samples/s.

Time	X	Y	Distance moved
0	-8.7393	-26.1678	–
0.08	-6.8267	-26.9699	2.0740
0.16	-4.7220	-27.0748	2.1074
0.24	-3.2380	-26.6227	1.5513

Some dependent variables have discrete values. For example, **In zone** or **Mobility**. The following table shows the **In zone** variable calculated for a few samples. In zone=1, Not in zone=0. The subject's center point enters zone 1 at 0.16 s while its nose point is already in that zone. For clarity, X,Y coordinates have been removed from the table.

Time	In zone (zone1, nose-point)	In zone (zone1, center-point)
0	1	0
0.08	1	0
0.16	1	1
0.24	1	1

Statistics

Statistics are descriptive statistics of the values of Dependent Variables.

Example 1 – To quantify locomotor activity, choose the *Total* statistic for Distance moved, and the *Mean* statistic for Velocity.

Example 2 – To quantify spatial orientation behavior, choose the *Mean* and *Standard deviation* statistics for Heading.

For the complete list of Dependent Variables and their Statistics, see page 498 and page 558 respectively.

General procedure



The procedure below includes the instructions for selecting dependent variables and statistics before the actual calculations. If you have already done so, go to step 7, and make sure that the Analysis profile you want to apply is highlighted in blue in the Explorer.

- 1 Make sure that the Data profile specifying the data you want to analyze is active (that is, highlighted in blue in the Explorer).



- 2 From the **Analysis** menu, choose **Analysis Profile**.
- 3 In the **Analysis Profiles** window, select **New** and type a name for the new profile, then click **OK**.
- 4 Click the **Add** button next to the dependent variable you want to use for analysis (for an overview of dependent variables, see page 498).

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- 5 In the dependent variable-specific tab, select the properties of the dependent variable. In the **Statistics** tab, choose the statistics you want to calculate for that variable.
- 6 Repeat steps 4 and 5 to add more dependent variables.
- 7 From the **Analysis** menu select **Results** and then **Analysis Output** or press the **Calculate** button  on the component tool bar.
- 8 The statistics table appears on the screen.
 - To Interpret and customize your result, see page 484.
 - To export your result, see page 492.
 - To manage analysis profiles, see page 581.



Notes

- For more information on setting the dependent variables' properties, see page 482.
- Some dependent variables are available if you have one or more of the following add-ons: **Multiple Body Points**, **Social Interactions**, **Trial and Hardware Control**.
- You only can select specific body points if your experiment is set to **Center-point, nose-point and tail-base detection** (see page 102).
- You can also open an existing Analysis profile. In step 3 above, select **Open** and choose one from the list, or click that profile in the Experiment Explorer.
- For more information on Data profiles, see Chapter 11.
- You can change the properties or delete a dependent variable already selected. You can also add multiple instances of a dependent variables, with different settings (see below).

How to...

Change the properties of a dependent variable

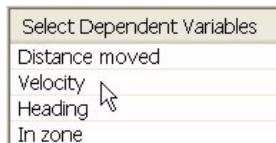
You can change the properties of a dependent variable at any time, for example to add one more statistic. Next, re-calculate the statistics.

In the Analysis profile

- 1 Open the Analysis profile that contains the dependent variable you want to change (see page 581).

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- 2 Double-click the dependent variable under **Selected Dependent Variables**, or right-click the dependent variable and select **Edit**.



- 3 Change the variable's properties.
- 4 From the **Analysis** menu select **Results** and then **Analysis Output** or press the **Calculate** button  on the component tool bar.

In the Analysis result

- 1 In the result table, right-click the dependent variable header and select **Properties**.
- 2 Change the properties of the variable.
- 3 Refresh the analysis result: from the **Analysis** menu select **Results** and then **Analysis Output** or press the **Calculate** button  on the component tool bar.



For more information on the variable's properties, see the *How to specify* section for that dependent variable, beginning from page 498.

Calculate multiple instances of a dependent variable

You can add multiple instances of a dependent variable to an Analysis profile. This is handy when you want to compare the effect of different settings on the same dependent variable (for example, different Mobility thresholds).

- 1 Open the Analysis profile that contains the dependent variable.
 - 2 Right-click the dependent variable under **Select Dependent Variables** and select **Duplicate**.
- Result** – A new row is appended under Select Dependent Variables.
- 3 Set the properties you require for the new instance of the dependent variable (see above).



Additional instances of a dependent variable are given names with a progressive number (for example, **Distance moved 2**, **Distance moved 3**, etc.). You can rename those variables (see below).

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Rename a dependent variable

- 1 Under **Selected Dependent Variables**, right-click the variable's name and select **Rename**.
- 2 Type the new name and press **Enter**.

Remove a dependent variable from your profile

To remove a dependent variable from a profile, under **Selected Dependent Variables** click the variable's name and press **Delete**, or right-click the variable's name and select **Delete**.

14.2 Customizing your results table

Interpreting the statistics result

By default, the statistics result is a table where each row corresponds to a subject tracked in one arena during a specific trial (see Figure 14.1.).

Result 1	Trial	Arena	Subject	Start-End	Variables		Distance moved		In zone			
					Object ID	Treatment	Center-point		Front Open 1		Nose-point Open 2	
							Total	cm	Frequency	s	Latency	s
Result 1	Trial 1	Arena 1	Subject 1	Start-End	A234S	Control	825.9	13	20.8	3	4.32	
	Trial 2	Arena 1	Subject 1	Start-End	A233S	Treated	239.9	7	11.04	8	2	
	Trial 4	Arena 1	Subject 1	Start-End	A324V	Control	292.9	4	6.64	2	14.16	
	Trial 5	Arena 1	Subject 1	Start-End	A221S	Treated	445.6	19	7.28	10	91.12	
	Trial 6	Arena 1	Subject 1	Start-End	A221D	Control	291	5	31.04	29	2.24	
	Trial 7	Arena 1	Subject 1	Start-End	A332S	Treated	507.6	22	39.6	12	156	
	Trial 8	Arena 1	Subject 1	Start-End	A434S	Control	317	5	148.7	13	46.16	
	Trial 9	Arena 1	Subject 1	Start-End	A3222	Treated	329.7	26	0.4	7	179.6	
	Trial 10	Arena 1	Subject 1	Start-End	A333S	Control	604.2	23	3.76	40	12.4	
	Trial 11	Arena 1	Subject 1	Start-End	A345S	Treated	3060	25	7.28	10	1.68	

Figure 14.1. An example of statistic result. A – Headers for Data Selection Results, Trials, Arenas, Subjects, Time bins considered. B – Headers for independent variables (by default, only user-defined variables are shown). C – Headers for the dependent variables and their statistics. D – Cells containing the value of independent variables for a specific item under A. E – Cells containing the statistics of a dependent variable for a specific item under A. See Showing and hiding results on page 487 to show/add columns and rows.

- The row headers (left-most columns) tell you which **Trial**, **Arena** and **Subject** a specific result corresponds to.

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Because of the hierarchical structure of Trials, Arenas and Subjects, **Trial** can contain one or more **Arenas**, and one or more **Subjects**.

- Other headers are shown on rows:
 - **Result** – The Data Selection Result used for analysis (this corresponds to the name of the **Result** box in your Data Selection; see page 387);
 - **Start-End** – The time bin considered for analysis. If you apply **Results per time bin** (see page 430), Start-End is replaced by the time interval for a specific row (for example, **0:01:00- 0:02:00**).
 - **Nest over zone** – The names of the zones you used for nesting (see page 406) are displayed on rows.
 - **Results per zone** – The names of the zones and body points you selected in the Results box in a data profile (see page 430) are displayed on rows.
- The column headers (at the top of the table) are divided in two parts:
 - **Independent Variables** (headers of gray columns) – Lists the independent variables associated to a result. By default, all the user-defined variables are shown.
 - **Dependent Variables** – Lists the dependent variable and statistic corresponding to a result. The dependent variables and statistics are selected in the Analysis profile you have used to run analysis.

Showing and hiding table headers

By showing/hiding headers of the table, you do not change the main structure of the table. For example, if you choose to remove the headers for **Arenas**, you still see the results obtained for each Arena. To remove the results, see **Showing and hiding results** below.

To show or hide a header, click the **Show/Hide** button  on the component tool bar and make sure that the corresponding option under **Column Headers** or **Row Headers** is selected or deselected respectively.

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Example

		Arena 1	Subject 1	Start-End	759.6
		Arena 2	Subject 1	Start-End	
Result 1	Trial 3	Arena 3	Subject 1	Start-End	496.3
		Arena 4	Subject 1	Start-End	541.2
		Arena 1	Subject 1	Start-End	675.4
		Arena 2	Subject 1	Start-End	773.3
	Trial 4	Arena 3	Subject 1	Start-End	501.7
		Arena 4	Subject 1	Start-End	396.5

You want to remove the following headers:

- Selection Result (named **Result 1** in this example), because there is only Result 1 in your Data Profile, and therefore that header does not add information to the table.
- Time bin (named **Start-End** in this example), because you analyze the whole length of each track (no Results per time bin defined), therefore that header does not add information to the table.

Solution – Click the **Show/Hide** button, then **Row Headers**, and de-select **Selection Result** and **Time bin**.

Result:

	Arena 1	Subject 1	759.6
Trial 3	Arena 2	Subject 1	620.1
	Arena 3	Subject 1	496.3
	Arena 4	Subject 1	541.2
Trial 4	Arena 1	Subject 1	675.4
	Arena 2	Subject 1	773.3
	Arena 3	Subject 1	501.7
	Arena 4	Subject 1	396.5

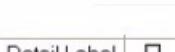


Notes

- If you de-select Independent Variables, the columns containing the values of independent variables are hidden.
- If you de-select **Trials**, Arenas or Subjects, statistics for multiple Trials, Arenas and Subjects are **not** lumped together.
- If you de-select Dependent Variables and Statistics, the results are not removed. Only the names of Dependent Variables and Statistics are removed from the headers.
- If you de-select **Detail Label** from the Column Headers, the name of the body point or zone used to calculate that dependent variable is removed. For

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example, remove **Center-point** from the header of the dependent variable **Distance moved**:



The diagram illustrates the process of hiding a table header. On the left, there is a table titled "Distance moved" with rows for "Center-point", "Total", "cm", "653.8", and "852.2". A blue arrow points to a "Detail Label" button, which is a small rectangular icon with a square checkbox and the text "Detail Label" next to it. To the right of the button is another table titled "Distance moved" with rows for "Total", "cm", "653.8", and "852.2". The "Center-point" row has disappeared.

Distance moved	
Center-point	
Total	
cm	
653.8	
852.2	

Detail Label



Distance moved	
Total	
cm	
653.8	
852.2	

- If you clear **Unit** from the Column Headers, the units for the variables as defined in the Experiment Settings (see page 102) are hidden.
- You can swap rows and columns of your table when you export the analysis output (see page 496).

Showing and hiding results

By showing/hiding results, you change the main structure of the table. If you want to remove only the headers, see the section above.

- 1 Click the **Show/Hide** button  on the component tool bar and select the corresponding category of results.
 - To have a result displayed in the table, in the window that appears, make sure that the item you want to have in the table is selected.
 - To hide a result, de-select the item you do not want to have in the table.
- 2 Click **OK**.

Example 1

A number of statistics has been calculated for a setup with multiple arenas. You want to remove the results for *Trial 3* and all results for *Arena 2*.

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Result 1	Trial 3	Arena 1	Subject 1	Start-End	759.6
		Arena 2	Subject 1	Start-End	620.1
		Arena 3	Subject 1	Start-End	496.3
		Arena 4	Subject 1	Start-End	541.2
	Trial 4	Arena 1	Subject 1	Start-End	675.4
		Arena 2	Subject 1	Start-End	773.3
		Arena 3	Subject 1	Start-End	501.7
		Arena 4	Subject 1	Start-End	396.5

Solution – Click the **Show/Hide** button, then **Trials** and clear **Trial 3**.



Next, click the **Show/Hide** button again, select **Arenas** and clear **Arena 2**.

Result – The rows for Trial 3 and Arena 2 are removed from the table.

Trial 2	Arena 1	Subject 1	Start-End	1974
	Arena 3	Subject 1	Start-End	1592
	Arena 4	Subject 1	Start-End	1934
Trial 4	Arena 1	Subject 1	Start-End	675.4
	Arena 3	Subject 1	Start-End	501.7
	Arena 4	Subject 1	Start-End	396.5

Example 2

You have obtained a results table containing the values of your user-defined variables: Subject ID, Treatment level, etc. You want to remove **Subject ID** and include the system variable **Start Time** in the table.

Independent Variables		Distance moved
Subject ID	Treatment	Total
		cm
A21003	Treated	653.8
A21345	Control	852.2
A21443	Treated	630.1
A21444		758.9
A21344	Control	1974
A21346		2026
A21347	Treated	1592
A21348		1934

Solution – Click **Show/Hide**, then **Independent Variables**, select **Start Time** and clear **Subject ID**.

Calculating Statistics

- Starttime
- Subject ID

Result:

Independent Variables		Distance moved
Starttime	Treatment	Total
		cm
19-Jan-05 12:25:28.0	Treated	653.8
	Control	852.2
	Treated	630.1
		758.9
	Control	1974
		2026
19-Jan-05 12:26:16.0	Treated	1592
	Treated	1934



Notes

- You can also show/hide results by changing the properties of the dependent variables. Right-click the dependent variable headers in your table and select **Properties**. Make the changes you require and re-calculate the statistics to refresh the table.
- You cannot lump or group results for Trials, Arenas and Subjects together in the table. Changing the layout has no effect on the calculations.
- If you add a user-defined variable to your Trial List (see page 117) after you have created your statistics result, and you re-do the calculations, the result does not include the new variable. You must click the **Show/Hide** button, select **Independent Variables** and select the new user-defined variable.
- If you want to add a new Dependent Variable, do this in the Analysis profile (see page 481) and then re-run the calculations.

Sorting row and column headers

To sort row and column headers, right-click one of the headers belonging to the category you want to sort, and select **Sort Ascending** or **Sort Descending**.

Example

To sort the columns for dependent variables, right-click the dependent variable header and select the appropriate **Sort** options.

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Distance moved				
Maximum	Mean	Minimum	Sort Ascending	Sort Descending
cm	cm	cm	Properties	
2.068	0.5939	0.0682		
1.466	0.7197	0.0593		
2.938	0.8595	0.01549	264.7	

Result – The columns for dependent variables are sorted accordingly. However, columns at lower levels, for example the statistics (see the picture above), are not sorted within each dependent variable column.



Notes

- When you sort column headers, you do not sort the cells contained in those columns. If you want to sort cells, see the next section.
- When you sort by a category, categories at lower and higher levels in the table are not sorted.
- You can swap rows and columns of your table when you export the analysis output (see page 496).
- See page 496 for the procedure to export for analysis in a statistical program such as SPSS.

Sorting cells

To sort cells, right-click one of the cells containing the values you want to sort, and select **Sort Ascending** or **Sort Descending**.

Example

You want to sort the table below according to the value of the independent variable **Treatment**, in such a way that the tracks for **Control** subjects appear in the upper half of the table.

Calculating Statistics

		Independent Variables	Distance moved
	Day	Treatment	Total
Trial 1	1	Arena 1	Treated 653.8
		Arena 2	Control 852.2
		Arena 3	Treated 630.1
		Arena 4	758.9
Trial 2	1	Arena 1	Control 1974
		Arena 2	2026
		Arena 3	Treated 1592
		Arena 4	1934
Trial 3	1	Arena 1	Control 759.6
		Arena 2	Treated 620.1
		Arena 3	496.3
		Arena 4	541.2
Trial 4	2	Arena 1	Control 675.4
		Arena 2	Treated 773.3
		Arena 3	Control 501.7
		Arena 4	Treated 396.5

Solution – right-click one of the Treatment cells and select **Sort Ascending**.



Result:

		Independent Variables	Distance moved
	Day	Treatment	Total
Trial 1	1	Arena 2	852.2
		Arena 4	758.9
		Arena 1	1974
		Arena 2	2026
Trial 2	1	Arena 1	759.6
		Arena 4	541.2
		Arena 1	675.4
		Arena 3	501.7
Trial 3	1	Arena 1	653.8
		Arena 3	630.1
		Arena 3	1592
		Arena 4	1934
Trial 4	2	Arena 2	620.1
		Arena 3	496.3
		Arena 2	773.3
		Arena 4	396.5

14.3 Exporting data and statistics

You have four options for exporting your data:

- **Exporting raw data and dependent variables** – To export the X,Y coordinates and subject's area, together with the value of the dependent variables for each sample (including the manually-scored data).
→ See the next page
- **Exporting the GLP log files** – You can export the General and Experiment log files.
→ See page 580
- **Exporting the manual scoring log** – To export the manually scored data in a text file for import into The Observer XT.
→ See page 495
- **Exporting analysis output** – To export the analysis output table.
→ See page 496



If you export data to Microsoft Excel 2003 or below, this has a file limit of 256 columns and 65536 rows. However, in this case you can create a export file (*.txt) longer than that in EthoVision XT and open it in another program, such as SPSS.

Exporting track data and dependent variables



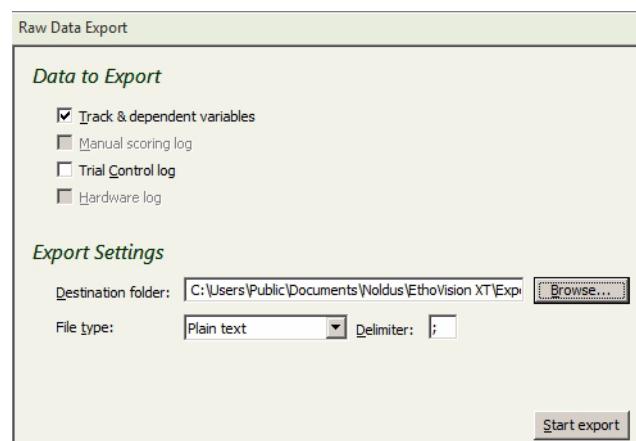
With this option you can also export the manually scored data in the same format as the other dependent variables, that is, each time point (sample) receives a value of the behavior ('Rearing' or 'Not Rearing'). If, on the other hand, you want to export manually scored behaviors to a more condensed format (for example, 5.12s Rearing, 5.88s Not Rearing) or you wish to export them to The Observer XT, then choose **Exporting the manual scoring log** (see page 495).

- 1 Make sure your Analysis Profile/Track Smoothing Profile/Data Profile, which specifies the dependent variables you want to export, is highlighted in blue under **Analysis Profiles** in the Experiment Explorer (If not: right-click the Analysis profile you require and select **Set as Current**).

Calculating Statistics



- 2 From the **Analysis** menu select **Export** and then **Raw Data**, or in the Experiment Explorer under **Analysis - Export** click **Raw Data**.



- 3 Select **Track & dependent variables**.
- 4 Click **Browse** next to **Destination folder** and navigate to the location where you want to save the export files.
- 5 Under **File Type**, select one of the following:
- **Plain text** – To export the data as a text (*.txt) files. Next, enter the character that you want to use to separate columns in the exported file in the **Delimiter** field.
 - **Excel** – To export the data as Excel files (*.xls or *.xlsx, depending on which Excel version you have on your computer).
- 6 Click **Start export**.



Notes

- If you export to **Plain text**, each track is exported as a separate file. Each file name is unique, and is formed by, in the following order:
 - Export type (Raw Data, or Manual Scoring, Trial Control or Hardware, depending on what option you have chosen);

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- Experiment name;
- Trial number and Arena name.

For example **Raw Data-Open Field-Trial 1-Arena 1.txt**

- If you export to **Excel**, the file contains all tracks of a trial. The file name is unique and formed by the following elements:
 - **Raw data** (independent of the type of export);
 - Experiment name;
 - Trial number.
- For example **Raw Data-Plus Maze-Trial 1.xlsx**: Data of each subject is written in a separate tab of the worksheet, named as follows: Export type (Track, Manual Scoring or Trial Control, depending on what option you have chosen) + Arena name + Subject name.
- A warning might appear that the file is in a different format than specified by the file extension when you open the *.xls file in MS Office 2007. When you next click **Yes**, the file opens without problems.
 - If you export the same data multiple times to the same location, EthoVision attaches a number at the end of the file name: (1), (2), etc.
 - The files' suggested location is the **Export Files** folder located in your experiment folder.
 - It is not necessary to calculate statistics and have a statistics result displayed on the screen in order to export dependent variables.
 - The values exported have a variable number of decimals, depending on the number of integers. The total number of significant digits exported is always six (for example, 1.23456 or 1234.56), or seven if the unsigned number is smaller than one (0.123456).
 - Both missing coordinates of body points and values of dependent variables that could not be calculated for a specific sample are exported as '-'.
 - **Difference between Trial time and Recording time – Trial time** is the time elapsed from the start of the trial (basically, the moment when you clicked the green **Start** button). Recording time is the time elapsed from the start of the track. For more information on trials and tracks, see page 203.
 - Choose **Track & dependent variables** also if you want to export Trial Control events and states defined in your Analysis profile (see page 571). For example, to export the state 'from *Cue light on* To *Subject in Feeder zone*'. The state is exported as a series of 0s and 1s (0= state not active, 1= state active) in the corresponding column of the export file.

Calculating Statistics

- Choose **Trial Control log** if you want to export Trial Control events and states with their own time stamp, for example 6.8 s - *Cue light on - Activated*. For more information, see page 571.
- Choose **Hardware log** if you want to export the complete log of events of hardware devices as a separate file. See the EthoVision XT Trial and Hardware Control Manual on your installation DVD.

Exporting the manual scoring log

Exporting the manual scoring log means that the manually-scored data will be saved to a file with the following structure:

Time stamp - Subject - Behavior - Event type (start or stop)

For example:

6.8 Subject 1 Grooming Head State start
8.12 Subject 2 Grooming Head Start stop

- 1 From the **Analysis** menu, select **Export** and then **Raw Data**, or in the Experiment Explorer under **Analysis - Export** click **Raw Data**.
- 2 Make sure that **Manual scoring log** is selected.
- 3 Select the export format. If you export to The Observer XT, select **Plain text**, and select semicolon (;) as **Delimiter**.
- 4 Click **Export**.

For more options see page 492.

Notes



- To import data into The Observer XT, see page 575.
- If you export to Excel, the manually scored data are stored in a separate tab of the Excel worksheet. The tab is named **Manual scoring - <arena name>**.
- If you export to Plain text, data of different arenas are exported to different files.
- For more information on file names and formats, see page 493.

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Exporting statistics

- 1 Make sure your statistics result is open on your screen and shows the layout you require. If the table's layout does not fit the program you want to export the result to, change it accordingly (see page 484).
- 2 From the **Analysis** menu, select **Export** and then **Analysis Output**, or in the Experiment Explorer under **Analysis - Export**, click **Analysis Output**.
The **Export analysis results** window appears.

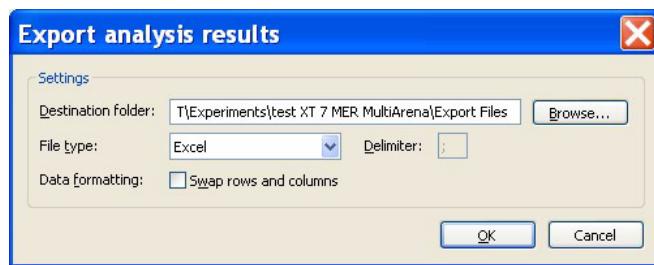


Figure 14.2. The **Export analysis results** window.

- 3 Click the **Browse** button to select the location for your export file. Its suggested location is the **Export Files** folder located in your experiment folder.
- 4 Under **File Type**, select one of the following:
 - Plain text** – To export the table as a text (*.txt) file. Next, enter the character that you want to use to separate columns in the exported file in the **Delimiter** field.
 - Excel** – To export the table as an Excel file (*.xls or *.xlsx, depending on which Excel version you have on your computer).

You have the additional option:

- Swap rows and columns** – Select this option if your statistical program requires this.

- 5 Click **OK**.

Notes



- The name of the export file is **Analysis Output-[Experiment name]**.
- If you export results multiple times to the same location, EthoVision attaches a number at the end of the file name: (1), (2), etc.
- The exported file contains the rows and columns that are displayed on the analysis result currently on the screen. Hidden rows and columns (see page 487) are not exported.
- Cells for statistics that could not be calculated (for example, the velocity of a subject that was never found in a zone) are exported as '0'.

Export settings for specific programs

SPSS

- Recommended format: Text or Excel.
- Web site: <http://www.spss.com/spss>



See page 573 for a description of how to export statistics to SPSS.

Systat

- Recommended format: Text or Excel.
 - Each case must be written in one line.
 - Separator (for text files): space or comma.
- Web site: <http://www.systat.com>.

SAS

- Recommended format: Text or Excel.
- For text files: use the DELIMITER option to specify the text delimiter if this is other than space.
- For Excel files: use the PROC IMPORT procedure with the SHEET option to specify the worksheet to import. Use the GETNAMES option to read variable names from the first row of the worksheet.
- You can also use the Import Wizard from the File menu (however, this can be time consuming when used repeatedly).

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Web site: <http://www.sas.com>

Genstat

- Recommended format: Text or Excel.
- Data must be arranged in rectangular format, with one variable per column.

Web site: <http://www.vsni.co.uk/software/genstat>.

Statistica

- Recommended format: Excel or Text.
- You can also copy and paste data tables from the EthoVision XT statistics table to a Statistica file.

Web site: <http://www.statsoft.com>.

GB STAT

- Recommended format: Excel or Text.
- For Excel files, Variable names can be imported if they are placed in the first row of the spreadsheet.
- For Text files, the number of variables must be known. Alphabetic information will be skipped over, but embedded numeric information will be picked up as data values.
- Special characters and dates may be misinterpreted by GB STAT.

Web site: <http://www.gbstat.com>.

14.4 Dependent Variables



Some dependent variables are only available if you have the **Multiple body points** add-on, the **Trial and Hardware Control** add-on or the **Social Interactions** add-on installed.

For the statistics available, see page 558.

Distance and Time

- Distance moved (page 500)

Calculating Statistics

- Velocity (page 501)

Location

- In zone (page 502)
- Distance to zone (page 504)
- Distance to point (page 507)
- Zone transition (page 508)
- Heading to point (page 510)

Path Shape

- Head direction (page 512)
- Heading (page 514)
- Turn angle (page 517)
- Angular velocity (page 522)
- Meander (page 524)

Individual Behavior

- Movement (page 526)
- Elongation (page 528)
- Mobility (page 530)
- Rotation (page 536)
- Head directed to zone (page 539)

Social Behavior

- Distance between subjects (page 541)
- Proximity (page 542)
- Relative movement (page 545)
- Net weighted movement (page 549)
- Weighted movement from (page 550)
- Weighted movement to (page 553)

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Manually Scored Behavior

This section lists the behaviors (and their groups) that you defined for scoring manually behavioral data (see page 555).

Trial Control

- Trial Control state (page 556).
- Trial Control event (page 557).

Distance moved

Definition

The distance traveled by the center, nose or tail-base point of the subject from the previous sample to the current one. This variable is also known as 'trip distance'.

Distance moved is calculated as:

$$DM_n = \sqrt{(X_n - X_{n-1})^2 + (Y_n - Y_{n-1})^2}$$

where DM_n = Distance moved from sample $n-1$ to sample n , X_{n-1} , Y_{n-1} = X,Y coordinates of the center, nose or tail-base point at sample $n-1$, X_n , Y_n = X,Y coordinates of the center, nose or tail-base point at sample n .

In the **Distance moved** window, click the **Subject** tab and specify the body points Distance moved should be calculated for.

How to specify Distance moved

- 1 Click **Add** next to **Distance moved** (see page 481), then click the **Body points** tab and select the body points for which you want to calculate the distance. By default, **Center-point** is selected.
- 2 Click the **Statistics** tab to choose the statistics for distance (see page 558 for details). Next, click **Add**.



If your experiment is set to **Only center-point detection** or **Color marker tracking**, the **Body points** tab is absent. Calculations are based on the center point.

See the Remarks on page 501 about how sample rate and missing samples might influence Distance moved.

Calculating Statistics

Application

Distance moved is often used to give a general measure of activity. It is also used as the basis for calculating other parameters such as velocity (see below).

Velocity

Definition

The distance moved by the center, nose or tail-base point of the subject per unit time. It is also known as 'Linear velocity', 'Locomotor rate' and 'Speed of movement'.

Velocity is obtained by dividing Distance moved, calculated as on page 500, by the time difference between a sample and the previous one:

$$V_n = \frac{DM_n}{t_n + t_{n-1}}$$

where V_n = velocity at sample n (expressed in the unit you have defined in the Experiment Settings) and DM_n = Distance moved at sample n .

How to specify Velocity

- 1 Click the **Add** button next to **Velocity** (see page 481) and click the **Body points** tab. Select the body points for which you want to calculate the velocity. By default, **Center-point** is selected.
- 2 Click the **Statistics** tab to choose the statistics for velocity (see page 558 for details). Next, click **Add**.



If your experiment is set to **Only center-point detection** or **Color marker tracking**, the **Body points** tab is absent. Calculations are based on the center point.

Remarks

- **Sample rate influences the calculation of Velocity/Distance moved** – When tracking at too low a sample rate, parts of the actual path are cut off, resulting in an underestimation of per-sample Velocity/Distance moved. If, on the other hand, the sample rate is too high, EthoVision XT catches the wobbling of the body's center point of the walking animal, causing extra apparent movement, and therefore an overestimation of Velocity/Distance moved.

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See Track Smoothing (page 378) for a description of how to filter out small movements.

- **Missing samples do not affect the calculation of Velocity/Distance moved**
 - Velocity/Distance moved are calculated only for adjacent non-missing samples; missing samples are ignored. However, make sure that the proportion of missing samples is low (less than 1%).



You can view the proportion of missing samples as one of the System Variables in the Trial list.

Application

Apart from the obvious applications of this dependent variable, the mean velocity is sometimes used as a measure of general activity (for example, Nilsson *et al.* 1993, *J. Exp. Biol.* **180**, 153-162; Winberg *et al.* 1993, *J. Exp. Biol.* **179**, 213-232).

In zone

Definition

A discrete (state) variable with two possible states, **In zone** and **Not in zone**, depending on whether the body point chosen is within a zone (or group of zones).

The state for a specified zone is determined for each sample by comparing the coordinates of the chosen body point with the coordinates that make up the zone of interest.



- When you export In zone as a dependent variable (see page 492), the values are exported as 0 (Not in zone) and 1 (In zone).
- When a body point is missing, the **In zone** state does not change from the previous sample.

How to specify In zone

- 1 Click the **Add** button next to **In zone** (see page 481) and click the **In zone** tab.
- 2 Under **In the following zones**, select the zones you want to analyze. For example, if you want to calculate the total time the animal was in Zone 1, select Zone 1.

Calculating Statistics

If you have chosen two or more zones, select how body points should be analyzed:

- **For each selected zone** – The body points are analyzed in each zone separately.
 - **When in any of the selected zones** – The body points are analyzed when in any of the selected zones.
 - **When in all selected zones** – The body points are analyzed when in all those zones simultaneously.
 - **When not in any of the zones** – The body points are analyzed that are in none of the selected zones.
- 3 Under **From following body points**, select the points you want to consider for the calculation. For example, select **Nose-point** if you want to calculate the statistics of the time the nose point was in a specific zone. By default, **Center-point** is selected.
- If you have chosen two or more body points, select one of the following from the list:
- **For each selected point** – Statistics are calculated for each point separately.
 - **If any point is in zone** – Statistics are calculated for when any of the selected points is in the zone.
 - **When all points are in zone** – Statistics are calculated for when all the selected points are in a zone simultaneously.
- 4 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.



If your experiment is set to **Only center-point detection** or **Color marker tracking**, the In zone variable is calculated for the center-point.

Application

The dependent variable In zone is a standard variable for any study involving the usage of space by animals. For example:

- **Open field** – How much time did the animal spend by the walls, and how long did it take to cross the open center? (for example, Berendsen *et al.* 1994, *Behav. Pharm.* 5 (Suppl. 1): 81).
- **Maze studies** – How many errors did the animal make? (for example, Ploeger G.E. 1995, PhD thesis, Utrecht University) How long did it take to get to the target (Ploeger *et al.* 1994, *Behav. Neurosci.* 108, 927-934) How many times did

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the animal enter the open arms in a plus maze? (Law *et al.* 2003. *J. Neurosci.* 23: 10419-10432).

- **Four-way olfactometer** – How much time did the animal spend in the treated odor field? When did it first enter one of the arms? (Kaiser and de Jong 1994, *Behav. Proc.* 30: 175-184).
- **Water-maze** – How much time does the animal spend in an 18-cm wide path (Whishaw's corridor) from the starting location to the platform, designated as the correct route? If a rat deviated from this route, it received a maximum of one error on that trial (Whishaw's error, Whishaw 1985, *Behav. Neurosci.* 99(5): 979-1005), indicating that it did not show a direct swim path.

Distance to zone

Definition

The shortest distance between a subject's body point (or selection of points) and a zone (or group of zones). You can calculate the distance regardless of where the body point is, or assuming that the point is always outside the zone (in the latter case, the distance is set to zero when the body point enters the zone).

The calculation of this variable is performed in two steps:

- 1 The coordinates of the point on the zone border that is closest to the coordinates of the body point for the current sample are found.
- 2 The distance in a straight line between the two coordinates is calculated.

How to specify Distance to zone

- 1 Click the **Add** button next to **Distance to zone** (see page 481) and click the **Distance to zone** tab.
- 2 Under **To the following zones**, select the zones you want to consider for the calculation. For example, if you want to calculate the mean distance to Zone 1, select Zone 1. By default, **Arena** is selected.
If you have chosen two or more zones, select how the zones should be analyzed:
 - **For each of the selected zones** – Zones are analyzed separately.
 - **Shortest distance to any zone** – For each sample, EthoVision XT chooses the zone that is currently closest to the point(s) you have chosen, and uses the resulting distances for calculating the statistics.

Calculating Statistics

- 3 Select the **Include if in zone** option if you want to calculate the distance to the border of a zone of interest, regardless of whether the subject is outside or inside the zone. If you want to calculate the distance to the border of the zone when the subjects is outside the zone, leave this option cleared. See one of the Remarks below.
- 4 Under **From following body points**, select the points you want to consider for the calculation. For example, select **Nose-point** if you want to analyze the distance between the nose-point and a specific zone. By default, **Center-point** is selected.

If you have chosen two or more body points, select one of the following from the list:

- **For each selected point** – Statistics are calculated for each point separately.
 - **Shortest distance to any point** – For each sample, EthoVision XT chooses the body point that is currently closest to the zone, and uses the resulting distances for calculating the statistics.
- 5 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.



If your experiment is set to **Only center-point detection** or **Color marker tracking**, the body point options are not available. Calculations are based on the center point.

Remarks

If you have selected **Include if in zone** in the **Distance to zone** tab of the variable's properties window, and the body point's coordinates lie inside the zone, the Distance to zone > '0' (see also Figure 14.3.).

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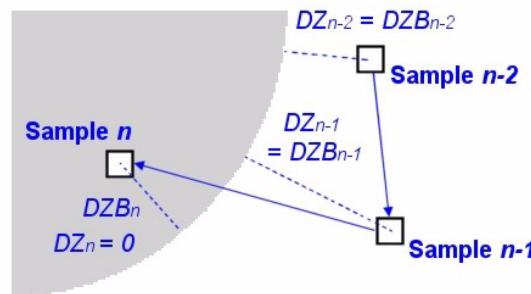


Figure 14.3. The Distance to zone (DZ, dotted lines) for three consecutive samples. The zone is shown in gray. When the sample is within the zone, DZ is zero. When you select **Include if in zone** in the variable properties (see text), the Distance to zone border (DZB) is calculated. In this case, when the sample is within the zone, DZB is greater than zero. In all other cases, DZ is equal to DZB.

Application

Two examples of how you can use Distance to zone (with **Include if in zone** not selected, see above):

- In a Morris water maze test with the hidden platform defined as a zone, Distance to zone can measure the animal's progress towards the platform. You can select the **Total** statistic to give a measure of the cumulative distance to zone (for training trials) and the **Mean** statistic to give a measure of average proximity (for probe trials; Gallagher *et al.* 1993. *Behav. Neurosci.* **107**: 618-626). You can also divide the maze into quadrants to give a more fine-grained analysis of behavior during the trial.
- In a study of territorial behavior, the territory of one animal could be defined as a zone, allowing you to measure how close the other animal comes to the area occupied by its rival.

One example of how you can use Distance to zone with **Include if in zone** selected (see above) is the following:

- In a study of anxiety, one could define an entire open field as a zone, and then use Distance to zone to measure to what extent animals dare to move away from the wall. More generally, if you are interested in the distance between a subject and the edge of an open field or the border of a plus-maze, you can select the complete arena as a zone.+

Distance to point

Definition

The shortest distance between a subject's body point and one or more points.

The calculation of this variable is performed in two steps:

- 1 The coordinates of the defined point(s) and the body point(s) for the current sample are found.
- 2 The distance in a straight line between the coordinates is calculated.



If your experiment is set to **Only center-point detection** or **Color marker tracking**, the body point options are not available. Calculations are based on the center point.

How to specify Distance to point

- 1 Click the **Add** button next to **Distance to point** (see page 481) and click the **Distance to point** tab.

- 2 Under **To following points**, select the points you want to consider for the calculation. For example, if you want to calculate the mean distance to Cue 1, select Cue 1. By default, **Arena** (that is, the center of the Arena) is selected.

If you have chosen two or more points, select how the points should be analyzed:

- **For each of the selected points** – Points are analyzed separately.
 - **Shortest distance to any points** – For each sample, EthoVision XT chooses the point that is currently closest to the body point(s) you have chosen, and uses the resulting distances for calculating the statistics.
- 3 Under **From following body points**, select the points you want to consider for the calculation. For example, select **Nose-point** if you want to analyze the distance between the nose-point and a specific point. By default, **Center-point** is selected.

If you have chosen two or more body points, select one of the following from the list:

- **For each of the selected points** – Statistics are calculated for each point separately.
- **Shortest distance to any point** – For each sample, EthoVision XT chooses the body point that is currently closest to the point, and uses the resulting distances for calculating the statistics.

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- 4 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.



If your experiment is set to **Only center-point detection** or **Color marker tracking**, the body point options are not available. Calculations are based on the center-point.

Remark

The center point of a zone can lie outside the zone itself. This occurs when the zone is ring-shaped or very asymmetrical.

Application

Below are two examples of how Distance to point is of particular use in studies of spatial orientation:

- When analyzing the flight behavior of an insect in an odor plume, the plume itself can be defined as a zone, while the upwind odor source is regarded as a point. Using Distance to point, you can measure the insect's progress towards the source at any moment in time.
- In an open field test, using the center point of the central area of the arena, Distance to point can be used to measure how far the animal ventured into the central area.

Zone transition

Definition

This is the number of times an animal moves from one Zone to another.

A Zone transition is counted if the time of entering the **From** Zone is before the time of entering the **To** Zone.

How to specify Zone transition

- 1 Click the **Add** button next to **Zone transition** (see page 481) and click the **Zone transition** tab.
- 2 Select a Zone both in the **From** list and in the **To** list.
- 3 Depending on which option you select (**Allow intermediate zone visits** or **Only count direct transitions**) you can get different results.

Calculating Statistics

Example - An animal moves from Zone A, via Zone B to Zone C (see Figure 14.4. below).

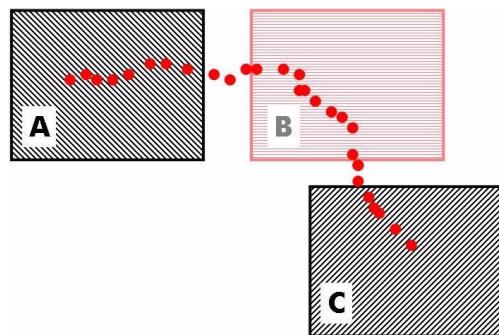


Figure 14.4. Sample points of an animal moving from Zone A to B to C. Zone B is in a different Zone group.

Zone B is in a different Zone group than Zones A and C. You select Zone A in the **From** list and Zone C in the **To** list. Depending on the option you select, you get the following results:

- If you select **Allow intermediate zone visits**, the calculated number of zone transitions is '1', because it does not matter that the animal visits other zones between moving from zone A to zone C.
 - If you select **Only count direct transitions**, the calculated number of zone transitions is '0', because the animal did not move directly from Zone A to C.
- 4 In the **Body points** tab, select the body point(s) you want to use for calculation.
- 5 Click **Add**.



The **Body Points** tab is only available if your experiment is set to **Center-point, nose-point and tail-base detection**.

Application

The dependent variable **Zone transition** can be used to investigate how an animal explores the environment. For example, in studies on foraging behavior, you can determine whether an animal visited food patches in a certain order by calculating Zone transitions for different combinations of food patches.

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Excel macro Zone transition

Included on the EthoVision XT installation DVD is an Excel file that contains a macro that calculates additional statistics based on the dependent variable In zone.

To use this macro, do the following:

- 1 Export the track data, including the dependent variable In Zone.
- 2 Copy the Zone transition.xls file from the **Utilities** folder on the EthoVision XT installation DVD to a folder on your computer.
- 3 Open the Zone transition.xls file and follow the instructions in this file. When opening this file, click **Enable Macros**.

Heading to point

Definition

The direction of movement of the nose, center or tail-base point of the current sample relative to a point of interest. Heading ranges from -180° to +180°. 'Compass heading' and 'compass angle' are synonyms for Heading.



If your experiment is set to **Only center-point detection** or **Color marker tracking**, calculations are based on the center point.

Calculation

Heading to point is calculated in a way similar to Heading (page 514). The difference is that for Heading to point the reference line is the line that connects the previous sample and the point of interest. For Heading the reference line is the line parallel to the x axis (see Figure 14.5. and Figure 14.8.).

How to specify Heading to point

- 1 Click the **Add** button next to **Heading to point** (see page 481).
- 2 In the **Heading to point** tab, under **For following body points**, select one or more body points for which you want to calculate Heading to point.
- 3 From the **Point of interest** list, select the center of the Arena, center of gravity (COG) of a zone or a point you defined in the Arena Settings.

Calculating Statistics

- 4 Click the **Statistics** tab to choose the statistics for this dependent variable (see page 558 for details). Next, click **Add**.

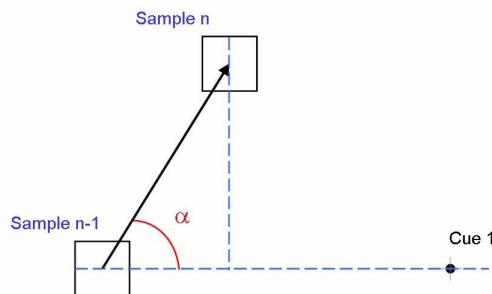


Figure 14.5. Relation between angle α and the dependent variable Heading to point relative to the reference line that connects sample $n-1$ and the point of interest ('Cue 1').

Application

Heading to point can be used to calculate the animal's orientation relative to a point of interest. For example, in the Morris water maze, Heading to point can be used to determine the Heading angle error. The Heading angle error is usually determined after the animal has traveled a minimum distance. The Heading angle error at this point is the deviation from a direct line from starting point to center of the platform (see Figure 14.6.).

Heading to point can also be used to analyze the animal's movement relative to a novel object; for example, the assumption could be that an animal is interested in a novel object when it is heading towards the center of the object.

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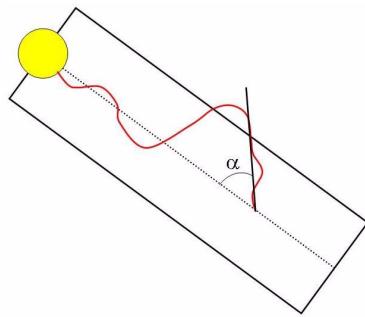


Figure 14.6. The Heading angle error (α).

Head direction

Definition

The smallest angle formed by the Head direction line of the current sample relative to a line parallel to the x axis in the coordinate system. Head direction ranges from -180° to +180° (see Figure 14.7.). See also Head directed to zone (page 539).

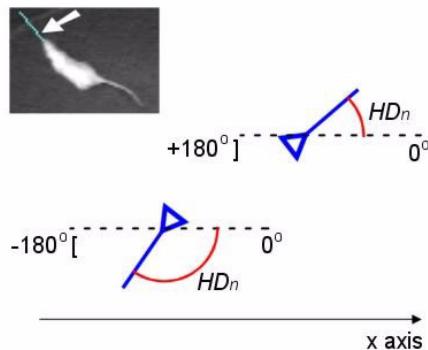


Figure 14.7. Two examples of how Head direction (HD) is calculated for a generic sample n. The triangle indicates the nose-point and the line departing from it is the Head direction line. Note that the position of the axis origin does not influence the calculation of the heading.



- Head direction is not available if your experiment is set to **Only center-point detection** or **Color marker tracking**.
- You can check the Head direction during data acquisition. Click the **Show/Hide** button on the component tool bar, select **Track Features** and make sure **Head direction** is selected. Next, let the animal move in the arena or play the video file. The real time Head direction is shown in the **Analysis Results and Scoring** pane (see page 276).

How to specify Head direction

Click the **Add** button next to **Head direction** (see page 481). Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.

Remarks

- During acquisition, the Head direction line is calculated from the Subject's contour, therefore its value also depends on the shape of the Subject on that sample.
- Head direction is always calculated relative to the orientation of the x axis you have chosen in the Arena Settings used for that trial (see page 160). By default, the x axis is horizontal and pointing to the right. If the x axis is not horizontal or is pointing to another direction, Head direction is calculated

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accordingly. Therefore, the same Head direction line may result in different Head direction values if it is tracked with different axes.

- The range of Head direction values is $[-180^\circ; +180^\circ]$. Values from 0° to $+180^\circ$ occur when the Head direction line is left to reference axis. Values from 0° to -180° (excluded) occur in the other cases (see Figure 14.7.).
- If you swap nose-point and tail-base, the Head direction value is removed and cannot be recovered for the samples that are swapped.

Application

Head direction is useful for studies of spatial orientation and searching behavior. For this you can, for example, use the direction of gaze or the variation in Head direction.

Heading

Definition

The direction of movement of the nose, center or tail-base point of the current sample relative to a line parallel to the x axis in the coordinate system. Heading ranges from -180° to $+180^\circ$ (see Figure 14.8.). 'Compass heading' and 'compass angle' are synonyms for Heading.

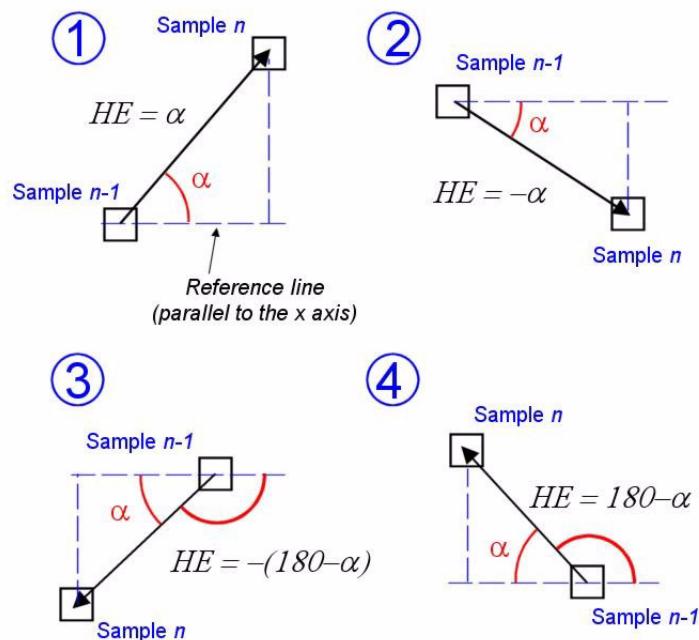


Figure 14.8. Relation between angle α and the dependent variable Heading (HE) for the four directions an animal's body point can be moving relative to the x axis. Here, it is assumed the x axis is horizontal and pointing to the right, and the y axis is pointing upward.

See also Heading to point (page 510).

Calculation

Heading is calculated in three steps:

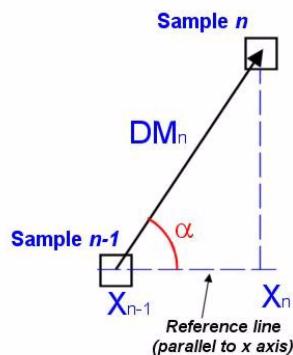
- 1 The smallest angle α is found between the reference line and the vector connecting the samples $n-1$ and n (see Figure 14.8.).

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- 2 The value of α is calculated according to the formula:

$$\alpha = \arccos \frac{|X_n - X_{n-1}|}{DM_n}$$

Where DM_n is the distance moved at sample n and X_n and X_{n-1} the x coordinates of the center, nose or tail-base point at sample n and $n-1$ respectively.



- 3 How to convert α to Heading depends on the direction of movement between samples $n-1$ and n (Figure 14.8.). The relation between Heading and α is determined by the following rules.

- If $\Delta X > 0$ and $\Delta Y \geq 0$, then Heading = α (Situation 1 in Figure 14.8.).
- If $\Delta X \geq 0$ and $\Delta Y < 0$, then Heading = $-\alpha$ (Situation 2).
- If $\Delta X < 0$ and $\Delta Y \leq 0$, then Heading = $-(180 - \alpha)$ (Situation 3).
- If $\Delta X \leq 0$ and $\Delta Y > 0$, then Heading = $180 - \alpha$ (Situation 4).

Where $\Delta X = X_n - X_{n-1}$ and $\Delta Y = Y_n - Y_{n-1}$.



- Heading and the dependent variables based on Heading (Turn angle and Angular velocity) are calculated in a different way than in EthoVision 3. It is so that they are consistent with the calculation of Head direction.
- Because α is defined as the smallest angle between the reference line and the vector from sample $n-1$ to sample n , it can only range from 0 to 90 degrees.

Calculating Statistics

How to specify Heading

- 1 Click the **Add** button next to **Heading** (see page 481) and click the **Body points** tab. Select the body points for which you want to calculate the velocity. By default, **Center-point** is selected.
- 2 Click the **Statistics** tab to choose the statistics for heading (see 14.5 for details). Next, click **Add**.



If your experiment is set to **Only center-point detection** or **Color marker tracking**, the **Body points** tab is absent. Calculations are based on the center point.

Remarks

- Heading is always calculated relative to the orientation of the x axis you have chosen in the Arena Settings used for that trial (see page 160). By default, the x axis is horizontal and pointing to the right. If the x axis is not horizontal or is pointing to another direction, Heading is calculated accordingly. Therefore, the same movement may result in different Heading values if it is tracked with different axes (that is, different Arena Settings).
- In EthoVision 2/3, Heading ranges from 0° to 360° relative to the user-defined reference line (see the corresponding Reference Manual). Therefore, be careful when comparing Heading values calculated with EthoVision 2/3 and EthoVision XT:
 - A value x between 0° and +180° in EthoVision XT corresponds to 360-x in EthoVision 2/3.
 - A value x between 0° and -180° in EthoVision XT corresponds to -x in EthoVision 2/3.

Application

Heading is used in studies of spatial orientation. For example, you can use it to determine the direction of flight of a moth relative to the direction of the air flow in a wind tunnel. In a Morris water maze test, you can use this variable to measure initial heading of the path after releasing the animal in the basin.

Turn angle

Definition

The change in direction of the nose, center, tail-base point or Head direction line

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between two consecutive samples.



Turn angle is calculated in a way different from that in EthoVision 3. This is because Turn angle is based on Heading, which is set consistent with Head direction.

Calculation

You can calculate Turn angle in two ways, based on body points or the Head direction line:

- **Based on body points** – Turn angle is calculated as the difference between two subsequent values for Heading (page 514) of the specified body point:

$$\Delta\text{Heading} = \text{Heading}_n - \text{Heading}_{n-1}$$

- If $\Delta\text{Heading} < -180^\circ$ then relative Turn angle = $\Delta\text{Heading} + 360$.
- If $\Delta\text{Heading} \geq +180^\circ$ then relative Turn angle = $\Delta\text{Heading} - 360$.
- Else relative Turn angle = $\Delta\text{Heading}$.

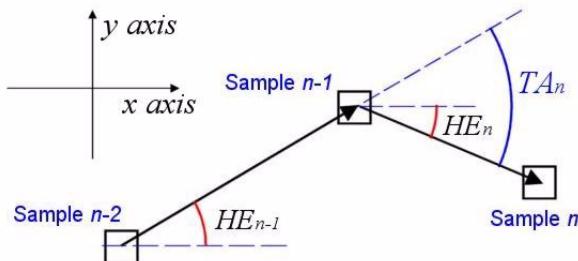


Figure 14.9. The Turn angle (TA) is the change in Heading (HE) of a body point from the previous sample (HE_{n-1}) to the current one (HE_n). In this example, HE_{n-1} is positive, while HE_n is negative (see the x-axis orientation). As a result, the difference $\text{HE}_n - \text{HE}_{n-1}$ is negative. For this axis orientation, a counterclockwise turn corresponds to a negative Turn angle.

- **Based on Head direction** – Turn angle is calculated as the difference between two subsequent values for Head direction (see Figure 14.10.). This value is independent of the position of the body points:

$$\Delta\text{Head direction} = \text{Head direction}_n - \text{Head direction}_{n-1}$$

- If $\Delta\text{Head direction} < -180^\circ$ then relative Turn angle = $\Delta\text{Head direction} + 360$.
- If $\Delta\text{Head direction} \geq +180^\circ$ then relative Turn angle = $\Delta\text{Head direction} - 360$.

Calculating Statistics

- Else relative Turn angle = Δ Head direction.



This option is available only if your experiment is set to **Center-point, nose-point and tail-base detection** (see page 102).

Note that calculating the Turn angle from Head direction is not the same as calculating it from the nose-point. This is because the Turn angle at sample n calculated for a body point depends on the last three samples ($n-2$, $n-1$ and n ; see the picture above), while Turn angle based on Head direction depends only on the samples $n-1$ and n . Consider the following example:

At any sample, the Head direction does not necessarily coincide with the direction of movement of a body point (in the example above, the nose-point). This is caused by the fact that Head direction is calculated from the contour of the subject, independent of movement.

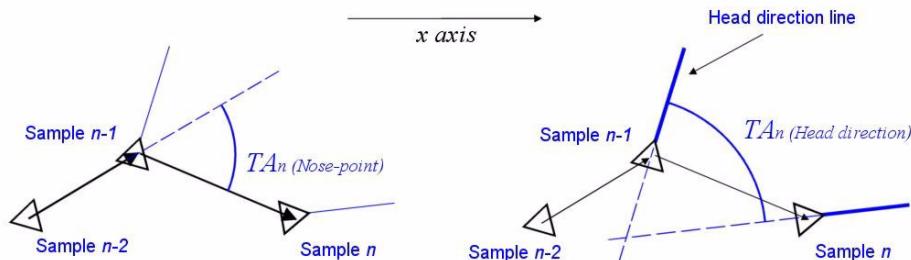


Figure 14.10. An example of the difference between Turn angles (TA) calculated from the nose-point (left) and those calculated from the Head direction (right). Triangles represent nose-point samples. Thick segments departing from the triangles are the Head direction lines.

Absolute vs. Relative Turn angles

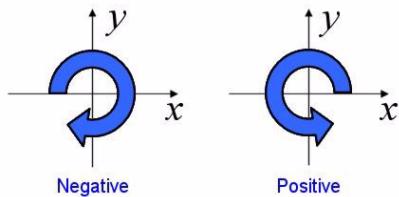
- Absolute Turn angle** – The difference in direction is unsigned. Absolute Turn angle ranges from 0° to $+180^\circ$.
- Relative Turn angle** – The difference in direction is signed. Depending on the orientation of the x axis, a clockwise turn is scored as positive or negative value, a counterclockwise turn is scored with opposite sign (see below). Therefore, the relative Turn angle ranges from -180° to $+180^\circ$.



Use the picture below to know the sign of a turn. Re-orient the axes and the circle arrows according to the orientation of the axes in the Arena Settings.

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How is the turn relative to your axes?



How to specify Turn angle

- 1 Click the **Add** button next to **Turn angle** (see page 481) and click the **Turn angle** tab. Select **Absolute** or **Relative** (see above). Select **Head direction turn angle (body point is ignored)** if you want to calculate turn angle in the way specified on page 518.
- 2 Click the **Body points** tab. Select the body points for which you want to calculate the turn angle. By default, **Center-point** is selected.



If your experiment is set to **Only center-point detection** or **Color marker tracking**, this tab is absent. Calculations are based on the center point.

- 3 Click the **Statistics** tab to choose the statistics for turn angle (see page 558 for details). Next, click **Add**.

Remarks

- The relative Turn angle depends on the orientation of the x and y axes in the Arena Settings used for that trial (see page 160). By default, the x axis is horizontal and pointing to the right. A clockwise turn is scored as a negative value, and a counterclockwise is scored as a positive value. If the x axis is pointing to the left, a clockwise turn is scored as a positive value, and a counterclockwise turn is scored as a negative value. See other examples in Figure 14.11.
- The Turn angle should not be confused with turn bias or turning rate. These are actually synonyms for the dependent variables Relative Angular velocity and absolute Angular velocity, respectively (see page 522).

Calculating Statistics

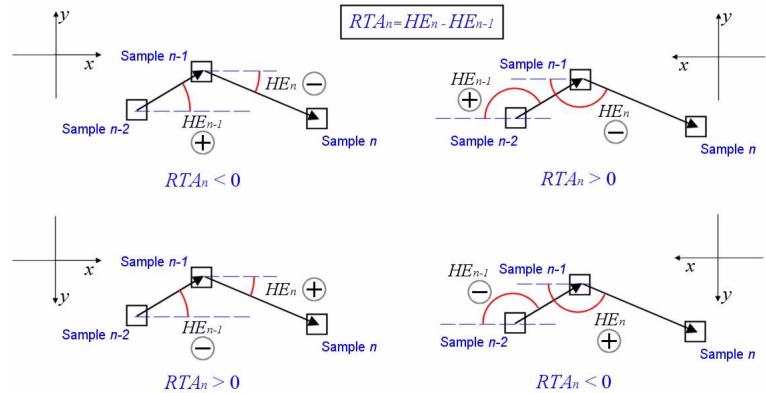
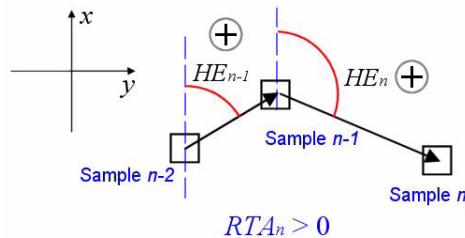


Figure 14.11. The sign of the relative Turn angle (RTA) depends on the orientation of the x and y axes. The examples illustrate the effect of four different axis orientation on a clockwise turn. The sign inside the circles indicates the sign of Heading (HE) values. Note: When the difference $HE_n - HE_{n-1}$ is larger than $+180^\circ$ or smaller than -180° (examples on the right), the rules on page 517 apply.

- Note that if the axes are swapped, the reference line is now vertical. the relative Turn angle is calculated accordingly. Compare the following picture with the example in the top left corner of Figure 14.8.:



Application

Assessing turn angles can be helpful for detecting stereotypic movements. In this case, consecutive turn angles tend to have large values (for example, in circling behavior of rodents), or show repeating patterns (for example, rocking or waving).

Cumulative Turn angles are used to calculate rotations (see page 536).

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Angular velocity

Definition

The change in direction of movement of the nose, center, tail-base point or Head direction line between two consecutive samples, calculated per unit time. Angular velocity is expressed in degrees/second (°/s).



- Note that Angular velocity is calculated in a way different from that in EthoVision 3. This is because Angular velocity is based on Heading, which is set consistent with Head direction.
- Angular velocity ranges from 0°/s to a maximum value that is depending on the time between two samples. For example, if the sample rate is 25, the maximum attainable turn (180°, see Turn angle) results in an angular velocity of $180/0.04 = 4500^{\circ}/s$.

Calculation

$$RAV_n = \frac{RTA_n}{t_n - t_{n-1}}$$

where RAV_n is the relative Angular velocity for sample n , RTA_n is the relative Turn angle for sample n (see page 517), and $t_n - t_{n-1}$ is the time difference between the current and the previous sample.

You can calculate Angular velocity in two ways, based on body points or the Head direction line:

- **Based on body points** – For the body points nose, center or tail-base, the angular velocity is calculated from the turn angle (see page 517) of the specified body point.
- **Based on Head direction** – For the Head direction line, the angular velocity is calculated from the turn angle based on Head direction (see page 518; see also Figure 14.10.). Its value is independent of the position of the body points.



This option is available only if your experiment is set to **Center-point, nose-point and tail-base detection** (see page 102).

Note that calculating the Angular velocity from Head direction is not the same as calculating it from the nose-point. This is because the Turn angle (and therefore Angular velocity) at sample n calculated for a body point depends on the last three samples ($n-2$, $n-1$ and n ; see Figure 14.10.), while the Turn angle based on Head direction depends only on the samples $n-1$ and n .

Calculating Statistics

Absolute vs. Relative Angular velocity

- **Absolute Angular velocity** – The rate of change in direction is unsigned. Also known as Turning rate.
- **Relative Angular velocity** – The rate of change in direction is signed. Depending on the orientation of the x and y axes, a clockwise turn is scored as positive or negative value, a counterclockwise turn is scored with opposite sign (see the relative Turn angle). *Turn bias* (degrees/s) and *circling tendency* are synonyms for this variable.

How to specify Angular velocity

- 1 Click the **Add** button next to Angular velocity (see page 481) and click the **Angular velocity** tab. Select **Absolute** or **Relative** (see above). Select **Head direction angular velocity (body point is ignored)** if you want to calculate angular velocity based on head direction (see above and also page 518).
- 2 Click the **Body points** tab. Select the body points for which you want to calculate the velocity. By default, **Center-point** is selected.
- 3 Click the **Statistics** tab to choose the statistics for angular velocity (see page 558 for details). Next, click **Add**.



If your experiment is set to **Only center-point detection** or **Color marker tracking**, the **Body points** tab is absent. Calculations are based on the center point.

Remarks

- If the body point turns more than 180° between one sample and the next, the direction of turning is calculated incorrectly. For instance, if the subject makes a counterclockwise turn of 210°, the program interprets this as a 150° clockwise turn. As a result the Angular velocity gets a value smaller than expected, and in case of relative Angular velocity a false sign. To prevent this kind of error, set the sample rate at such a level that it is practically impossible for the subject to make a turn more than 180° between two subsequent samples.
- The difference between relative and absolute Angular velocity is best explained by looking at the mean values of the two dependent variables in the following example:

Time	Angular velocity	
	Absolute	Relative
0.04	10	-10
0.08	45	45
0.12	35	-35
Mean	30	0

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The mean absolute Angular velocity better reflects the amount of turning, whereas the mean relative Angular velocity gives a better indication of the overall direction of turning.

Application

The absolute Angular velocity is used to express the amount of turning per unit time. Generally, a high value of this dependent variable is associated with local search, for instance in response to non-volatile semiochemicals (Bell W.J. 1991, *Searching Behaviour: The Behavioural Ecology of Finding Resources*. London: Chapman & Hall).

The relative Angular velocity measures the speed of change in direction of movement. The mean of this dependent variable can be used to assess turn bias or circular tendency, the tendency of a subject to turn to a specific direction. Studying this helps detecting peculiarities or abnormalities of behavior (for instance, stereotypic movements, reaction to toxic substances, etc.).

Meander

Definition

Meander is the change in direction of movement of a subject relative to the distance moved by that subject. Meander can be calculated in two different ways:

- **Relative Meander** – The difference in direction is signed. With a default position of the Calibration axes (see page 160), a clockwise turn is scored as positive or negative value, a counterclockwise turn is scored with opposite sign (see below). The relative meander ranges from -180°/cm to +180°/cm.
- **Absolute Meander** – The change in direction is unsigned. The absolute Meander ranges from 0°/cm to 180°/cm.

Calculation

$$RM_n = \frac{RTA_n}{DM_n}$$

where RM is relative Meander, RTA is relative Turn angle (see page 517) and DM is Distance moved (see page 500).

Absolute Meander is the absolute value of the relative Meander.

You can calculate Meander in two ways, based on body points or the Head direction line:

Calculating Statistics

- **Based on body points** – Meander is calculated from the turn angle (see page 517) of the specified body point.
- **Based on Head direction** – Meander is calculated from the turn angle based on the Head direction line (see page 518; see also Figure 14.10.). Its value is independent of the position of the body points.



This option is available only if your experiment is set to **Center-point, nose-point and tail-base detection**.

How to specify Meander

- 1 Click the **Add** button next to **Meander** (see page 481) and click the **Meander** tab. Select **Absolute** or **Relative** (see above). Select **Head direction meander (body point is ignored)** if you want to calculate meander based on the head direction line (see above and also page 518).
- 2 Click the **Body points** tab. Select the body points for which you want to calculate meander. By default, **Center-point** is selected.
- 3 Click the **Statistics** tab to choose the statistics for meander (see page 558 for details). Next, click **Add**.



If your experiment is set to **Only center-point detection** or **Color marker tracking**, the **Body points** tab is absent. Calculations are based on the center point.

Application

- The relative Meander is a measure for the direction of turning per unit distance. This dependent variable can be of additional value to other turn bias variables, such as relative Turn angle and relative Angular velocity, since the turn bias is 'corrected' for the distance moved. For instance, if two individuals move at different speeds, the two can have very different values for the mean relative Turn angle, but at the same time have identical values for the mean relative meander.
- The absolute Meander is often used in combination with the dependent variables absolute Turn angle and absolute Angular velocity to study turning rates. Bell (1991) reports that in most studies, when plotting the values, absolute Meander generates a smoother curve than absolute Angular velocity. This is caused by the fact that the latter dependent variable is influenced both by speed as well as by real turning rate.

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Movement

Definition

A discrete variable, related to one of the body points, with two possible states, **Moving** and **Not moving**:

- The state is **Moving** if the running average velocity exceeds the user-defined **Start velocity**.
- The state remains **Moving** until the running average velocity drops below the user-defined **Stop velocity**.
- The state then becomes **Not moving** until the running average velocity reaches the **Start velocity** again.

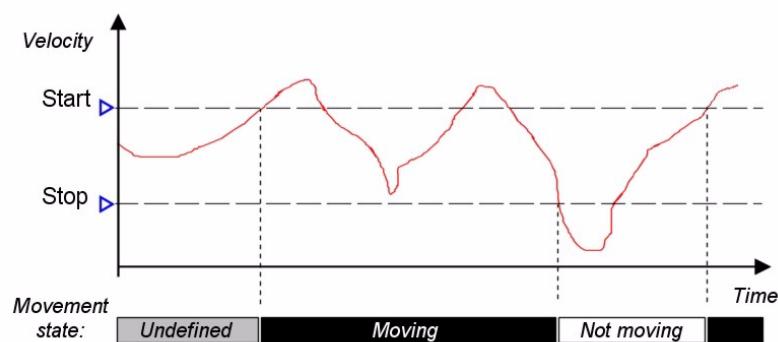


Figure 14.12. An example of how the Movement dependent variable is calculated.

Because the velocity initially lies between the Stop velocity and the Start velocity, the state is undefined. When velocity exceeds the Start velocity value, Movement is given the value Moving. When velocity drops below the Stop velocity value, Movement is given the value Not moving.

In order to reduce the sensitivity of this dependent variable to brief changes in velocity, the data can be smoothed by taking the running average of the last n samples. This number is referred to as the **averaging interval**.



When a body point is missing, the current **Movement** state does not change from the previous sample.

Calculating Statistics

How to specify Movement

1 Click the **Add** button next to **Movement** (see page 481) and click the **Movement** tab. Enter the following:

- **Averaging interval** – The number of samples over which the running average velocity is based. The default value is **1**, that is, velocity is not smoothed before calculating the **Movement** variable.
- **Start velocity** – The velocity above which the subject is considered to be moving.
- **Stop velocity** – The velocity below which displacements of the subject's body points are no longer attributed to locomotion but to system noise, body wobble or pivoting on the spot.

Under **Calculate statistics for**, you have the following options:

- Moving** – Select this option to calculate statistics for when the subject is considered to be moving.
- Not moving** – Select this option to calculate statistics for when the subject is considered not to be moving.

You can select either one of them, or both.

2 Click the **Body points** tab. Select the body points for which you want to calculate the velocity. By default, **Center-point** is selected.
3 Click the **Statistics** tab to choose the statistics for movement (see page 558 for details). Next, click **Add**.



If your experiment is set to **Only Center-point detection** or **Color marker tracking**, the **Body points** tab is absent. Calculations are based on the center point.

Remarks

- By increasing the averaging interval, you can increase the reliability of movement detection. A running average velocity based on more samples diminishes the effect of random errors. However, a drawback of increasing the Averaging interval is that it causes a delay in the determination of a state transition, proportional to the length of the interval.
- In some cases the number of samples available for smoothing can be less than the averaging interval entered. For example, when there are missing samples or at the beginning of the track. In such cases EthoVision XT uses the samples available in the specified interval.
- The use of **Start velocity** and **Stop velocity** is illustrated in Figure 14.12.. Values of velocity between Start velocity and Stop velocity result in no change in the

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current state of the subject (moving or not moving). Note that the smaller the difference between the two threshold velocities, the more transitions between the states Moving and Not moving will be scored. By defining such a buffer, you prevent overestimation of transition rates because of a velocity joggling just around the movement threshold.

Without this buffer, a Not moving period lasting a short time, say 0.12 s, could be recorded, although such a short time span can obviously not be regarded as resting, nor can it be sufficient for the animal to orientate itself.

Application

Like Velocity, the Movement dependent variable provides information on the subject's locomotor activity.

Elongation

Definition

A discrete (state) variable with three possible states: **Stretched**, **Normal** and **Contracted**, depending on where the running average elongation measure (E) of the subject's shape calculated for the current sample lays relative to two user-defined thresholds:

- If the running average elongation percentage is greater than the **Stretched above** value, the state is **Stretched**.
- If the running average elongation percentage is smaller than the **Contracted below** value, the state is **Contracted**.
- In all the other cases, the state is **Normal**.

The running average elongation is calculated for each sample, over the number of samples specified by the **Averaging interval**.



When the subject missing, the **Elongation** state does not change from the previous sample.

Calculation

The elongation measure is calculated as follows:

Calculating Statistics

$$E = \frac{\sqrt{\left(\sum_x (x - x_c) - \sum_y (y - y_c)^2 \right)^2 - 4 \left[\sum_{x,y} (x - x_c) \cdot (y - y_c) \right]^2}}{\sum_x (x - x_c)^2 - \sum_y (y - y_c)^2}$$

where x, y are the coordinates of the pixels of the subject's contour in the current sample, and x_c and y_c are the coordinates of the center of the subject.

Elongation is expressed as a percentage and ranges from 0 (when the subject's shape is perfectly circular) to 100% (when the subject's shape is a line).

How to specify Elongation

- 1 Click the **Add** button next to Elongation (see page 481) and click the **Elongation** tab.
- 2 Enter the following:
 - **Averaging interval** – The number of samples over which the running average elongation is based. The default value is 1, that is, the elongation measure is not smoothed before calculating the Elongation variable.
 - **Stretched above** – The elongation measure above which the subject is considered to be Stretched.
 - **Contracted below** – The elongation measure below which the subject is considered to be Contracted.

Under **Calculate statistics for**, select at least one of the following options:

- Stretched** – To calculate statistics for when the subject is considered to be Stretched.
 - Normal** – To calculate statistics for when the subject is considered not to be Stretched or Contracted.
 - Contracted** – To calculate statistics for when the subject is considered to be Contracted.
- 3 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.



- To find the optimal Stretched above and Contracted below, run a few test trials and check in the **Analysis Results and Scoring** pane (see page 276) the

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values of **Elongation** when the animal shows such behavior. These values are calculated real-time during acquisition.

- The Elongation thresholds set in Acquisition are not used in analysis. When you specify the Elongation dependent variable in your Analysis profile, enter the new values in the appropriate fields.
- Values of elongation percentage between Stretched above and Contracted below result in the subject being considered as Normal.

Remarks

- The elongation measure is independent of video size and the subject's position and orientation.
- By increasing the averaging interval, you can increase the reliability of detection of stretching and contracting. A running average elongation based on more samples diminishes the effect of random changes in elongation measure between consecutive samples that would be detected as state transitions. However, a drawback of increasing the Averaging interval is that it causes a delay in the determination of a state transition, proportional to the length of the interval.
- In some cases the number of samples available for smoothing can be less than the averaging interval entered. For example, when there are missing samples or at the beginning of the track. In such cases EthoVision XT uses the samples available in the specified interval. For example, the value of Elongation for the first sample of the track is always calculated over one sample.
- If you use the Advanced Model-Based (XT6) method, we suggest to use the **Dilation** and **Erosion** filter (see page 249) to remove the animal's tail from the detected image of the subjects.

Application

The Elongation variable can help you assess the frequency and duration of stretch attend postures in a more objective way.

Mobility

Definition

A discrete (state) variable with three possible states: **Highly mobile**, **Mobile** and **Immobile**, depending on where the changed pixels of the detected subject

Calculating Statistics

between current sample and previous sample (referred to as **changed area**) lay relative to two user-defined thresholds.

- See page 534 for the difference between Mobility and Movement.



When the subject missing, the **Mobility** state does not change from the previous sample.

Step 1 - Calculation of the changed area

All pixel coordinates of the subjects are determined immediately after they have been detected. Those coordinates are compared with the previous sample to determine the number of changed pixels between the two. The changed pixels are

- The subject pixels found in the current sample but not in the previous sample AND
- The subject pixels found for the previous sample but not for the current sample.

This can be expressed in the following formula:

$$CA_n = (A_n - A_{n-1}) + (A_{n-1} - A_n)$$

Where CA_n is the changed area for the current sample n , A_n is the area for the sample n , and A_{n-1} the area for the sample $n-1$.

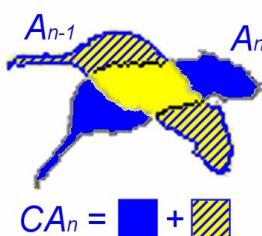


Figure 14.13. An example of changed area for Mobility detection.

The changed area (in number of pixels) is available when you export your raw data (see the column named **Changed Area** in the track file).

Mobility is calculated by taking *every* pixel identified as the subject and comparing it between the current image and the previous one. If all the pixels are the same, there is zero mobility. If all the pixels are different, there is 100% mobility. If the animal is moving and increases its velocity (whilst keeping the same shape) there will be an increase in mobility, because the pixels belonging to the animal are

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increasingly different as it moves faster.

Step 2 - Calculation of Mobility

The formula for Mobility is simply the changed area for the current sample divided by the sum of the current area and the previous area:

$$Mobility = \frac{CA_n}{A_{n-1} + A_n} \times 100$$

Mobility ranges from 0 (the sample is identical to the one before it) to 100 (no pixel overlap).

Step 3 - Running average

To smooth the Mobility parameter, an **Averaging interval** is used. This gives you the option to specify the number of samples for calculating a running average of Mobility. The Mobility percentage (see above) is summed over the number of samples that you specify, and divided by the number of samples, to create an average Mobility for these samples. This way, sudden changes in surface area caused by such factors as the animal entering a shadowed area and not being identified correctly, or a reflection being identified momentarily as the animal, are smoothed out.



When you export data, you do not export any information on the averaging interval.

Step 4 - Calculation of the Mobility dependent variable

The Mobility state variable is established for each sample, according to the value of running average Mobility relative to the thresholds:

- Below the **Immobile threshold**, the state is **Immobile**.
- Between the **Immobile threshold** and the **Highly mobile threshold**, the state is **Mobile**.
- Above the **Highly mobile threshold**, the state is **Highly mobile**.

How to specify Mobility

- 1 Click the **Add** button next to **Mobility** (see page 481) and click the **Mobility** tab.
- 2 Enter the following:

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- **Averaging Interval** – The number of samples over which the running average mobility is based. The default value is 1, that is, the mobility measure is not smoothed before determining the Mobility state variable.
- **Highly mobile threshold** – The percentage of change in body area above which the subject is considered to be Highly mobile.
- **Immobile threshold** – The percentage of change in body area below which the subject is considered Immobile.



You can enter a number with up to two decimals.

Under **Calculate statistics for**, select at least one of the three following options:

- Highly mobile** – Statistics are calculated for when the subject is considered Highly mobile.
- Mobile** – Statistics are calculated for when the subject is considered Mobile.
- Immobile** – Statistics are calculated for when the subject is considered Immobile.

3 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.



- To find the optimal Highly mobile and Immobile threshold, run a few test trials and check in the **Analysis Results and Scoring** pane (see page 276) the values of Mobility when the animal shows such behavior. These values are calculated real-time during acquisition.
- Mobility detection thresholds set in Acquisition are not used in analysis. When you specify the Mobility dependent variable in your Analysis profile, enter the new values in the appropriate fields.

Remarks

- Since Mobility is calculated on the detected subject, the grey-scale threshold values used in detection also have an influence on the mobility variable. If your detection settings are such that only part of the animal is detected, then only the mobility for that part is calculated.
- In some cases the number of samples available for smoothing can be less than the averaging interval entered. For example, when there are missing samples or at the beginning of the track. In such cases EthoVision XT uses the samples available in the specified interval. For example, the value of Mobility for the first sample of the track is always calculated over one sample.

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- You set the thresholds during acquisition (see page 276), but you can override them when calculating statistics to produce new values for Mobility. To see what the original values of Mobility thresholds were (unless you have changed them while acquiring data), open the Acquisition module and click the button next to **Mobility** in the **Analysis results and Scoring** pane.

Frequently asked questions about mobility detection

1 What is the difference between Mobility and Movement?

Mobility can be defined as the degree of movement of an animal's body independent of spatial displacement of the center or any other body point, which is measured by Movement. 'Independent' does not mean that mobility is corrected for the centre point position, it means that the calculation does not use the x,y coordinates of the animal (they are not in the equation used to calculate it). Mobility is calculated 100% independent of movement of the coordinates identified as the centre point (or the nose/tail point). That means that the centre point can have zero movement but high mobility.

For example, imagine that you are tracking a rat in an open field. When the rat stands still and grooms, the center of gravity does not move, therefore there is no spatial displacement of the subject (the current state for the Movement variable is **Not moving**), however the rat's head and forelimbs move, resulting in changes in the surface area. Although there is no spatial displacement of the body, the current state of the Mobility variable is **Mobile** (depending on the threshold value).

2 Does Mobility detection depend on the size of the subject vs. arena?

No. It is dependent on the size of the subject only. Keeping resolution constant, the smaller the subject, the smaller the number of pixels that form its image, the more likely that any small movement results in a change in area that is detected as Mobility.

3 Does Mobility detection depend on the video resolution?

Yes. The higher the video resolution, the greater the number of pixels that form the image of the subject. Therefore, the less likely that a small movement of the subject results in an abrupt change in area.

However, this effect is present at very low resolution (for example, when the subject is less than 100 pixels large). At resolutions provided by MPEG media files, this effect is negligible.

4 Does Mobility detection depend on sample rate?

Yes. Since Mobility is detected from the change in area of the subject between two samples, and the change in area depends on how frequently the subject area is acquired (that is, the sample rate), Mobility depends on the sample rate. All being equal, the higher the sample rate, the smaller the change in subject area.

Calculating Statistics

In the example of Figure 14.14., the **Immobile threshold** was set to 2%, and the **Highly mobile threshold** was set to 10%. A certain movement pattern detected with a sample rate x determines a change in area of around 15%, which causes EthoVision consider the subject **Highly mobile**.

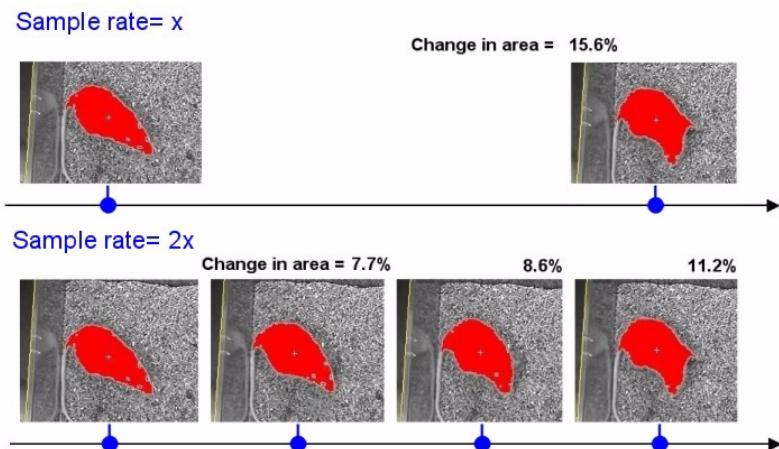


Figure 14.14. The effect of sample rate on mobility detection. For explanation, see text

If the sample rate doubles, more samples are captured in the same time interval, therefore the same movement results to a smaller change in area between samples. For the intermediate samples, EthoVision XT considers the subject **Mobile** since the change in area is smaller than 10%. The proportion of samples where the subject is considered Mobile increases relative to the proportion of samples where the subject is considered Highly mobile.

As a general rule, the higher the sample rate, the lower the **Immobile** and **Highly mobile** thresholds must be.

Application

Mobility can be used to assess general activity, and changes in behaviors in specific paradigms. For example, in Porsolt swim tests (for example, Russig *et al.* 2003, *Behav. Pharm.* 14: 1-18) it allows to detect changes in behavior, for example from swimming to floating, more objectively than when observing directly. You can download an application note on the Porsolt swim test from our web site www.noldus.com.

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You can also use Mobility to detect freezing behavior in which case you need to set a very low value of **Immobile threshold**. Mobility can also be used to quantify movement of zebra fish embryos within their eggs in a 24-well plate with back-lighting.

Rotation

Definition

This is the number of turns (either clockwise or counterclockwise) of 360° .

There are two methods available to calculate rotations:

- **Rotation** - The first method is based on the turn angle from one sample to the next, based on only one body point. This method is suitable for when your animal walks around in circles.

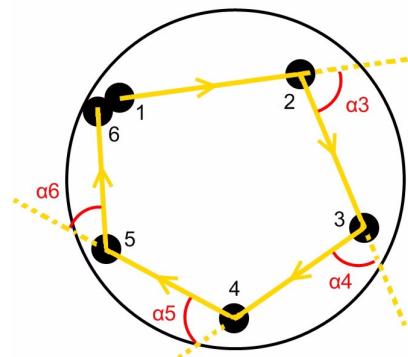


Figure 14.15. A simplified example of 6 samples from an animal walking around in a circular arena. **Rotation** is based on the accumulation of the turn angles (α) between heading of the body point (center, nose or tail-base) of the current and previous sample. Also see page 517.

- **Body axis rotation** - The second method is based on the turn angle between one sample axis (the line between center-point and nose-point or between tail-base and center-point) and the next. Choose this method if your animal is spinning around its own axis.

Calculating Statistics

This method is also more robust for micro rotations; an animal that makes small body rotations, without actually really moving around the arena. If you do not want these micro rotations of the body points to be calculated as actual rotations, use this method to ignore them.

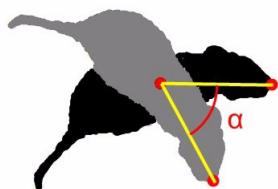


Figure 14.16. Body axis rotation is based on the accumulation of the turn angles between the axis from center-point to noise point of the current and previous sample. It is also possible to use the axis from tail-base to center-point (not shown in this picture).



The **Body axis rotation** method is only available if your experiment is set to **Center-point, nose-point and tail-base detection**. If you are using center-point detection only, you can use Track Smoothing (see page 378) to reduce the counting of micro rotations.

Calculation

- 1 The cumulative Turn angle of consecutive samples is determined.
- 2 As soon as the cumulative Turn angle exceeds the corresponding Rotation count (default value = '1', which corresponds to a turn of 360°), one Rotation is counted.
- 3 Next, the cumulative Turn angle is reset and consecutive Turn angles are summed until the cumulative Turn angle, again, exceeds the corresponding Rotation count.



Occasional turns in the other direction can be taken into account by setting the **Threshold** value (see step 4 in How to specify Rotation below) to a specific angle.

How to specify Rotation

- 1 Click the **Add** button next to **Rotation** (see page 481).

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- 2 Select whether EthoVision XT should count rotations in a clockwise or counterclockwise direction.
- 3 If you want to use the second method for **Body axis rotation**, select the check box and choose if you want to use the **Axis: Center-point to Nose-point** or the **Axis: Tail-base to Center-point**. If you want to use the first method for “normal” rotation, leave the check box unselected.
- 4 In the **Count every ... rotation** list, you specify how rotations should be counted.

The default value of ‘1’ means that every 360° turn in the specified direction counted as one rotation. If you select a value of ‘0.5’ in this list, every 180° turn is counted as one rotation.

- 5 By entering a threshold angle in the **Threshold** box, you can compensate for turns in the direction opposite to the one you selected in step 2.

Example – You are interested in clockwise rotations of your animal but these are occasionally interrupted by counterclockwise turns. Depending on the value of the Turn angle in the counterclockwise direction, one of the following occurs:

- The Turn angle in the counterclockwise direction **does not exceed** the **Threshold** angle – EthoVision XT continues to calculate the cumulative Turn angle until it exceeds the corresponding Rotation count.
- The Turn angle in the counterclockwise direction **does exceed** the **Threshold** angle – the cumulative Turn angle is reset and a new sequence of samples is used to calculate the cumulative Turn angle until the cumulative Turn angle exceeds the corresponding Rotation count.

Visualize data to validate your choice of threshold value.

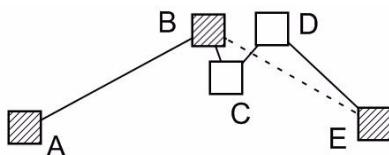
- 6 Enter a value of **Minimum Distance** if you want to remove the samples for which the distance from the last sample selected in the track is too short to represent actual movement. By default this is 2 cm/0.78 inch. Turn angles and Rotation are re-calculated according to this filter. If you do not want to apply any filter, enter 0.
- 7 If you selected **Center-point, nose-point and tail-base detection** in the Experiment settings, and you did not select the **Body axis rotations** check box (second method), you can now click the **Body Points** tab. Select the body points for which you want to calculate Rotation.
- 8 In the **Statistics** tab, select the statistics you want to use (see also page 558).

Calculating Statistics



If you want to count both clockwise and counterclockwise rotations, or if you want to use both methods, you can add the dependent variable **Rotation** several times. Rename the variables to be able to distinguish between them. (Right-click the variable in the Selected Dependent Variables list and choose Rename).

Applying the **Minimum Distance Moved** filter – Samples are filtered with the **Direct** method for Minimal Distance Moved (page 380). However, contrary to Minimal Distance Moved available in Track Smoothing, this filter changes the values of turn angle at a specific sample. In the example below, samples C and D are excluded since their distance from B is shorter than the threshold entered. The turn angle for sample E is recalculated according to the samples A, B and E.



Application

Circling or rotational behavior is used in rats as an indicator of cerebral asymmetry. For example, striatal asymmetries in dopamine characteristics, such as dopamine levels, metabolites, release and uptake, have been functionally related to an increase in rotational behavior (e.g., Carlson and Click, 1989; Schirmer *et al.*, 2007). Amphetamine, a dopamine releaser, induces rotations in animals with striatal asymmetries, which can be blocked by haloperidol. The animal usually turns away from the side of higher dopaminergic activity.

Head directed to zone

Definition

The duration the animal's head is directed towards a Zone or a circular area around a Point.



Head directed to zone is not available if your experiment is set to **Only center-point detection** or **Color marker tracking**.

Calculation

Head directed to zone is calculated in two steps:

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- 1 The Head direction line is calculated based on the Subject's contour.
- 2 The Head direction line is extrapolated and the samples for which this line crosses a Zone or the circular area around a Point are used to calculate the duration of Head directed to zone (see Figure 14.17.).

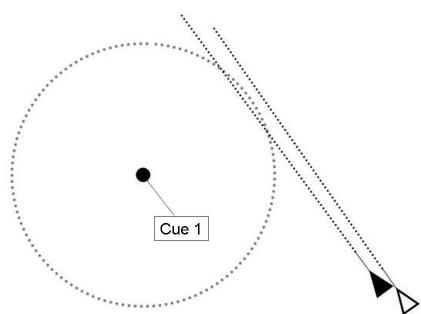


Figure 14.17. Example of how Head directed to zone is determined. The circle indicates an area around a Point labeled Cue 1. The open triangle indicates a Head directed to zone that is not directed to Cue 1, the closed triangle indicates a Head directed to zone that is directed to Cue 1.

How to specify Head directed to zone

- 1 Click the **Add** button next to **Head directed to zone** (see page 481).
- 2 In the **Head directed to zone** tab, under **Zone of interest** you can select:
 - Zone** – Select a Zone from this list.
 - Point** – Select either a Point you defined in the Arena Settings or the center of a Zone.
Because a Point has an infinitely small surface area, you need to define a circular zone around the Point. The default radius is 0.1 cm. The smaller the radius around a Point, the less likely it is that the animal's head is exactly directed at this Point.
- 3 Next, you can specify when Head directed to zone should be calculated, depending on the location of the animal:
 - Calculate when** – From this list you select the body point that should be in the Zone selected in the **In** list. When you select **All detected body points** from the **Calculate when** list, Head directed to zone is only calculated when all three body points are in the Zone selected in the **In** list.

Calculating Statistics

- **In** – Select one of the Zones from this list.
- 4 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.

Application

Head directed to zone is especially designed for use in the novel object test. Exploration of a novel object is normally defined as directly attending to the object when the head is within a 2 cm radius of the object (Ennaceur and Delacour, 1988). In EthoVision XT, you calculate Head directed to zone when the Nose-point of the animal is within a 2 cm radius from the center of the zone you have drawn around the novel object.

Distance between subjects

Definition

The distance between a body point of a subject and a body point of another subject.

Distance is calculated for each Subject (*Actor*) relative to other subjects (*Receivers*).

Calculation

Formula:

$$DS_n = \sqrt{(X_{a,n} - X_{r,n})^2 + (Y_{a,n} - Y_{r,n})^2}$$

Where DS_n is the distance between Actor and Receiver at sample n , $X_{a,n}$ and $Y_{a,n}$ the X,Y coordinates of the selected body point of the Actor at sample n , and $X_{r,n}$ and $Y_{r,n}$ the X,Y coordinates of the selected body point of the Receiver at sample n .

How to specify Distance between subjects

- 1 Click the **Add** button next to **Distance between subjects** (see page 481).
- 2 Click the **Body points** tab and select the body points of the focal subject (*Actor*) you want to use to calculate distance.
- 3 Click the **Receiver** tab. Here, you specify the other subjects (*Receiver*).
 - Under **Select**, choose the subjects you want to calculate the distance from.

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- Under **Body points**, select the body points of the subjects selected above.
- 4 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.



Distance is calculated for each combination of Actor's body points and Receiver's body points. Each row of the result table shows the results for an Actor. For example: The row heading shows **Subject 1**. The column heading **Nose point / Subject 2 / Center point**. This can be read as 'the cell contains the distance between the nose point of Subject 1 and the center point of Subject 2'.

Application

This parameter forms the basis for the Proximity dependent variable used in studies of social or aggressive behavior.

Proximity

Definition

A discrete variable with two possible states, **In proximity** and **Not in proximity**:

- The state is **In proximity** when the distance between the selected body points of the focal subject (*Actor*) and the body points of another subject (*Receiver*) is lower than a user-defined *In proximity* threshold.
- The state is **Not in proximity** when the distance between the selected body points of the Actor and the body points of another subject Receiver is greater than a user-defined *Not in proximity* threshold.
- The state does not change from the previous sample when the distance stays between the two thresholds.



If at least one of two subjects' selected body points is missing, the **Proximity** state does not change from the previous sample.

Calculation

For each sample, the program calculates first the *Distance between Subjects* (see above), then it compares this value with the *In proximity* and *Not in proximity* thresholds to establish the state at that sample.

How to specify Proximity

- 1 Click the **Add** button next to **Proximity** (see page 481).

Calculating Statistics

- 2 In the **Proximity** tab, enter the **In proximity** and **Not in proximity** distance values that specify when the two subjects are considered in proximity to each other (see the Definition above).
- 3 Under **Calculate statistics for**, select the state you want to analyze. For example, select **In proximity** if you want to know how often or how long the subjects were close to each other. Select **Not in proximity** if you want to analyze when the animals were far from each other.
- 4 Click the **Body points** tab. Select the body points of the focal subject (Actor) you want to use to calculate proximity.
If you select two or three points, a drop-down list becomes available. Choose:
 - **All selected points** – A state is assigned only when all selected points are in that state relative to the Receiver (proximity or not in proximity). If body points are in different states, that sample is not used in analysis.
 - **Any selected point** – A state is assigned when at least one selected body point is in that state relative to the Receiver (proximity or not in proximity).
 - **Each point** – A state is defined for each point of the Actor. Results are displayed for each point separately.
- 5 Click the **Receiver** tab. Here, you specify the other subjects (Receiver).
 - Under **Select**, choose the subjects you want to calculate the distance from. If you select two or more subjects, select one of the available options from the list:
 - **All selected subjects** – A state is assigned only when the Actor is in that state for all selected Receiver (In proximity / Not in proximity). If the Actor is in different states relative to different Receivers (for example, Subject 1 *In proximity* of Subject 2 and *Not in proximity* of Subject 3), that sample is not used in analysis.
 - **Any selected subject** – A state is assigned when the Actor is in that state for at least one Receiver (In proximity/Not in proximity).
 - **Each subject** – A state is assigned to each combination Actor*Receiver. Results are displayed for each Receiver.
 - Under **Body points**, select the body points of the Receivers you want to use to define proximity. If you select two or three points, select one of the available options:
 - **All selected points** – A state is assigned only when all selected points are in that state relative to the Receiver (in proximity/not in proximity). If different body points are in different states, the sample is not used in the analysis.

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- **Any selected point** – A state is assigned when at least one selected body point is in that state relative to the Receiver (in proximity/not in proximity).
 - **Each point** – A state is defined for each point of the Actor. Results are shown for each point separately.
- 6 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.



Notes

- If the experiment is set to **Only center-point detection** or **Color marker tracking**, the **Body points** tab is absent. Calculations are based on the center point.
- **Any selected points** – At any sample time, it is possible that the Actor's body points are in different states relative to the body points of the Receiver. For example, the Actor's nose point being *In proximity* of the Receiver's center point, and the Actor's center point *Not in proximity* of the Receiver's center point. In such cases when you select **Any selected point** (step 4 and 5 above), multiple states can be assigned to that sample.
- **Any selected Subjects** – At any sample time, it is possible that the Actor is in different states relative to different Receivers. For example, Subject 1 being *In proximity* of Subject 2 and *Not in proximity* of Subject 3. In such cases when you select **Any selected Subject** (step 5 above), multiple states can be assigned to that sample.
- You can check multiple states occurring at one sample time when exporting the dependent variable (see page 492). At a specific sample time, the value of the variable is **1** in more than one column. In the example above, the columns for **in proximity** and **not in proximity** will both show **1**.

Application

Proximity can be used to study the behavioral interactions between individual animals, for instance the effects of individual housing vs. group housing on social behavior of rats (Spruijt *et al.* 1992. *Physiology & Behavior* **51**: 747-752), or social isolation as a symptom of schizophrenia (Sams-Dodd, 1995. *J. Neuroscience Methods* **59**: 157-167).

Relative movement

Definition

A discrete variable with four possible states, **Moving to**, **Moving from**, **No relative movement**, and **No interaction**.

- The state is **Moving to** when the focal subject (*Actor*) is moving towards another subject (*Receiver*).
- The state is **Moving from** when the Actor is moving away from the Receiver.
- The state is **No relative movement** when two subjects are not moving relative to each other.
- The state is **No interaction** when the distance between subjects is great enough that they can be considered as not interacting.

Calculation

- 1 The middle point Q is determined between the body points of the Receiver for two consecutive samples $n-1$ and n (see Q in Figure 14.18.).

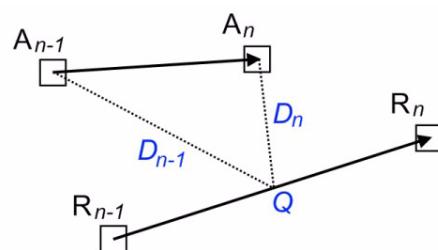


Figure 14.18. Illustration of the dependent variable Relative movement. A is the body point of the Actor, R of the Receiver. Q is the interpolated position between the Receiver's body points at samples n and $n-1$.

- 2 The distance between the Actor's body point and Q is calculated for samples n and $n-1$.
- 3 The state of Relative movement is determined:
 - If $D_n > D_{n-1}$ – **Moving from**
 - If $D_n < D_{n-1}$ – **Moving to**
 - If $D_n = D_{n-1}$ – **No relative movement**

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- If $D_n >$ Maximum interaction distance (user defined) – **No interaction.**



From Figure 14.18. it can be seen that the outcome depends on which animal is considered as Actor and which as Receiver. In Figure 14.18., A is clearly **Moving to R**. If R was the Actor, the point Q would be defined for the other subject in the middle of the An-1-An segment. In that case D_n would be longer than D_{n-1} , thus R would be considered **Moving from A**.

How to specify Relative movement

- 1 Click the **Add** button next to **Relative movement** (see page 481).
- 2 In the **Relative Movement** tab:
 - Under **Maximum interaction distance**, enter the distance above which you do not want to consider the subjects as interacting (Default: 50 cm/ 16.69 inches).
 - Under **Calculate statistics for**, select the states you want to consider (see the Definition above). By default, all states are selected.
- 3 Click the **Body points** tab. Select the body points of the focal subject (Actor) you want to use to calculate relative movement.

If you select two or three points, a drop-down list becomes available. Choose:

 - **All selected points** – A state is assigned only when all selected points are in that state relative to the Receiver (moving to/from/no movement/no interaction). If body points are in different states, the state is not assigned to that sample.
 - **Any selected point** – A state is assigned when at least one selected body point is in that state relative to the Receiver (moving to/from/no movement/no interaction). See the note below.
 - **Each point** – A state is defined for each point of the Actor. Results are shown for each point separately.
- 4 Click the **Receiver** tab. Here, you specify the other subjects (Receiver).
 - Under **Select**, choose the subjects. If you select two or more subjects, select one of the available options from the list:
 - **All selected subjects** – A state is assigned only when the Actor is in that state for all selected Receiver (moving to/from/no movement/no interaction). If the Actor is in different states relative to different subjects (for example, Subject 1 *Moving to* Subject 2 and *Moving from* Subject 3), the state is not assigned to that sample.

Calculating Statistics

- **Any selected subject** – A state is assigned when the Actor is in that state for at least one Receiver (moving to/from/no movement/no interaction).
- **Each Subject** – A state is assigned to each selected subject as a separate *Receiver*. Results are shown for each Receiver.
- Under **Body points**, select the body points of the subjects selected above. If you select two or three points, select one of the available options:
 - **All selected points** – A state is assigned when the Actor is in that state relative to all selected points of the Receivers (moving to/from/no movement/no interaction). If the Actor is in different states relative to different Receiver's body points, the state is not assigned to that sample.
 - **Any selected point** – A state is assigned when the Actor is in that state relative to at least one selected body point (moving to/from/no movement/no interaction).
 - **Each point** – A state is defined for each body point of the Receiver. Results are shown for each Receiver body point separately.

- 5 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.



Notes

- **All / Any selected points** – At any sample, body points of one subject can be in different states relative to another subject's point. Consider the example of Figure 14.19.. The center point of the Actor A is moving to the Receiver R, while the nose point of A is moving from the Receiver. Selecting **All selected points** will give no state for that sample, which is not used in analysis. In the export file, this will be marked by 0 in all columns for relative movement. Selecting **Any selected point** will give both states **Moving to** and **Moving from** for that sample (Figure 14.19.; see also the last note).
- **Any selected subject** – At any sample time, it is possible that the Actor is in different states relative to different Receivers. For example, Subject 1 being Moving to Subject 2 and Moving from Subject 3. In such cases when you select **Any selected Subject** (step 5 above), multiple states are assigned to that sample (see the last note).

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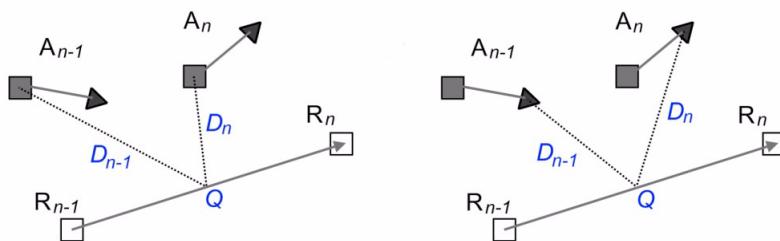


Figure 14.19. Relative movement with multiple body points. Squares: center point of Actor (A) and Receiver (R). Triangles: nose point of Actor (A). For clarity, the Receiver is represented by the center point only. Left picture: The Actor's center point is **moving to** (D_n is shorter than D_{n-1}). Right picture: The Actor's nose point is **moving from** (D_n is longer than D_{n-1}). In this case, selecting **All selected points** (step 3) gives no unique state for Relative movement at sample n . Selecting Any selected points gives the state **Moving to** for center point and **Moving from** for nose point at sample n .



- If your experiment is set to **Only center-point detection** or **Color marker tracking**, the body point options are absent. Calculations are based on the center point.
- You can check multiple states being assigned to one sample time when exporting the dependent variable (see page 492). At a specific sample time, the value of the variable is **1** in more than one column of the export file. In the example above, the columns for **moving to** and **moving from** will both show **1** in the corresponding sample row.

Application

Relative movement can be used to study the effects of individual housing vs. group housing on the social behavior of rats (Spruijt *et al.* 1992. *Physiology & Behavior* **51**: 747-752; Hol *et al.* 1999. *Behavioural Brain Research* **100**: 91-97), or for studying the behavioral interactions between individually recognized animals.

Net weighted movement

Definition

The signed, distance-weighted change in distance between two subjects from one sample to the next.

Net weighted movement is weighted by the distance between two subjects. Changes in positions of subjects which are at a large distance from each other have a lower weight, so they can be distinguished from movements at close distance, which have a different biological meaning.

Unlike Relative movement, this is a continuous variable (in distance units). The Net weighted movement is positive if the subject (*Actor*) is getting closer to another subject (*Receiver*), negative in the other case.

Calculation

Formula:

$$NWM_n = (D_{n-1} - D_n) * \text{abs}(DS_n - DS_{n-1}) / (\max(DS_n, DS_{n-1}))$$

Where:

D_{n-1} , D_n is the distance between the Actor's body point and the interpolated point of the Receiver calculated for two consecutive samples.

DS_{n-1} , DS_n is the distance between subjects for two samples (see page 541).



From Figure 14.18. it can be seen that the outcome depends on which subject is considered as Actor and which as Receiver. In Figure 14.18., Net weighted movement of subject A is **positive** relative to R. If R was the Actor, the point Q would be defined for the other subject in the middle of the $A_{n-1}-A_n$ segment. In that case D_n would be longer than D_{n-1} , thus Net weighted movement of subject R would be **negative** relative to A.

How to specify Net weighted movement

- 1 Click the **Add** button next to **Net weighted movement** (see page 481).
- 2 In the **Net Weighted Movement** tab, under **Maximum interaction distance**, enter the distance above which you do not want to consider the subjects as interacting. (Default: 50 cm/16.69 inches)
- 3 Click the **Body points** tab. Select the body points of the focal subject (*Actor*) you want to use to calculate net weighted movement.
If you select two or three points, results are calculated for each point separately.

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4 Click the **Receiver** tab.

- Under **Select**, choose the subjects you want to consider as Receivers.
- Under **Body points**, select the body points of the subjects selected above. If you select two or more subjects and points, results are calculated for each combination separately.

5 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.

Notes

- 
- The dependent variable is calculated for all the subjects selected in the Data profile. Each subject displayed on the rows of your result table is considered as Actor. The subjects displayed on the columns are the Receivers.
 - If your experiment is set to **Only center-point detection** or **Color marker tracking**, the body point options are absent. Calculations are based on the center point.

Application

Net weighted movement can be used as an objective measure for the intensity of approach and avoidance behavior (Spruijt *et al.* 1992. *Physiology & Behavior* 51: 747-752). The advantage of this variable relative to Weighted movement to/from is that it integrates both. This means that you can analyze, for instance, the movement of subjects regardless of the direction towards or away from each other.

Weighted movement from

Definition

The distance-weighted change in distance between subjects, when a subject (*Actor*) moves away from another subject (*Receiver*).

Weighted movement from is a continuous variable, and is always positive. It is calculated only when the state of the Actor is **moving from** the Receiver (see also **Relative movement** on page 545).

This variable was named *Speed of moving from* in EthoVision 3.

Calculating Statistics

Calculation

Formula:

- If $D_n - D_{n-1} > 0$:
 $WMF_n = (D_n - D_{n-1}) * \text{abs}(DS_n - DS_{n-1}) / (\max(DS_n, DS_{n-1}))$
- If $D_n - D_{n-1} \leq 0$ or $DS_n = 0$:
 $WMF_n = \text{missing value}$

Where:

D_{n-1}, D_n is the distance between the Actor's body point and the interpolated point of the Receiver calculated for two consecutive samples (see Figure 14.20.).

DS_{n-1}, DS_n is the distance between the subjects' body points for two samples (see page 541).

Weighted movement from is not calculated when $DS_n=0$, and $DS_n > \text{Maximum interaction distance}$.

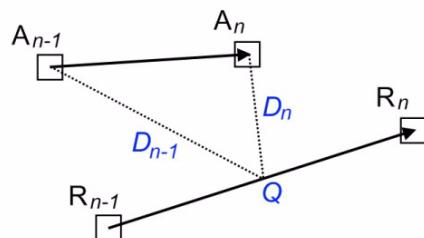


Figure 14.20. Illustration of the dependent variable Weighted movement to. A is the body point of the Actor, R of the Receiver. Q is the interpolated position between the Receiver's body points at samples n and $n-1$.



Notes

- Weighted movement from is equal to the absolute value of Net weighted movement, taken for those samples in which the value of NWM is negative (the focal subject moves away from another subject).
- Weighted movement from is weighted by the distance between two subjects. Changes in positions of subjects which are at a large distance from each other have a lower weight, so they can be distinguished from movements at close distance, which have a different biological meaning.

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- From Figure 14.20, it can be seen that the outcome depends on which of the two interacting subjects is considered as Actor and which as Receiver. This is because formula takes possible differences in speed of approach of the subjects into account. In Figure 14.20., the difference $D_n - D_{n-1}$ is positive, therefore **Weighted movement from R** is calculated. If R was the Actor, the point Q would be defined for the other subject in the middle of the $A_{n-1} - A_n$ segment. In that case D_n would be shorter than D_{n-1} , thus $D_n - D_{n-1} < 0$, and the dependent variable would not be calculated.
- This dependent variable is not a speed, as time is not involved in its calculation. However, the parameter is quadratically sensitive to movement of the subject.

How to specify Weighted movement from

- 1 Click the **Add** button next to **Weighted movement from** (see page 481).
- 2 In the **Weighted movement From** tab, under **Maximum interaction distance**, enter the distance above which you do not want to consider the subjects as interacting. (Default: 50 cm/16.69 inches)
- 3 Click the **Body points** tab. Select the body points of the focal subject (*Actor*) you want to use to calculate the dependent variable.
If you select two or three points, results are calculated for each point separately.
- 4 Click the **Receiver** tab.
 - Under **Select**, choose the subjects you want to consider as Receivers.
 - Under **Body points**, select the body points of the subjects selected above. If you select two or more subjects and points, results are calculated for each combination separately.
- 5 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.



Notes

- The dependent variable is calculated for all the subjects selected in the Data profile. Each subject displayed on the rows of your result table is considered as Actor. The subjects displayed on the columns are the Receivers.
- If your experiment is set to **Only center-point detection** or **Color marker tracking**, the body point options are absent. Calculations are based on the center point.

Application

Weighted movement from can be used as an objective measure for the intensity of avoidance (Spruijt *et al.* 1992. *Physiology & Behavior* 51: 747-752).

Weighted movement to

Definition

The distance-weighted change in distance between subjects, when a subject (*Actor*) moves towards another subject (*Receiver*).

Weighted movement to is a continuous variable, and is always positive. It is calculated only when the state of the Actor is **moving to** the Receiver (see also **Relative movement** on page 545).

This variable was named *Speed of moving to* in EthoVision 3.

Calculation

Formula:

- If $D_n - D_{n-1} < 0$:
 $WMT_n = (D_{n-1} - D_n) * \text{abs}(DS_n - DS_{n-1}) / (\max(DS_n, DS_{n-1}))$
- If $D_n - D_{n-1} \geq 0$ or $DS_n = 0$:
 $WMT_n = \text{missing value}$

Where:

D_{n-1} , D_n is the distance between the Actor's body point and the interpolated point of the Receiver calculated for two consecutive samples (see Figure 14.20.). DS_{n-1} , DS_n is the distance between the subjects' body points for two samples (see page 541).

Weighted movement to is not calculated when $DS_n=0$, and $DS_n >$ Maximum interaction distance.

Notes



- Weighted movement to is equal to the absolute value of Net weighted movement, taken for those samples in which the value of NWM is positive (the focal subject moves towards another subject).
- Weighted movement to is weighted by the distance between two subjects. Changes in positions of subjects which are at a large distance from each other

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have a lower weight, so they can be distinguished from movements at close distance, which have a different biological meaning.

- From Figure 14.18. it can be seen that the outcome depends on which of the two interacting subjects is considered as Actor and which as Receiver. This is because formula takes possible differences in speed of approach of the subjects into account. In Figure 14.18., the difference $D_n - D_{n-1}$ is negative, therefore **Weighted movement to R** is calculated. If R was the Actor, the point Q would be defined for the other subject in the middle of the $A_{n-1}-A_n$ segment. In that case D_n would be longer than D_{n-1} , thus $D_n - D_{n-1} > 0$, and the dependent variable would not be calculated.
- This dependent variable is not a speed, as time is not involved in its calculation. However, the parameter is quadratically sensitive to movement of the subject.

How to specify Weighted movement to

- 1 Click the **Add** button next to **Weighted movement to** (see page 481).
- 2 In the **Weighted movement To** tab, under **Maximum interaction distance**, enter the distance above which you do not want to consider the subjects as interacting. (Default: 50 cm/16.69 inches)
- 3 Click the **Body points** tab. Select the body points of the focal subject (*Actor*) you want to use to calculate the dependent variable.
If you select two or three points, results are calculated for each point separately.
- 4 Click the **Receiver** tab.
 - Under **Select**, choose the subjects you want to consider as Receivers.
 - Under **Body points**, select the body points of the subjects selected above.
If you select two or more subjects and points, results are calculated for each combination separately.
- 5 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.



Notes

- The dependent variable is calculated for all the subjects selected in the Data profile. Each subject displayed on the rows of your result table is considered as Actor. The subjects displayed on the columns are the Receivers.
- If your experiment is set to **Only center-point detection** or **Color marker tracking**, the body point options are absent. Calculations are based on the center point.

Application

Weighted movement to can be used as an objective measure for the intensity of avoidance (Spruijt *et al.* 1992. *Physiology & Behavior* 51: 747-752).

Manually scored behavior

Definition

Behaviors defined in the manual scoring settings are analyzed the same way as the behavioral states (In zone, movement etc.)

How to specify a manually scored behavior

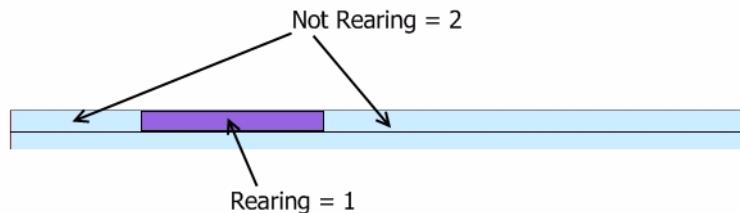
- 1 Click the **Add** button next to the name of the behavior (or behavior group) you want to calculate (see page 481).
- 2 In the tab named as the behavior (or the behavior group), select the behavior you want to calculate statistics for, and clear the options for the behaviors you do not want to include in the table.
 - For **Mutually-exclusive** behaviors, the window lists all behaviors of the group selected by default. The analysis result will show the statistics for each behavior separately. Clear the option for the behavior you do not want to analyze.
 - For **Start-Stop** behaviors, the window lists the behavior (selected by default) and its opposite, indicated by **Not <behavior>**. Selecting **Not <behavior>** means that the program calculates the statistics for the time that the behavior was not active (see below).
- 3 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.



Notes

- **Not <behavior>** – For example, if you define *Rearing* as a Start-Stop behavior and score it one time during the test (not at the start of the trial), then the results will be (see also the figure below):
 - Frequency of Rearing = 1.
 - Frequency of "Not Rearing" = 2. The first occurrence of "Not Rearing" is at the start of the trial, and the second occurrence is after you press the Stop code for Rearing.

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The duration of Not <behavior> is the sum of all the periods of time that the behavior was not active.

- To export raw data of manually scored behaviors, see page 492 and page 495.

Trial Control state

Definition

A time interval specified by two events of Trial and Hardware Control occurred during the trial. The interval may also occur in two or more instances if the events that mark its start and end occur repeatedly during the trial.

If an interval occurs in more instances during a trial, you can choose to analyze either each occurrence or the sum up the results for all occurrences. See the **Calculate statistics per interval** option below.

How to specify a Trial Control state

- 1 Click the **Add** button next to **Trial Control state** (see page 481).
- 2 Next to **From**, from the **Element** list select the Trial and Hardware Control element that makes the criterion for the start of the interval. From the **Event** list, select the state of that element that makes the start of the interval.
- 3 Next to **To**, from the **Element** list select the Trial and Hardware Control element that makes the criterion for the end of the interval. From the **Event** list, select the state of that element that makes the end of the interval.

Choose which occurrence of the ending event you want to consider.

Calculating Statistics

- 4 An interval may occur several times in a trial. If you want to have statistics for each occurrence, select the **Calculate statistics per interval** option. Next to **For consecutive intervals**, choose the range of occurrences you want to have in the results.
- 5 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.



Notes

- Do not select **Calculate statistics per interval** when you want to sum up the results from the occurrences of the state interval in the trial. For example, to calculate the total duration of the state **From** condition 'In Cue zone' becomes true **To** condition 'In Feeder zone' becomes true.
- Statistics of Duration and Latency can only be a multiple of the sample interval (=1/sample rate). For example, when you create a condition 'Subject in zone A for >= 3 s', this condition is met when the time elapsed from its activation exceeds 3 s. If the sample rate is 12.5 frames/s (thus the sample interval is 1/12.5 = 0.08 s), the condition is met at the first multiple of 0.08 greater than 3 s, that is 3.04 s. This affects data analysis, for example the duration of the state 'From condition active to condition true' is 3.04 s.
- Please note that the Frequency of a Trial Control state is determined by the start of the state. This means that at the end of a trial, a Trial Control state is counted even if there is no stop event.
- For more information on Trial Control state, see the EthoVision XT Trial and Hardware Control manual on your installation DVD.



Application

You can use Trial control states to test whether trial control works as expected, and for analyzing learning behavior. For example, calculate the duration of the Trial control state 'From *Cue light ON* to *Subject in Feeder zone*' to see whether this interval decreases during a trial.

Trial Control event

Definition

A point event, with no duration, defined by an element of Trial and Hardware Control (condition, action, rule/sub-rule and reference).

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A Trial Control event has no duration. For Trial Control events you can only calculate frequency and latency of first or last occurrence.

How to specify Trial Control event

- 1 Click the **Add** button next to **Trial Control event** (see page 481)
- 2 From the **Element** list select the Trial and Hardware Control element to be analyzed. For example, if you want to analyze the hardware-base action 'Drop pellet' select **Action: Drop pellet**.
- 3 From the **Event** list, select the state of the element. The options available depend on what you have chosen as Element.
- 4 Click the **Statistics** tab to choose the statistics for that dependent variable (see page 558 for details). Next, click **Add**.



Statistics of Duration and Latency can only be a multiple of the sample interval (=1/sample rate). For example, when you define a condition 'Subject in zone A for >= 3 s', this condition is met when the time elapsed from its activation exceeds 3 s. If the sample rate is 12.5 frames/s (thus the sample interval is 1/12.5 = 0.08 s), the condition is met at the first multiple of 0.08 greater than 3 s, that is 3.04 s. This affects data analysis, for example the latency of the event 'Condition becomes true' is 3.04 s.



For more information on Trial Control event, see the EthoVision XT Trial and Hardware Control manual.

Application

You can use Trial control events to test whether trial control works as expected. For example, visualize the Trial control event 'condition true' and plot it together with the video to check that the condition is met at the correct time. You can also define a Trial control event like 'Action Drop pellet' to calculate its frequency.

14.5 Statistics available

Statistics are a summary of the values of the dependent variable calculated for all the samples in your tracks (or the segments you have selected in the active Data profile).

To select a statistic for a dependent variable, click the **Statistics** tab of the variable's properties (see page 481) and select the corresponding box.

Calculating Statistics

The following statistics are available:

- **Latency to first** – The time from the start of the track (or time window) till the first occurrence of the behavior (for example, the first time in the track that the animal is in a zone).
- **Latency to last** – The time from the start of the track (or time window) till the last occurrence of the behavior.
- **Duration** – The duration of the state variable. For example, the time that the animal is in zone.
- **Standard Error** – The standard deviation divided by the square root of the number of samples.
- **Standard Deviation** – The population standard deviation of the mean, defined by the formula:

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{N-1}}$$

where x is the individual variable value, \bar{x} the mean and N the number of samples.

- **Variance** – The square of the standard deviation.
- **Mean** – The sum of all variable values divided by the number of samples.
- **Minimum** – The smallest variable value.
- **Maximum** – The largest variable value.
- **Total** – The sum of all the values.
- **Frequency** – The total number of occurrences of a state (only for variables with discrete values).
- **Number of samples** – The total number of valid values of that variable (only for continuous variables).



Notes

- Not all statistics listed are available for every dependent variable. To view the list of statistics for a specific variable, double-click its name under **Selected Dependent Variables** and click the **Statistics** tab.
- Values of **Latency** and **Duration** can only be a multiple of the sample interval (= 1/sample rate).
- Variables used in a Trial Control condition are based on the time that the condition becomes active, not from the start of the track. For example, if you

define a condition based on **Latency to first**, latency is counted from the time the condition becomes active. For more information, see the EthoVision XT Trial and Hardware Control Manual on your installation DVD.

- To add or remove statistics for a dependent variable, double-click that variable under Selected Dependent Variables and change your selection.

15

File management



You must carry out all your file management in EthoVision XT. If you delete, move or rename files using the Windows Explorer, EthoVision may not be able to find the files again (even after restoring the files to their original location), because EthoVision files contain references to other files. The only exceptions are backup files which are designed to be moved to a different location.

File types that can be managed in EthoVision XT are:

- **Experiment files (*.evxt):**
 - Creating a new experiment – See page 562.
 - Opening an existing experiment – See page 563.
 - Saving an experiment – See page 564.
 - Renaming/copying an experiment – See page 564.
- **Experiment backup files (*.evz):**
 - Backing up an experiment – See page 565.
 - Restoring an experiment from a backup file – See page 566.
- **Export files (*.txt, *.xls):**
 - Exporting track files – See page 568.
 - Exporting analysis results – See page 570.
 - Exporting manually scored data – See page 570.
 - Exporting Trial Control data – See page 571.

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- Importing your export files into other programs – See page 573.
- **Media files (*.mpg, *.mpeg)** – See page 579.
- **Import profiles for external data (*.eip) and external data files** – See page 579.
- **General and (GLP) Experiment Log File** – See page 580.
- **Log file with error messages** – See page 22.
- **Managing Settings and Profiles** – See page 581.

15.1 Experiments

An experiment contains the settings and data from one series of trials. All the trials in one experiment have the same independent variables (but normally not the same independent variable values).

When you want to create a new experiment, you have two options:

- **New template experiment** - Selecting this option guides you through the steps necessary to set up an experiment for a number of standard tests, such as, the Morris Water Maze test or a Radial Maze test. Based on a number of choices you make (for example, video source, type of animal, type of arena, number of subjects per arena, etc), EthoVision XT creates an experiment that you can use as a basis for your specific test. You can also use an existing experiment as a template for a new experiment.



For details how to create a new template experiment, see page 92 in Chapter 5 **Setting up an Experiment**.

- **New default experiment** (see below) - This is the way you created an experiment in previous versions of EthoVision XT.

Creating a new default experiment

1 Do one of the following:

- In the EthoVision Startup window, under Create a new experiment, click **New default experiment**.
- From the **File** menu, choose **New Default Experiment** (or press <Ctrl+N>).

File management

The **New Experiment** window appears (Figure 15.1).

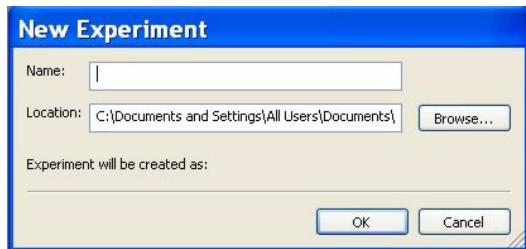


Figure 15.1. The **New Experiment** window.

2 Enter a name for your experiment.



An experiment file name cannot contain any of the following characters: / : * ? \ " < > | . ; . If any of these characters is used for an experiment name, a warning appears.

3 Browse to the location in which you want to store your experiment. See page 586 for the default file locations.



- You can specify the default experiment location during installation. If you like, you can change it by setting preferences. See page 127.
- Select **GLP Experiment** if you want EthoVision to support you in making a GLP-compliant experiment. You need the GLP add-on module for this (see Chapter 4 "EthoVision XT and GLP").

4 Click **OK**. The **New Experiment** window closes and the experiment is created. You can find the experiment file (*.evxt) in a folder with the same name as the experiment.

Opening an existing experiment

To open an experiment:

1 Do one of the following:

- In the EthoVision Startup window, under **Open an existing experiment**, click an experiment from the list or click **Browse** to open another one.
- From the **File** menu, select **Open Experiment** or press **Ctrl+O**.

2 Browse to the location where your experiment is stored and click **Open**.

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When you try to open an experiment from an older version a warning appears, informing you that you cannot open upgraded experiments in the old version of the software. This is particularly import when you try to open an EthoVision XT 7 experiment in EthoVision XT 8 because:

- **Hardware logs are irrecoverably lost.**
- **Statistics results must be recalculated.**
- **The Minimum Distance Moved nesting criterion is removed from the Data Selection. You can select the Minimal Distance Moved method in your Track Smoothing profile. See page 380 for more information.**



We strongly recommend to make a backup of your experiment in EthoVision XT 7 before opening it in EthoVision XT 8. To make a backup, from the File menu, select Make Backup. If you uninstalled EthoVision XT 7, you can also make a copy of your experiment using Windows Explorer before you open it in EthoVision XT 8. Always copy the complete experiment folder and not only the *.evxt file otherwise your experiment may get corrupt!

Saving an experiment

To save an experiment, from the **File** menu, select **Save Experiment** or press **<Ctrl+S>**.

You can also use auto-save to save an experiment:

- 1 From the **File** menu, select **Preferences**.
- 2 Click the **Auto save** tab, select **Enable auto save** and set the interval.



When you make changes to your experiment, e.g., changes in settings, these are saved with save or auto-save. During data acquisition and track editing, data are saved to file immediately.

Renaming/copying an experiment

You can rename or copy an experiment by saving it under a different name.

- 1 From the **File** menu, select **Save Experiment As** and enter the new experiment name.



Make sure that the name does not contain any of the following characters:
/ : * ? \ " < > | . ; .

File management

- 2 Browse to the location in which you want to store your experiment and click **OK**. A new experiment directory is made with the same name as the experiment.

Deleting an experiment

If you want to delete an existing experiment, open the **File** menu and click **Open Experiment**. This opens a browser. Click the experiment folder you want to delete and press the **Delete ** key on your keyboard. A warning message opens in which you are asked if you really want to delete the experiment. If you click **Yes**, the experiment including all the tracks, videos and all other related files are deleted. You can also use the Windows Explorer to browse to the experiment folder and delete it.

Backing up an experiment

If you want to keep a safe copy of your data, or transfer an experiment to a different computer, you can make a backup of the experiment with all its associated files:

- 1 From the **File** menu, select **Make Backup**. The **Backup Experiment** window opens (Figure 15.2).



Figure 15.2. The **Backup Experiment** window.

- 2 Enter a name for your experiment backup.



Make sure that the name does not contain any of the following characters:
/ : * ? \ " < > | . ; .

- 3 Browse to the location in which you want to store your experiment backup file.

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4 Select whether you want to:

- Include Media Files.**
- Include Export Files**
- Include External (physiological data) Files**
- Make backup read-only** – When you select this option you get a warning in case you (accidentally) delete the backup file.

5 Click **OK**. The backup file is created (*.evz).



Your backup files can get very big if you include your media files!



Notes

- Make sure that your media files, export files and external data files are within the experiment folder, otherwise they will not be included in the backup.
- You should store the backup file on a secure medium (CD, DVD, flash drive or network drive), in a separate location.
- **Windows XP users** – To store your backup file on a CD or DVD, you can use CDBurnerXP. If you bought your computer from Noldus IT, CDBurnerXP has already been installed on your computer. Do not install another CD/DVD burning program as they often install codecs which can interfere with EthoVision's video handling.
- **Windows 7 users** – CD/DVD burning is built into Windows 7. After you insert a CD or DVD into your computer's CD/DVD drive, a dialog box appears. Click **Burn files to disk** and follow the instructions on the screen.
- Keep multiple copies of your backup file and every three years make a new copy of your CD/DVD. Shield the CD/DVD from light and take care that it does not get scratched.

Restoring an experiment

To restore an experiment:

1 Do one of the following:

- In the EthoVision Startup window, under **Restore a backup experiment**, click **Restore backup**.
- From the **File** menu, select **Restore Backup**.

File management

- 2 Browse to the location where your backup file is stored, select it and click **Open**.
- 3 Browse to the location where you want to restore the experiment and click **OK**.

The experiment directory and all associated files of the experiment are now restored.

Deleting a backup

If you want to delete an existing backup, open the **File** menu and click **Restore Backup**. This opens a browser. Click the backup file you want to delete and press the **Delete** (Del) key on your keyboard. A warning message opens in which you are asked if you real want to delete the file. If you click yes the file is deleted. If you delete an entire folder, the contents of the folder including all the sub folders are deleted. You can also use the Windows Explorer to browse to the experiment backup file (*.evz) and delete it.

Exporting/importing experiments

You can import experiments from previous EthoVision XT versions (4/5/6/7/8.0) into EthoVision XT 8.5. Upon opening, these experiments are converted to EthoVision XT 8.5 experiments. Experiments from EthoVision 1,2 and 3 cannot be imported into EthoVision XT.



EthoVision XT 4/5/6/7/8.0 experiments converted to EthoVision XT 8.5 Experiments or experiments created in EthoVision XT 8.5, cannot be re-opened in EthoVision XT 4/5/6/7/8.0. If you try to do so, this may corrupt your experiment. See also the warning on page 564.

15.2 Track files

Importing track files

You cannot import track files from one EthoVision XT experiment into another. This means that **data which you want to analyze together in EthoVision must be acquired in the same experiment**.

As a result, experiments will contain a large amount of data. It is therefore important that you regularly backup your data and store the backup in a separate location. See backing up an experiment on page 565.

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15.3 Export files

EthoVision XT offers a comprehensive set of descriptive analytical tools. However, if you want to perform tests to see if, for instance, your treatment has a significant effect on the dependent variables (such as velocity or distance moved), or if you want to perform other types of analysis, you must export your data to another program. EthoVision allows you to export your data (before or after analysis) in formats suitable for spreadsheets, databases and statistical packages.

For importing files to other programs, see page 573.

Exporting files

Exporting track files

Exporting track files means that you export the x,y coordinates and the subject size for each sample together with the values of the dependent variables which you selected in your analysis profile and the independent variables that you defined in the **Trial List**. See page 492 for the procedure to export your track data and Figure 15.3 for an example of a track file.

The track files that you export are, by default, saved in the **Export Files** subfolder of your experiment folder. For the default experiment location, see page 586.

File management

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	
13	Start time	14-11-2006 14:49:56													
14	Trial Duration	00:01:00													
15	Recording after	00:00:02													
16	Recording duration	00:00:58													
17	Track	Track file0000a00000000000_0001.trk													
18	Tracking source														
19	Video file	C:\Documents and Settings\All Users\Documents\Noldus\EthoVision XT\Experiments\Open field XT 60\Media Files\Open field1.mpg													
20	Video start time	14-11-2006 14:49:56													
21	Trial status														
22	Acquisition status	Acquired													
23	Track status	Acquired													
24	Video file status	External													
25	Sync status	Not available													
26	Reference duration														
27	Reference time														
28	Soft file														
29	External data Independent Variable														
30	Heart rate	HR													
31	User-defined Independent Variable														
32	Rat ID	1													
33	Treatment	drug													
34															
35	Trial time		Recording time	X center	Y center	Area	Areachang	Elongation	Velocity	Distance r	In zone{b1}	In zone{c1}	Movement	Distance t	Result 1
36		2	0	-45.6744	12.24915	42.52506	-	0.566958	-	-	1	0	8.611514	1	
37		2.2	0.2	-46.1703	6.351053	43.40641	6.169439	0.628786	29.59456	5.918912	1	0	1	7.68348	
38		2.4	0.4	-46.0822	0.777878	46.49111	21.15236	0.856012	27.86935	5.573781	1	0	1	6.76849	
39		2.6	0.6	-45.4043	-4.47473	52.2191	48.03349	0.834809	26.48089	5.296178	1	0	1	5.978689	
40		2.8	0.8	-44.2409	-9.40688	50.67754	56.18596	0.810187	25.37758	5.067515	1	0	1	5.280633	
41		3	1	-42.6997	-14.018	52.66057	53.10124	0.879901	24.30939	4.861877	1	0	1	4.582937	
42		3.2	1.2	-40.8594	-18.3077	53.32158	50.4572	0.830969	23.33867	4.667735	1	0	1	4.03105	
43		3.4	1.4	-38.7709	-22.275	55.30462	53.98259	0.847322	22.41744	4.483488	1	0	1	3.73625	
44		3.6	1.6	-36.4659	-25.9189	52.66057	42.30473	0.81778	21.55847	4.311694	1	0	1	3.482803	
45		3.8	1.8	-33.9629	-29.2372	51.99956	22.25405	0.860497	20.7822	4.15644	1	0	1	3.357782	

Figure 15.3. Part of a track file.



Notes

- The track file export takes into account your data selection for entire tracks, but not for within tracks. So, if you make a data selection such that you only have track 1 and only zone 1, EthoVision will export track 1, but the entire track. If you want to filter out zone 1 in your track data, you should include the zone as a dependent variable and then filter on that column in Excel. The result column in the export file has values 0 and 1 dependent on the fact if the data selection criteria were met (1) or not met (0).
- Trial time** is the time elapsed from the start of the trial (basically, the moment when you clicked the green **Start** button). Recording time is the time elapsed from the start of the track. The difference between the two time values is also shown next to the header **Recording after**. For more information on trials and tracks, see page 203.
- The values of the x,y coordinates and the subject size in the exported track files are expressed in calibrated units, (square) millimeters, (square) inches, etc., not in pixels.
- Samples that were **not found** are indicated by "-" instead of the coordinate values. Note that sometimes the center point is found, not the nose point or

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the tail base (or neither of the two). After acquisition you can see the proportion of samples that were not found in column Subject not found the Trial list. If this column is not visible, click **View Settings** on the component tool bar, select **Variables** and then select **Subject not found**.

- Excel can import a limited number of rows and columns. For Excel 2003 this is 256 columns and 65536 rows. For Excel 2007 and newer versions this is 16384 rows and 1048576 columns. EthoVision XT gives a warning if the number of exported rows exceeds the limit. Excel will only import the maximum number of rows. The exported EthoVision XT files can still be imported in another program like SPSS.

Exporting analysis results

See page 496 for the procedure to export your analysis results and Figure 15.4 for an example of an analysis output file. The analysis output files that you export are, by default, saved in the **Export Files** subfolder of your experiment folder. For the default experiment location, see page 586.

	A	B	C	D	E	F	G	H	I
1					Distance n	In zone	In zone	In zone	I
2					Center-poi	Nose-point	Nose-point	Nose-point	Nose-point
3	Rat ID	Treatment	Dose	Total	Duration	Frequency	Latency	Duration	F
4				cm	s		s	s	
5	Trial 1	1	1	0	336,8281	33,2	4	0	5,36
6	Trial 2	2	2	0,1	339,1545	33,2	4	0	5,52
7	Trial 3	3	1	0	339,8803	33,2	4	0	5,68
8	Trial 4	4	2	0,5	211,4661	17,44	3	0	1,12
9	Trial 5	5	1	0	302,2497	28,96	4	0	4,4
10	Trial 6	6	2	0,1	225,3102	22,4	3	0	1,12
11	Trial 7	7	1	0	397,5319	37,04	5	0	6,64
12	Trial 8	8	2	0,5	159,9551	12,64	3	0	1,12
13	Trial 9	9	1	0	347,0023	33,2	4	0	5,04
14	Trial 10	10	2	0,1	245,2527	25,28	3	0	3,2

Figure 15.4. Part of an analysis output file.



You can also include Trial Control data in the analysis results.

Exporting manually-scored behaviors

You can export the behaviors scored manually as raw data or as a **Manual Scoring log**.

- As **raw data** – See page 492.
- As a **Manual Scoring log** – See page 495.

File management

Exporting Trial Control data

You can export Trial Control data in a **raw data** file (see below), in an analysis results sheet (see page 496 for the procedure to export your analysis results) or in a Trial Control log (see below).

- As **raw data** – Data are saved to additional columns of the track data file, and each sample point receives a “0” or “1” depending on whether a state (or event) is active at that time.

To export Trial Control data as raw data, define them first in the Analysis Profile (see page 557) and then from the **Analysis** menu select **Export** and then **Raw Data**. Make sure that the **Track & dependent variables** option is selected and click **Start export**.

See Figure 15.5 for an example of Trial Control data exported as raw data.

25	Sync statu	Not available												
26	Reference	+ 0:02:12.1												
27	Reference	56:02.0												
28	Sof file													
29	User-defined Independent Variable													
30	Animal ID													
31	Day													
32	Dose													
33														
34	Trial time	Recording	X center	Y center	X nose	Y nose	X tail	Y tail	Area	Frequency	Light on	Duration	Light on	Interva
536	40.08	40.08	-39.1441	-7.90048	-42.475	-7.1493	-37.4353	-13.0462	27.841	0	0	0	0	
537	40.16	40.16	-39.1443	-7.21036	-42.475	-7.1493	-37.4353	-13.0462	28.45053	0	0	0	0	
538	40.24	40.24	-39.1196	-6.359	-42.475	-7.1493	-37.4353	-13.0462	29.93849	0	0	0	0	
539	40.32	40.32	-39.1171	-5.26601	-38.0493	-1.01125	-40.4126	-9.18471	29.57994	0	0	0	0	
540	40.4	40.4	-39.1235	-4.30661	-38.7624	1.013074	-40.0025	-7.88315	30.74521	0	0	0	0	
541	40.48	40.48	-39.1372	-3.35561	-38.9161	2.922175	-40.2511	-6.17149	31.71328	1	1	1	1	
542	40.56	40.56	-39.1508	-2.05771	-38.9161	2.922175	-40.2511	-6.17149	30.04605	0	1	0	1	
543	40.64	40.64	-39.1889	-0.37162	-38.9161	2.922175	-40.2511	-6.17149	31.56987	0	1	0	1	
544	40.72	40.72	-39.2659	0.434588	-40.3744	6.698809	-38.535	-2.95356	34.29481	0	1	0	1	
545	40.8	40.8	-39.4444	1.210378	-40.3744	6.698809	-38.535	-2.95356	27.80795	0	1	0	1	

Figure 15.5. Part of a raw data file containing Trial Control data. The columns **Frequency Light on** and **Duration Light on** are dependent variables defined in an Analysis profile. **Frequency Light on** is a Trial Control event defined by the action “*Light on*”. Duration Light on is a state interval that goes as long as the light is on. At trial time 40.48, a light device switches on. This is marked by **Frequency Light On** which gets the value 1. From that moment, the variable **Duration Light On** gets value 1.

- As a **Trial Control log** – Data are written to a separate file, where all events occurred during the trial (also those not included in the Analysis Profile) are stored as lines each with a time stamp.

To export Trial Control data as Trial Control log, from the **Analysis** menu, select **Export** and then **Raw Data**. Select **Trial Control log** and click **Start export**.

See Figure 15.6 for an example of Trial Control log. The file contains the following columns:

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- **Trial Time and Recording time** – See page 492 for the difference.
- **Event** – The type of trial control event. (see page 176)
- **Rule, Condition, Action, Operator, Reference** – The Trial control element that is involved in the event described in the corresponding **Event** cell.
- **Variable, Value** – The variable that is subject to an action, and the new value assigned to the variable.
- **Reference1...4, Repeat1...4** – Marks the time that a sub-rule is active. **Repeat** indicates the number of times that sub-rule has been repeated up to that time.



To export log files of the hardware devices operated during the trial, select also the **Hardware log** option.



For more information see **Analysis of Trial Control data** in the EthoVision XT Trial and Hardware Control Manual on your installation DVD.

	A	B	C	D	E	F	G	H	I	J	K	L
19	Video file D:\SDC\Media files\10 min C57Bl6 4 kooien mpg2.mpg											
20	Video start	53:54.0										
21	Trial status											
22	Acquisition Acquired											
23	Track stat: Acquired											
24	Video file External											
25	Sync stat: Not available											
26	Reference + 00:02:14.6											
27	Reference	53:54.0										
28	Sof file											
29												
30	Trial time	Recording	Event	Rule	Condition	Action	Operator	Reference	Variable	Value	Reference1	Repeat1
31	1	0						Var1		0		
32	1	0 becomes active		Start-stop trial								
33	1	0 becomes active			Start track							
34	1	0 becomes inactive			Start track							
35	1	0 becomes active		Sub-rule (1)				Reference (1)				
36	1	0 makes sub-rule active		Sub-rule (1)				Reference (1)				
37	1	0 becomes active		Sub-rule (1)						Reference (1)	0	
38	1	0 becomes active			In zone (2)					Reference (1)	0	
39	1	0 becomes false			In zone (2)					Reference (1)	0	
40	1.04	0.04 becomes true			In zone (2)					Reference (1)	0	
41	1.04	0.04 becomes inactive			In zone (2)					Reference (1)	0	
42	1.04	0.04 becomes active				Variable act (1)				Reference (1)	0	

Figure 15.6. Part of a Trial Control log. Activation of conditions and actions is written as an event with a time stamp. The event is written in the **Event** column. The next columns describe which type of Trial Control element becomes true/false/active/inactive.

Importing files into other programs

Importing your export files into Excel

You can copy the analysis results and paste them into Excel. You can also export your EthoVision data to Excel in *.xls or *.xlsx format, depending on what version of Excel you have installed. Excel must be installed on your EthoVision computer. Otherwise create a text export and open that in Excel on another computer.

Importing your export files into SPSS (version 15.0/16.0 for Windows)

Before you can import your export files into statistical programs such as SPSS, you have to manually edit them and make sure that the names of your variables (dependent and independent) are in the first row of the data file and the data in the subsequent rows. To edit your export files, open them in Excel. Below you can find the procedure for creating and exporting an analysis output file, editing the file and importing it into SPSS to carry out a one-way ANOVA.

In EthoVision:

- 1 Create a new analysis profile: from the **Analysis** menu, choose **Analysis Profile**.
 - 2 Click the **Add** button next to the dependent variables that you want to use and choose the statistics that you want to calculate.
-  See page 498 for a description of the available dependent variables and their statistics.
- 3 Make sure that the data profile that you want to use is active (that is, highlighted in blue in the Experiment Explorer).
 - 4 From the **Analysis** menu, select **Results** and then **Analysis Output**. Your analysis report is made.
 - 5 From the **Analysis** menu, choose **Export** and then **Analysis Output**. Browse to the location where you want to store your export file. By default, export files are stored in the experiment's **Export Files** folder (see page 586).
 - 6 Select the file type for your export file (either **Plain text** or **Excel**) and click **OK**. Your export file is created.

In Excel:

- 1 From the **File** menu, select **Open** and browse to the location where your export file is stored. Select the appropriate file and click **Open**.

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- 2** Rename the dependent variable names. For instance, in the example in Figure 15.4 rename the dependent variable 'Total' (Total distance moved of the center-point in cm) to, e.g., 'Total distance moved center-point' (Figure 15.7).

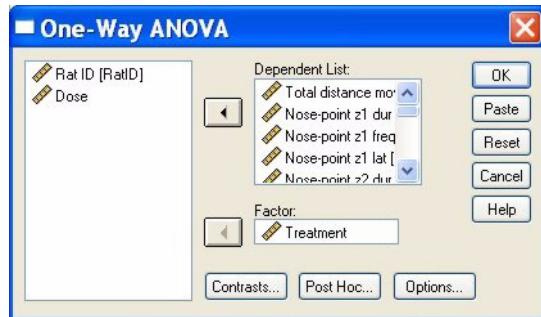
	A	B	C	D	E	F	G	H	I
1	Rat ID	Treatment	Dose	Total distance move	Nose-point z1 dur	Nose-point z1 freq	Nose-point z1 lat	Nose-point z2 dur	
2	Trial 1	1	1	0	336,8280575	33,2	4	0	5,36
3	Trial 2	2	2	0,1	339,1544847	33,2	4	0	5,52
4	Trial 3	3	1	0	339,8803038	33,2	4	0	5,68
5	Trial 4	4	2	0,5	211,4661154	17,44	3	0	1,12
6	Trial 5	5	1	0	302,2496858	28,96	4	0	4,4
7	Trial 6	6	2	0,1	225,3102236	22,4	3	0	1,12
8	Trial 7	7	1	0	397,5319106	37,04	5	0	6,64
9	Trial 8	8	2	0,5	159,9551233	12,64	3	0	1,12
10	Trial 9	9	1	0	347,0022792	33,2	4	0	5,04
11	Trial 10	10	2	0,1	245,2526641	25,28	3	0	3,2

Figure 15.7. The analysis output file of Figure 15.4 after editing. The border zone has been renamed to 'z1' and the center zone to 'z2', dur = duration, freq = frequency and lat = latency.

- 3** When you have renamed all the dependent variables, delete rows 1, 2 and 4.
4 Make sure that all your independent variable values are numerical values. If you used, for example, 'drug' and 'saline' as the values for the independent variable 'Treatment', you must change them, e.g., into '1' and '2'.
5 Save the data file: from the **File** menu, select **Save As** and give it a different name. For instance, 'original name_edited.xls'.

In SPSS:

- 1 Open the edited data file: from the **File** menu, select **Open** and then **Data**. In the **Files of type** field, select **Excel (*.xls)** and browse to the location where your edited data file is stored, select the file, and click **Open**.
- 2 In the **Opening Excel Data Source** window click **OK**. The data file opens.
- 3 From the **Analyze** menu, choose **Compare Means** and then **One-Way ANOVA**. The **One-Way ANOVA** window opens (Figure 15.8).

Figure 15.8. The **One-Way ANOVA** window in SPSS.

- 4 In the **Dependent List**, select the dependent variables that you want to analyze.
- 5 In the **Factor** box, select the independent variable that you want to test by.
- 6 Click **Post Hoc** to define the significance level for the test. Click **OK** to carry out the test.



It is possible that your version of SPSS does not have the same specifications as the version described above. If in doubt, check the SPSS manual or online help of your version of the software.

Importing manually scored data into The Observer XT

The procedure below refers to the Observer XT versions 7/8/9/10.

- 1 In EthoVision XT, export the data in form of Manual scoring log (see page 495).
 - Select **Manual Scoring log** only before clicking **Start Export**.
 - Select **Plain text** as export format and select semicolon (;) as **Delimiter**.
 - To export a subset of tracks or subjects, filter the data (see page 393) before exporting.
- 2 Open the file with the Windows **Notepad**.
- 3 Save the file under a different name.



Name the file including information on trial name, arena name, and values of the independent variables (for example, **Trial1 Arena2 Treated Male Apomorphine 05mg/kg.txt**).

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- 4 Remove the lines from “Experiment” to “ ” so that the first line of the file contains “Header Lines” and the second “Trial time”.

```
"Header Lines:","30";
"Trial time","Recording time","Subject","Behavior","Event";
1;0;"Subject 1";"No grooming","state start";
13.36;12.36;"Subject 1";"No grooming","state stop";
```

- 5 Change the number of Header lines to 2, then save and close the file.

```
"Header Lines:","2",
"Trial time","Recording time","Subject","Behavior","Event";
1;0;"Subject 1";"No grooming","state start";
```

- 6 In The Observer XT, create a project or open an existing one. Make sure that the project’s coding scheme contains exactly the same behavior names and that these are organized in the same groups (mutually-exclusive or start-stop) as the corresponding behaviors scored in EthoVision. See the note **Effects on the coding scheme** below.

- 7 From the **File** menu select **Import Observational Data**.

- 8 From the **Files of type** list, select **EthoVision XT Data File (*.txt)**.

 **EthoVision XT Data File** is an import profile made for manual scoring logs exported from EthoVision. It is installed automatically when you install EthoVision XT.

If you do not have this import profile, you can create one anew: see the note **Creating an import profile** below.

- 9 Select the file you want to import and click **Open**.

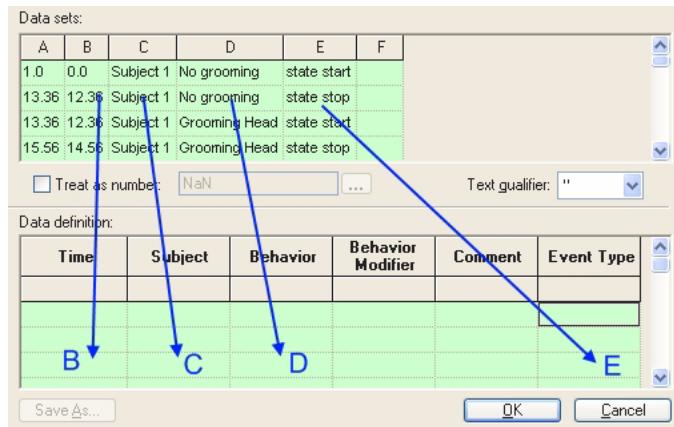
Result – The observation opens on your screen.

- 10 Repeat the procedure from step 2 for the remaining export files (remember that each trial and arena results in a separate export file).

- 11 Open the Independent Variables List (**Alt+F3**). Define the independent variables and enter the values of those variables for the trials you have imported.

- **Creating an import profile** – In the Import Profile Definition window, drag the columns of the Data Sets box as follows:

File management



- **Time** – Drag the column **B** (Recording time) to the **Time** column. This is the time of manually scored behaviors relative to the time that tracking started. For the difference between Trial and track, see page 203.

Note – Do not drag the column **A** (Trial time) as this provides the same timing as column **B** when imported into The Observer.

The predefined import profile **EthoVision XT Data File** uses the Recording time as time code for the manually scored behaviors.

If you want to import video in The Observer, you must keep the correct sync between events and video. See the note **Importing video into The Observer** below.

- **Event Type** – Drag the **E** column and click **OK** in the windows that appears.
- **Effects on the coding scheme** – If you import manually scored data without creating a coding scheme in advance, at import of the first file The Observer creates a coding scheme with all behaviors contained in that file. By default, all behaviors are put in a start-stop group named **Behavior1**. Rearrange the coding scheme by creating the same groups as those in EthoVision XT. Next, move the behaviors of **Behavior1** to the groups they belong to.
- **Independent variables** – The Observer does not import the independent variables from EthoVision XT. You must define those variables in the Observer's **Independent Variables List** and assign the values of those variables to each imported file. You can find those values in the headers of the original export files.
- **Multiple arenas** – If your setup includes multiple arenas, for each trial you import one file per arena. If you want to keep data from different arenas (in the same trial) in the same observation, you can import the files of the

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second, third, etc. arena in the same observation as that of the first arena. To do so, do the following:

- Follow the procedure on page 575 for the first arena. Right-click the name of the resulting observation and select **Properties**. Edit the observation name by giving a name that specifies the trial only, not the arena (for example **Open Field Trial 12**).
- For the second, third, etc. arena file, instead of following step 6 of the procedure above, in the Project Explorer right-click the observation name and select **Import Observational Data**, then follow the rest of the procedure. The files are imported in the observation of the first arena as separate event logs. Rename each event log in such a way it clearly indicates the arena from which it was obtained.
- **Importing video in The Observer** – Open the observation, and from the **Observe** menu select **Media File**, then **Open in Current Observation** and select the video file.

Choose the option below that applies:

- If the video file was obtained with programs other than EthoVision, or if you tracked from video previously recorded with EthoVision, take note of the difference between the **Start time** and the **Video start time** (you can find these in the headers of the export file). This difference is 0 only if you start the trial from the first video frame. Also, take note of the value of **Recording after** (also available in the export file). Calculate the value of $\text{Offset} = \text{Start time} - \text{Video start time} + \text{Recording after}$.
- If the video file was recorded with EthoVision while doing live tracking, take note of the value of **Recording after** (you can find this in the headers of the export file). In your case $\text{Offset} = \text{Recording after}$.



Next, in The Observer XT, click the **Offset** button, then **Numerical Offset** and locate the imported event log. In the corresponding cell, enter the Offset calculated as described above, and make sure that the sign stays positive (+).

Open the observation, click the start line of the event log. The video is positioned to the point that the tracking started.

For the difference between Trial and track, see page 203.



- For more information on creating an import profile for The Observer XT, see **Importing other observational data** in Chapter 12 of The Observer XT Reference Manual.

15.4 Media files

Please see Appendix B for information about using MPEG and H.264 files and the media file types that EthoVision XT supports.

EthoVision XT comes with a Picolo Diligent frame grabber and encoder board or a Picolo U4 H.264 frame grabber and encoder board. You can use these boards to do live tracking. You can also use it to save your live video to a digital media file. The Picolo Diligent board records your video in MPEG-4 format. The Picolo U4 H.264 board records your video in H.264 format. The location for these media files is the **Media files** subfolder in the experiment folder (see page 586).

15.5 Import profiles for external data and external data files

When you have the EthoVision XT **Physiology module**, you can import external data that have been acquired with a separate Data AcQuisition (DAQ) system. This can be, for example, physiological data (e.g. EEG, blood pressure, heart rate) or environmental data (e.g. room temperature, humidity). You can import all external data in ASCII format that have been sampled with a constant sample rate. EthoVision XT offers import profiles for a number of DAQ systems (Data Sciences, Minimitter, BIOPAC, Polar). If there is no predefined import profile for your system, you can create your own import profile. For the location of the import profiles, see page 586.



It may be that this folder is hidden. If so, then do the following:

- **Windows XP** – Open the Windows Explorer, browse to C:\ and from the **Tools** menu, select **Folder Options**. Click the **View** tab and make sure that **Display the contents of system folders** is selected.
- **Windows 7** – Open the Windows Explorer, browse to C:\ and from the **Tools** menu, select **Folder Options**. Click the **View** tab and make sure that under **Hidden Files and Folders** the option **Show Hidden Files, Folders and Drivers** is selected.

For information about opening, creating and editing import profiles, see Chapter 9.

15.6 General and Experiment log files

Exporting the General and Experiment log file

1 Do one of the following:



- In the Experiment Explorer, under **Export**, click **GLP Log**.
- From the **File** menu, select **GLP Log Files**, and click the **Export** button.

2 Select the option for the type of log file you want to export (**General log file** or **Experiment log file**)

3 Browse to the folder where you want to export the log file to and click **OK**.



- If your current experiment is a non-GLP experiment, there is no Experiment log file present.
- If you choose **Experiment log file**, the suggested export location is the experiment's **Export Files** folder.
- The export format is HTML (*.html). The name of the export file is:
 - For general logs, **GLP-EthoVision(n)**
 - For experiment logs, **GLP-[Experiment name](n)**

Where **n** is a progressive number.

Printing the General and Experiment log file

1 From the **File** menu, select **GLP Log Files**.

2 In the **GLP Log** window, select the option for the log file you want to print and click **Print**.

3 In the **Print Preview** window, you can:

- Change the page setup and the page orientation (portrait, landscape).
- Change the font size in the **Change Print Size** drop-down box (<Alt+S>).

4 Click the **Print Document** button or press **Alt+P**.

Deleting the Experiment log file



You can only clear the Experiment log file, you cannot delete it. You can only clear the Experiment log file when you have the user management right to **Delete Trials and Clear Log**.

1 From the **File** menu, select **GLP Log Files**.

File management

- 2 Make sure the **Experiment log file** radio button is selected and click the **Clear Log** button.

The log is cleared and a message is added that this was done.

15.7 Managing settings and profiles

You can store your favorite options in Settings and Profiles that apply to specific procedures. You can have an overview of your settings and profiles in the Experiment Explorer (see Figure 15.9).

- **Arena Settings** – Specify the arenas and zones you work with, and the calibration units.
- **Trial Control Settings** – Specify how you start and stop data acquisition automatically.
- **Detection Settings** – Specify how the animals are distinguished from the background.
- **Track Smoothing Profiles** – Specify the half window size of the Smoothing (Lowess) method.
- **Data Profiles** – Specify what tracks and data points to analyze.
- **Analysis Profiles** – Specify what dependent variables and what statistics to calculate.

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Figure 15.9. Settings and Profiles in the Experiment Explorer.



Contrary to all other settings and profiles, you can only have one set of Manual Scoring Settings.

General information

Default settings/profiles are created automatically for each new experiment.

- **Manual Scoring Settings** – Contains no behaviors.
- **Arena Settings 1** – Contains an arena that covers the entire video image, no zones and no calibration.
- **Trial Control Settings 1** – Contains the default start-stop trial rule. Acquisition starts when the center-point of the subject (or of any subject, in the case of an arena with multiple subjects) has been detected in the arena for 1 second after you started the trial. The trial stops when you manually give the Stop command or the time exceeds the Maximum trial duration (when this has been set).

File management

- **Detection Settings 1** – Default detection settings with Gray scaling as detection method.
- **Track Smoothing Profile 1** – By default, Lowess track smoothing and Minimal Distance Moved are not selected.
- **Data Profile 1** – Contains all data currently in the experiment.
- **Analysis Profile 1** – Contains the dependent variables Distance moved and Velocity.

You can create as many settings or profiles as you require. However, only one is active at a time. When carrying out a procedure, EthoVision XT uses the settings and profiles currently active. Active settings and profiles are highlighted in blue under the corresponding folder in the Explorer.

Example – Create Detection Settings for tracking mice of two strains, C57/BL6 and DBA2. Mouse strains have different fur color, so they need different gray scale settings.



Viewing the settings used in a trial

Once you have carried out a trial, you can check which settings were used to acquire the data (**Arena Settings**, **Detection Settings**, **Trial Control Settings**).

- 1 In the Experiment Explorer click **Trial List**.
- 2 Click the **Show/Hide** button on the component tool bar and select **Variables**, then select the type of settings you want to view.
- 3 The settings column is added to your Trial List. For a specific trial, the column shows the name of the settings used to acquire the data.
- 4 In the Experiment Explorer, open that settings profile.



Profiles that have been used to acquire data are marked with a lock symbol (see the next page).

Creating new settings/profiles

- 1 Do one of the following:

- For **Arena Settings**, **Trial Control Settings** or **Detection Settings**, from the **Setup** menu select the item you require, then **New**.

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- For **Track Smoothing Profiles**, **Data Profiles** and **Analysis Profiles**, from the **Select** menu choose the item you require, then **New**.
 - For all settings/profiles, right-click the corresponding folder in the Experiment Explorer and select **New**.
- 2 Type the name for the new profile or accept the suggested name, then press **Enter**.

Opening settings/profiles



Opening settings/profiles also means that those settings/profiles are activated (see below).

Do one of the following:

- For **Arena Settings**, **Trial Control Settings** or **Detection Settings**, from the **Setup** menu select the item you require, then **Open**. Choose the settings/profile you require from the list.
- For **Track Smoothing Profiles**, **Data Profiles** and **Analysis Profiles**, from the **Select** menu choose the item you require, then **Open**. Choose the settings/profile you require from the list.
- For all settings/profiles, right-click the settings/profile you require under the corresponding folder in the Experiment Explorer and select **Open**.

Activating settings/profiles

In the Explorer, right-click the settings/profile you want to activate and select **Set as Current**.

Active profiles are highlighted in blue in the Experiment Explorer. Activating means that EthoVision XT uses that profile to carry out a procedure. For example, when acquiring the data it uses the Arena Settings active at that moment. When calculating statistics, it uses the active Track Smoothing profile.

Editing settings/profiles

To edit settings/profiles, open them (see above) and then make the necessary changes.



When at least one trial has been carried out, the Arena Settings, Trial Control Settings and Detection Settings used for that trial are **locked**. Locked settings are marked by a lock symbol in the Experiment Explorer:

- Detection Settings (2)
- Detection Settings 1
- **Detection Settings 2**

File management

You cannot edit locked settings. If you want to make changes to those settings, make a copy (see below) and edit this. Then, make sure that you carry out the next trial using the new profile.

If you delete all trials acquired with specific settings, those settings are unlocked, so you can edit them.

Copying settings/profiles

Do one of the following:

- Open the settings/profile folder in the Experiment Explorer. Right-click the name of the settings/profile you want to copy and select **Duplicate**.
- From the **Setup** or **Analyze** menu, select the type of settings/profile you want to copy. In the window that opens, select **Duplicate from**. Change the name of the new settings/profile if necessary. From the drop-down list, select the settings/profile you want to copy from.



Figure 15.10. In this example the new **Arena Settings 3** are copied from the existing **Arena Settings 2**.

Result – A new item is added to the folder. Type a new name or accept the suggested one, then press **Enter**.



The suggested name for a new settings/profile is **[Settings/Profile] N**, where N is the first integer not yet used in the name of the other settings/profiles in that folder.

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Renaming settings/profiles

- 1 Open the settings/profiles folder in the Experiment Explorer.
- 2 Right-click the name of the settings/profile you want to rename and select **Rename**.
- 3 The profile name is highlighted. Type the name you require and press **Enter**.



You cannot rename settings/profiles that were used to acquire at least one trial.

Deleting settings/profiles

- 1 Open the settings/profile folder in the Experiment Explorer.
- 2 Right-click the name of the settings/profile you want to delete and select **Delete**.



Notes

- You cannot delete settings/profiles that were used to acquire at least one trial. To delete those settings/profiles, you must first delete the trials (make sure you do not lose any important data!).
- You cannot delete settings/profiles if your experiment is GLP-set and you do not have specific rights (see page 80).
- If the currently-active settings/profile is deleted, the first of the remaining settings/profiles folder is activated automatically.
- If you delete the only settings/profile in the folder, a new default settings/profile is created automatically.
- If you delete a Data profile or an Analysis profile used to create an analysis result open on your screen (track plot or statistics table), the result is updated according to the first available Data profile, or, if no other profiles are available, a newly-created default profile.

15.8 File locations

The default application folder of EthoVision XT:

- **Windows XP:** C:\Program Files\Noldus\EthoVision XT.

File management

- **Windows 7:** C:\Program Files\Noldus\EthoVision XT.

Experiments folder

The default folder for EthoVision XT experiments is:

- **Windows XP:** C:\Documents and Settings\All Users\Shared Documents\Noldus\EthoVision XT\Experiments.
- **Windows 7:** C:\Users\Public\Public Documents\Noldus\EthoVision XT\Experiments.

Each experiment has its own folder, the experiment folder. An experiment folder contains the following subfolders:

- **Bitmap Files** – Contains the background images that were used in the **Arena Settings** and the reference images that were used in the **Detection Settings**.
- **Configuration Files** – Contains the configuration files that are created when you set the connection between EthoVision XT and hardware devices.
- **Data Files** – Contains the binary track files acquired during acquisition.
- **Export Files** – When you export your track data, manually scored behaviors, analysis results or the experiment's GLP log files, the export files are, by default, stored in this subfolder.
- **External** – When you import external data files, they are stored in this subfolder. In addition, the subfolder contains the sync-out files that were used for external data co-acquisition.
- **Intermediate** – This folder contains intermediate results.
- **Log** – Contains the log files if your experiment is set as a GLP Experiment.
- **Media Files** – This is the default location for media files that have been recorded during the experiment.

Import Profiles

The default folder for profiles for import of physiological data is:

- **Windows XP:** C:\Documents and Settings\All Users\Application Data\Noldus\Common\Profiles.
- **Windows 7:** C:\ProgramData\Noldus\Common\Profiles.

Chapter 15

15.9 Printing

In the current version of EthoVision XT there is no printing functionality implemented, except for the GLP log files. See below for some suggestions to print your EthoVision graphs and tables in other programs.



You can also use the **Print Screen** button on your keyboard. With this button you can copy your graphs and tables to the Windows clipboard. Paste them into another program to print them.

Printing track plots

To print track plots, save them first as pictures and import them into other software to print them. See page 463 for the procedure to save track plots as pictures.

Printing your analysis results

To print your analysis results, export them to another program. See page 496 for the procedure to export your analysis results.

A

Keyboard shortcuts

All the common functions in EthoVision XT have keyboard shortcuts. You can use the keyboard to activate all the functions in EthoVision XT that are on the menus. Press ALT plus the letter underlined in the menu and then select the desired function by scrolling down to the function or by pressing the underlined letter. For example, to go to **Preferences** on the **File** menu, press **ALT+F, f**.

Why Keyboard Shortcuts?

Keyboard shortcuts allow you to use EthoVision XT without taking your hands off the keyboard. Using keyboard shortcuts can also help in the prevention of repetitive strain injury.

Keyboard shortcuts are only available when the corresponding menu or window is active.

A.1 Windows – General

Some of these shortcut keys can also be used in the **Experiment Explorer**, the **View Settings**, the **Arena Setup**, the **Trial Control**, the **Information View** and the **Detection Settings**.

Appendix A

Esc	Cancel action
F1	Help
F2	Rename/ edit
F3	Find next
Shift+F3	Find previous
F4	Expand pull down list
Ctrl+F4	Close active window
Alt + F4	Close application
F5	Refresh
Ctrl+F6 or Ctrl+Tab	Cycle through windows
Alt+Enter	Open Properties of selected item
Shift+F10 or context menu key	Open context menu of selected item
Alt+Spacebar	Open shortcut menu of selected window
Ctrl+Esc	Display Start menu
Alt+underlined letter	Application: open corresponding main menu Dialog: carry out corresponding command
F10	Activate main menu bar
Main-menu: down arrow key	Open menu-item/ cycle thru sub-items
Main-menu: up arrow key	Open menu-item/ cycle thru sub-items
Main-menu: right arrow key	Open sub-items/ Cycle thru items
Main-menu: left arrow key	Close sub-items/ Cycle thru items
Spacebar	Select/ clear checkbox if active option is a checkbox
Arrow keys	Move/ nudge cursor (highlighted item in grid) or selected item
Alt+Tab	Switch between open applications
Alt+Esc	Cycle through applications in order they were opened

Keyboard shortcuts

A.2 Windows – Explorer

Some of these shortcut keys can also be used in the **Experiment Explorer** and the **View Settings**.

End	Display the bottom of the active window
Home	Display the top of the active window
* on numeric keypad	Display all subfolders under the selected folder
+ on numeric keypad	Display the contents of the selected folder
- on numeric keypad	Collapse the selected folder
Left arrow key	Collapse current selection if it's expanded, or select parent folder
Right arrow key	Display current selection if it's collapsed, or select first subfolder
Up arrow key	Previous item
Down arrow key	Next item

A.3 EthoVision XT – General

Ctrl+N	New experiment
Ctrl+O	Open (experiment or other file type)
Ctrl+S	Save experiment
Ctrl+Shift+S	Save experiment as...

Appendix A

A.4 EthoVision XT – Select and Edit

Ctrl+X	Cut
Ctrl+C	Copy
Ctrl+V	Paste
Ctrl+Delete or Delete	Delete
Ctrl+drag item	Copy selected item
Shift+drag item	Move selected item
Shift+arrow keys	Expand/ contract block selection in direction of arrow key

A.5 EthoVision XT – Normal Grids

Ctrl+left arrow key	Normal mode: Go to first cell of row Edit cell mode: Move insertion point to beginning of previous word
Ctrl+right arrow key	Normal mode: Go to last cell of row Edit cell mode: Move insertion point to beginning of next word
Ctrl+up arrow key	Normal mode: Go to first cell of column Edit cell mode: - do nothing -
Ctrl+down arrow key	Normal mode: Go to last cell of column Edit cell mode: - do nothing -
Arrow keys	Move highlight in direction of key
F2	Rename/ edit cell content (see Windows - general)
Ctrl+Insert	Insert row/ add new element
Tab	Go to next cell in row. If last cell, do nothing

Keyboard shortcuts

Shift+Tab	Go to previous cell in row. If first cell, do nothing
Enter	(Accept entry and) Go to same column, next row. If last row, go to first cell last row
Shift+Enter	(Accept entry and) go to same column, previous row. If first row, go to first cell first row
Home	Go to first cell of row
End	Go to last cell of row
Page Up	Go to row - no. of rows visible in window and highlight same location (and de-select)
Page Down	Go to row + no. of rows visible in window and highlight same location (and de-select)
Ctrl+Home	Go to first cell of first column
Ctrl+End	Go to last cell of last column

A.6 EthoVision XT – Row-select Grids

An example of a row-select grid is the components windows in the **Data Selection screen** and **Dependent Variables screen**. In these windows, individual selection of cells is not possible.

Up arrow key	Step one row up
Down arrow key	Step one row down
Enter	(Accept entry and) Go to same column, next row. If last row, go to first cell last row
Shift+Enter	(Accept entry and) go to same column, previous row. If first row, go to first cell first row

Appendix A

A.7 EthoVision XT – Playback Control / Acquisition

Ctrl+up arrow key	Jump to begin
Ctrl+down arrow key	Jump to end
Ctrl+left arrow key	Step -1 sample
Ctrl+right arrow key	Step +1 sample
Ctrl+9	Play forward
Ctrl+0	Pause / stop
Ctrl+.	Zoom x2
Ctrl+,	Zoom x1/2
Ctrl+1	Playback speed 1/25x
Ctrl+2	Playback speed 1/5x
Ctrl+3	Playback speed 1/2x
Ctrl+4	Playback speed 1x
Ctrl+5	Playback speed 2x
Ctrl+6	Playback speed 4x
Ctrl+7	Playback speed 8x
Ctrl+8	Playback speed 16x
Ctrl+F3	Add Trial (Acquisition only)
Ctrl+F5	Start acquiring (Acquisition only)
Ctrl+F6	Stop acquiring (Acquisition only)

A.8 EthoVision XT – Arena Setup

See also the **Windows – General** and **EthoVision XT – Normal Grids** shortcut keys.

Zone windows

Del	Delete selected element
Ctrl+J	Validate Arena Setup
V	Normal (arrow) Mode
R	Rotation Mode
P	Point Edit Mode
L	Draw Lines
S	Draw Rectangle
O	Draw Open Polyline
Y	Draw Closed Polyline
E	Ellipse
T	3-Point Circle
Z	Label Zone

Drawing

Up arrow key	Nudge selected element smallest resolution unit up
Down arrow key	Nudge selected element smallest resolution unit down
Left arrow key	Nudge selected element smallest resolution unit left
Right arrow key	Nudge selected element smallest resolution unit right
Shift+arrow key	Nudge*10

Appendix A

A.9 EthoVision XT – Edit Tracks

Toolbox	
Ctrl+E	Select... dialog
W	Swap nose and tail in selection
Ctrl+T	Fix Nose-Tail swaps
D	Swap subjects in selection
Ctrl+Del	Set selection to missing
Ctrl+I	Interpolate selection
Ctrl+Shift+I	Interpolate only center-points
Ctrl+M	Set tail-base points to missing
Ctrl+Shift+M	Set nose points to missing

A.10 EthoVision XT – Trial Control

See **Windows – General** and **Row-select Grids** shortcut keys.

A.11 EthoVision XT – Detection settings

See **Windows – General** and **Row-select Grids** shortcut keys.

Keyboard shortcuts

A.12 Analysis Profiles

Components window

See also **row-select Grids**

Variable Profile window

See **Normal Grids**

A.13 Trial List

See **Normal Grids** shortcut keys.

B

Using MPEG

B.1 Digital video

An analog camera produces a stream of images. Each image consists of a series of lines, and each line has a continually varying color intensity. The images of an analog camera can be digitized (encoded) to make a video file (just like the one made by a digital video camera).

The images in a digital video file consist of a grid of pixels (picture elements), each pixel having a particular color and brightness. An uncompressed video file consists simply of all these grids of pixels, one after another. In a compressed video file various calculations are carried out so that, for instance, only the changes from one image to the other are recorded, making the file smaller. There are many different formats of digital video files. Some of them fall under the MPEG standard. MPEG is a group of formats (MPEG-1, MPEG-2, MPEG-4...) developed by the Moving Picture Experts Group of the International Standards Organization (ISO).

For analog cameras there is a slightly different aspect ratio than digital video files (normally 4:3). This means that if you digitize a signal from an analog camera, either the aspect ratio will be slightly changed (giving a small distortion), or part of the image will be cropped. EthoVision ensures that this distortion is compensated for in the tracking.

Appendix B

B.2 TV standards: NTSC and PAL

Depending which country you live in, the camera you use (digital or analog) will have a different TV standard. The main difference is that NTSC cameras (North and Central America, together with parts of Asia) have a frame rate of 29,997 frames (images per second), whereas PAL cameras (rest of the world) have a frame rate of 25 images per second. NTSC analog cameras have 525 lines and PAL cameras 625 lines, so it is pointless to digitize video from those cameras at a higher horizontal resolution than that.

B.3 MPEG file formats

One of the difficulties with MPEG files is that the file extension does not indicate what the file type is. *.Mpg can mean any one of different type of formats, and an MPEG-4 movie can have the extension *.mpg, *.mpeg or *.avi. Whether an MPEG file plays on your computer depends on a variety of factors, including how powerful your computer is and what software is installed.

MPEG-1

MPEG-1 is the only really standard video format. MPEG-1 movies are compressed by comparing each frame with reference frames (I-frames), making the files a lot smaller than the original (about 10 Mb/minute). MPEG-1 movies can be played on any computer. The resolution is relatively small (288 x 352 for PAL and 240 x 352 for NTSC). EthoVision XT cannot make MPEG-1 movies but other software can. MPEG-1 is often used to digitize VHS tapes.

MPEG-2

MPEG-2 is not a standard in the same sense that MPEG-1 is. An MPEG-2 file created with a particular encoder will often need a decoder from the same manufacturer to play back correctly. EthoVision XT cannot make MPEG-2 videos but other hardware and software can. MPEG-2 movies can be created in sizes up to 720 x 576 (PAL) or 720 x 480 (NTSC). This is called full D1. It is also possible to create MPEG-2 movies with fractions of that size (for example half D1, 352 X 576). We guarantee that MPEG-2 movies created with the Noldus MPEG Recorder 2 will be correctly tracked. If you use other encoders then we strongly recommend that you validate the tracking yourself before carrying out experiments.

Using MPEG



Camcorders can record video to MPEG-2 files on their own hard disks. Such video files will often not work with EthoVision. Test those files in Acquisition before deciding to work with them or convert them to a supported format.

MPEG-4

MPEG-4 can achieve a very high rate of compression with good quality, because it separately codes the background (which does not change much from frame to frame) from the moving parts of the video. If you ordered a computer from Noldus Information Technology when you purchased EthoVision XT, it can come with a Picolo Diligent frame grabber and encoder board. You can use this board to do live tracking. You can also use the Picolo Diligent board to save your live video to a digital media file. The video is saved in MPEG-4 format. If you use other hardware or software for creating MPEG-4 files, we cannot guarantee that the tracking results will be correct.

H.264 AVC

H.264 AVC is a type of MPEG-4 and is also known under the names H.264/AVC, AVC/H.264, H.264/MPEG-4 AVC, MPEG-4/H.264 AVC, MPEG-4 Part 10 or x.264. It creates good video quality and uses previously-encoded pictures as references in a much more flexible way than in other standards, allowing the use of up to 16 reference frames. Because H.264 encoding and decoding requires significant computing power, software encoding is typically slow. With an H.264 encoder card you can create H.264 video files without slowing down your computer.

If you ordered a computer from Noldus Information Technology when you purchased EthoVision XT, it can come with a Picolo U4 H.264 frame grabber and encoder board. You can use this board to do live tracking. You can also use the Picolo U4 H.264 board to save your live video to a digital media file. The video is saved in H.264 AVC format. If you use other hardware or software for creating H.264 files, we cannot guarantee that the tracking results will be correct.

Video file formats supported by EthoVision XT

EthoVision XT supports playback of the following digital video file formats:

- MPEG-1
- MPEG-2 – this is a collection of formats, not all are supported.
- MPEG-4 – this is a collection of formats, not all are supported.
- H.264 AVC – This is a type of MPEG-4, which is supported.

Appendix B

- DV-AVI – This is uncompressed video from FireWire cameras. We do not recommend this format because of the large file size.

To play back MPEG-1 and MPEG-2 files, EthoVision XT uses the MainConcept Decoder which is automatically installed when you install EthoVision XT. To play back MPEG-4 files, EthoVision XT uses the standard Windows XP decoder, which is present on all computers with Windows XP installed. To play back H.264 files you need decoding software which is installed when you install EthoVision.



We cannot guarantee that all MPEG-2, MPEG-4, and H.264 files can be played back in EthoVision XT, only those created using the frame grabber and encoder boards supplied by Noldus. Our tests have shown that FireWire cameras work well with EthoVision XT. USB 2.0 webcams are supported for demonstration purposes only. They can be used for tracking but we strongly advise that you validate the tracking results yourself before carrying out any experiments.

The following formats are NOT supported in EthoVision XT:

- WMV.
- Quick Time.
- FLV (YouTube).
- Xvid variants of the MPEG-4 format.

B.4 Storage medium

You can store your digitized video files in a number of different ways. Take care to select the best for your needs.

Hard disk

Normally when you make a video file, it will automatically be stored on your hard disk. However, a hard disk has limited capacity (normally just a few hours at the most), and if the disk fails then your video is lost. It is, therefore, always wise to make a backup, either to an optical storage medium such as a CD or DVD or to your network.

CD-ROM

CDs can contain 650-700 MB of data. If you are using a CD for backup, you should

Using MPEG

always verify that the file is written properly, and at least every three years you should make a new copy. Shield the CD from light and take care that it does not get scratched. Do not keep important data on a rewritable CD (CD-RW).

DVD-ROM

DVD disks normally can contain up to 4.7 GB of data, though double-sided and/or dual layer disks with higher capacity are becoming more common. DVD disks come in various formats (DVD-R, DVD+R, etc). Not all DVD writers and readers can read all the formats (especially DVD-R). Before you buy disks, check which formats your computer can read and write. Most DVD recorders can only record in DVD format, not in other formats such as MPEG. Therefore, first make an MPEG file and store that file on a DVD. If you are using a DVD for backup, you should always verify that the file is written properly, and at least every three years you should make a new copy.

B.5 Recording digital video

Digital video requires a lot of storage capacity and always needs to be compressed before it can be stored on a disk medium. In order to compress a video recording you need an encoder, usually a plug-in board, which converts the video signal into a media file on disk at a fraction of the original size. The input signal can either be analog or digital.

EthoVision XT comes either with the Picolo Diligent frame grabber and encoder board or with the Picolo U4 H.264 frame grabber and encoder board. You can use these board not only for live tracking, but also for (simultaneously) encoding your live video (in MPEG-4 or H.264 format). If you use a FireWire camera, you can only track live. You cannot save your live video to a digital media file in EthoVision XT, but you can use the Media Recorder software to make digital video files and track from these videos in EthoVision XT.

How to create a digital recording with the Picolo Diligent board

Follow the instructions on page 39 to install the Picolo Diligent frame grabber board in your computer. Connect your video camera to the frame grabber (see page 42 for more information). Please note that your video camera must be connected to two BNC connectors on the frame grabber board (see page 46).

In order to record your live video to a digital video file, you have to make the following settings in EthoVision:

Appendix B

- In the **Experiment Settings**:

- 1 Select **Live tracking** as your **Video Source**.
- 2 In the **Source** field, select one of the inputs (either one) to which you connected your video camera.



- 3 On the right of the field under the Video source field, click **Edit**. The Video Source Settings window opens (Figure B.1.).
 - Select the **Save video file** check box and in the **Saving source** field select the video input that you want to use for recording. If you connected your camera to BNC connectors 1 and 2 of the frame grabber and you chose connector 1 in step 2, then now you must select connector 2.
 - Select the video standard of your camera (**PAL** or **NTSC**).

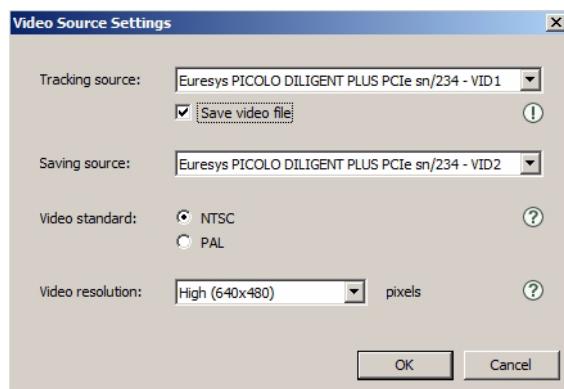


Figure B.1. The **Video Source Settings** window of the Picolo Diligent board.

- Select the video resolution that you want to use (**High** or **Medium**) and click **OK**.



See page 99 for more information about video (TV) standards and the video resolutions of the Picolo Diligent frame grabber.



You can also enter these settings when you use a template experiment. See chapter 5 for information on how to do this.

Using MPEG

- In the **Acquisition Method** window:

Choose one of the following options:

- Live tracking** – Select this option if you want to acquire data from the live image.
- Save video** – Select this check box to simultaneously save the live video to a digital video file.
- Only save video, track later** – Select this option if you want to record the live image to a video file without gathering the track data. You can do tracking at a later stage. Select this option if your computer is not fast enough to do tracking and recording at the same time.

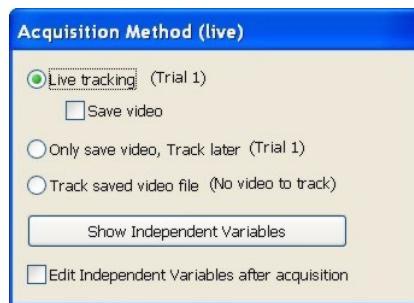


Figure B.2. The **Acquisition Method** window for live tracking.



The **Acquisition Method** window is one of the windows in the data acquisition screen. To open the data acquisition screen: from the **Acquisition** menu, choose **Open Acquisition**.

Your digital video files are saved in the **Media Files** subfolder of your experiment folder. By default in:

- **Windows XP:** C:\Documents and Settings\All Users\Shared\Documents\Noldus\EthoVision XT\Experiments\<experiment name> Media Files.
- **Windows 7:** C:\Users\Public\Public Documents\Noldus\EthoVision XT\Experiments\<experiment name> Media Files.

Appendix B

How to create a digital recording with the Picolo U4 H.264 board

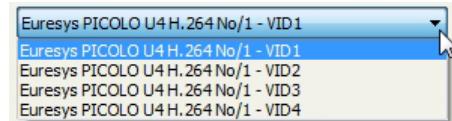
Follow the instructions on page 40 to install the Picolo U4 H.264 board in your computer. Connect your video camera to the frame grabber and encoder board (see page 43 for more information). Please note that your video camera must be connected to two BNC connectors on the frame grabber board (see page 46).

In order to record your live video to a digital video file, you have to make the following settings in EthoVision:

- In the **Experiment Settings**:

- 1 Select **Live tracking** as your **Video Source**.

- 2 In the **Source** field, select one of the inputs (either one) to which you connected your video camera.



- 3 On the right of the field under the Video source field, click **Edit**. The Video Source Settings window opens (Figure B.3.).

- Select the **Save video file** check box and in the **Saving source** field select the video input that you want to use for recording. If you connected your camera to BNC connectors 1 and 2 of the frame grabber and you chose connector 1 in step 2, then now you must select connector 2.

- Select the video standard of your camera (**PAL** or **NTSC**).

Using MPEG

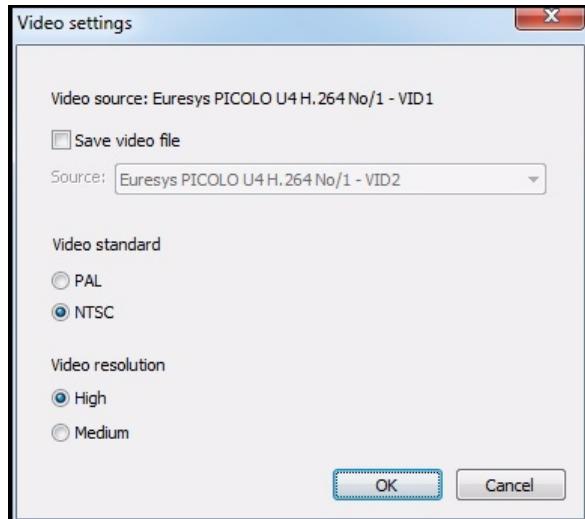


Figure B.3. The **Video Settings** window of the Picolo U4 frame grabber board.

- Select the video resolution that you want to use (**High** or **Medium**) and click **OK**.



See page 99 for more information about video (TV) standards and the video resolutions of the Picolo U4 H.264 frame grabber.

- In the **Acquisition Method** window:

Choose one of the following options:

- **Live tracking** – Select this option if you want to acquire data from the live image.
- **Save video** – Select this check box to simultaneously save the live video to a digital video file.
- **Only save video, track later** – Select this option if you want to record the live image to a video file without gathering the track data. You can do tracking at a later stage. Select this option if your computer is not fast enough to do tracking and recording at the same time.

Appendix B

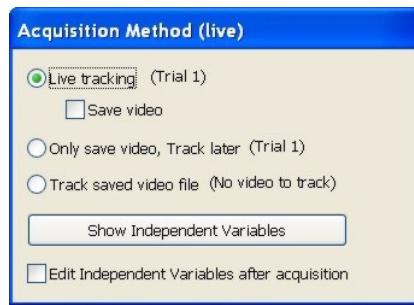


Figure B.4. The **Acquisition Method** window for live tracking.



The **Acquisition Method** window is one of the windows in the data acquisition screen. To open the data acquisition screen: from the **Acquisition** menu, choose **Open Acquisition**.

Your digital video files are saved in the **Media Files** subfolder of your experiment folder. By default in:

- **Windows XP:** C:\Documents and Settings\All Users\Shared Documents\Noldus\EthoVision XT\Experiments\<experiment name> Media Files.
- **Windows 7:** C:\Users\Public\Public Documents\Noldus\EthoVision XT\Experiments\<experiment name> Media Files.



You can also enter these settings when you use a template experiment. See chapter 5 for information on how to do this.

Recording video with other encoders

If you do not have a Picolo Diligent or a Picolo U4 H.264 frame grabber board, you can record your video using an encoding board and acquire data offline from that file (see page 293).

C

Settings overview

C.1 Thematic

Below you can find an overview of all the settings in EthoVision XT with a reference to a page number for more information. The overview is organized according to the workflow of the program.

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D

Technical Support

D.1 Knowledgebase

If you encounter a problem using EthoVision XT or any other Noldus system, you can search through hundreds of entries in a database of questions submitted by our customers to the Noldus support department with answers by our support staff.

You find the Knowledgebase at our website (www.noldus.com) in the **Support - Knowledgebase** section.

D.2 Help Desk

If you have any problems, questions, remarks or comments, please let us know. You can contact us via our web site (www.noldus.com) and fill out a Support Request Form (preferred), phone during normal working hours in two time zones or fax.



Please check the Reference Manual before contacting our support department. Press **F1** to start the help, then type in the index a keyword related to what you are having problems with.

Appendix D

Before you contact Technical Support, please have the following information available. To find this information, go to the **Help** menu and select **About EthoVision XT**:

- The version number of your copy of EthoVision XT.
- The name of the registered user of EthoVision XT (click **User Info**).
- The license number of your copy of EthoVision XT (click **User Info**).

Our Technical Support department may request a log file when answering your support question. Please see page 22.

Please refer to the **About Noldus - Contact us** on our web site (www.noldus.com) for other contact information.

D.3 Service contracts

Your licence of EthoVision XT comes with a standard service package of one year. This includes a one-year period of free technical support.

We can offer you even greater value and reassurance by providing comprehensive service contracts. Our Plus and Premium service contracts both extend the standard service you are entitled to as well as provide peace of mind at defined cost.

Please look on our web site (www.noldus.com) under **Animal Behavior** or **Human Behavior** in the **Services - Service Contracts** section for more information.

E

Glossary

AC adapter – A hardware device that transforms the AC mains voltage to a DC voltage that can be fed into an electrical apparatus, such as a video camera. Also referred to as a mains adapter.

Acquisition card – See *Frame grabber*.

ActiveMovie – ActiveMovie is a media file player, developed by Microsoft, that supports the MPEG digital video format.

Analog – A signal composed of a continually varying waveform. See also *Digital*.

Analog to digital converter – A hardware device that changes an analog signal into a digital quantity; a process known as digitization. An A/D converter is an essential component of a frame grabber.

Aperture number – Every lens has a number indicating the largest possible aperture opening (f/#). It is the ratio between the lens diameter and the focal length. Standard values are 1:1, 1:1.2, 1:2, 1:2.8, etc., with the second number being the aperture number. The smaller the aperture number, the larger the lens opening and vice versa. With a small aperture setting, images are rather dark, while by putting your aperture wide open, images become lighter.

Area – See *Surface area*.

Arena – The area in which one independent set of observations takes place. You can subdivide the arena into zones. Please see Chapter 6 for details.

Arena Settings – The definition of one or more arenas that can be used in an experiment together with all their zones and calibration. In EthoVision 3, it was called Arena definition. You can create different Arena Settings in the same experiment.

ASCII file – Commonly used term referring to a file that only contains ASCII characters higher than 31, that is no control codes such as those inserted by a word processing program. Also referred to as plain text file.

Aspect ratio – The relation of the horizontal and vertical sizes in computer graphics. An aspect ratio of, for example, 2:1 means that the width of the graphic

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is twice as large as the height. The current standard for a computer monitor is 3:4. It is important to maintain the aspect ratio constant to avoid distorting the display when resizing.

Auto focus – A mechanism that causes a lens to automatically focus on a subject. This is a standard feature on most camcorders and auto-focus lenses are also available for CCD cameras. Note that a change in focus also results in a change of the size of the scene observed by the camera. See also *Manual focus lens*.

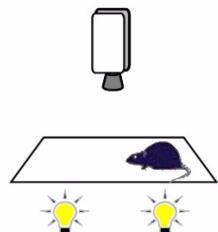
Automatic gain control – A mechanism to automatically control the contrast. You should disable the AGC when you use a camera with EthoVision XT, unless you use the Dynamic Subtraction method for detecting the subject (see Chapter 8).

AVI – A file format for storing video and audio information in files with the extension *.avi. AVI stands for Audio Video Interleave. The audio and video data are interleaved for each block of time. Note: EthoVision XT does not support AVI files. Some *MPEG-4* files have the extension *.avi, which EthoVision XT does support. See also *MPEG, QuickTime*.

Back-focusing – Increasing the distance between the CCD chip and the lens ('extension') beyond the normal focusing range of the camera. It has the same effect as using an extension ring which allows you to focus at a smaller lens-to-subject distance to obtain a greater magnification (magnification = extension/focal length).

Background – In EthoVision XT, the background is defined as everything in a scene which is not part of the subjects being tracked.

Backlighting – A method of illumination where the light source is positioned below/behind the subject, opposite the camera (see below).



Behavior – A category of the behavioral process that you want to measure through observation and event recording. In EthoVision XT, a behavior is a state with variable duration, specified by its start and stop events.

Bit – The smallest unit of data in a computer. A bit has a single value, either 0 or 1.

Glossary

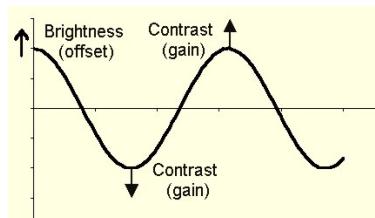
Bitmap – A file format with extension *.bmp where each element (pixel) and its attributes (for example, its color) is described by one or more bits. It is used by a number of graphics and page layout programs.

BNC cable – A Coax cable with a BNC connector on each end.

BNC connector – Professional video connector with a bayonet catch, attached to a coaxial cable. BNC connectors are suitable for low voltages and very high frequencies (up to 3 GHz).



Brightness – The value associated with a pixel representing its gray-level value. For example, in a monochrome 8-bit system, black=0 and white=255. If you increase the offset of a video image, the brightness increases. In the HSI color coding scheme, brightness is sometimes used instead of intensity or luminance, to represent the amount of light reflected by a colored surface.



Calibration – The process of converting a virtual image size (measured in pixels) into a real-world measurements (for example, cm or inches).

CCD – Charge-Coupled Device. The heart of the image sensor used in modern video cameras, which consists of more than 300,000 tiny light-sensitive elements on a surface area of about 1 cm². The scene projected on the CCD causes the elements to become electrically charged. The charge of each element is read out once every 1/25 second (the duration of an interlaced frame in CCIR cameras), resulting in an electrical current: the video signal. Professional color cameras have three CCDs: one for each primary color (red, green and blue). Important for applications under low-light conditions is that they are very light-sensitive and produce sharp images with as little as 5 lux (some cameras even go down to less than 0.1 lux).

CCD camera – Video camera based on a CCD image sensor. Virtually all modern video cameras are CCD cameras.

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Center-point – The point mathematically in the center of the shape detected as subject. In EthoVision 3, this was called Center of gravity. See also *Nose-point* and *Tail-base point*.

Chroma filter – A filter that removes the chrominance component from a color video signal. What is left is the luminance component, i.e. a black/white signal. A chroma filter can be practical when using a color camera in combination with a monochrome frame grabber.

Chrominance – The color component in a video signal, if the RGB fractions are combined. It is the colorimetric difference (dominant wavelength and purity) between any color and a reference "white" of equal luminance. In three-dimensional color space, chrominance is a vector that lies in a plane of constant *luminance*.

Cinch connector – See *Phono connector*.

Close-up filter (lens) – A lens attached in front of the normal lens, to give greater magnification. It works by shortening the focal length of a lens while keeping the lens-to-camera distance constant (magnification = x/f (where x = distance lens extended and f = focal length of lens)).

C-mount, CS-mount – See *Lens mount*.

CMYK – The colors used in the four-color printing process (process colors): cyan, magenta, yellow and black. Cyan, magenta and yellow are the negative image of the primary colors (red, green and blue).

Coax cable – Coaxial cable. Shielded cable for low-noise transmission of high-frequency video signals. A coax cable has a characteristic impedance (total effective resistance of an electronic circuit) which should be adapted to the equipment it is connected to. The standard for video connections is 75 ohm, although 50 ohm is acceptable for short distances (1-2 meter).

Codec – Codec stands for *code-decode* and refers to the process of creating (coding) and reading (decoding) a video file. Codec often also refers to an application that can both *compress* analog audio and video data into digital information and *decompress* a compressed incoming bitstream, splitting it into its audio and video components and converting them into analog signals for playback. Compressed data require far less storage space than usual. The extent to which data are compressed is expressed as a compression ratio.

Coding scheme – In The Observer XT, the complete set of information that defines how behavioral data are collected, including information about subjects, behaviors and modifiers, settings for keycodes, etc.

Color resolution – The number of intensity levels that can be represented in a pixel. The color resolution is typically a power of 2. For instance, an 8-bit monochrome frame grabber is able to digitize an image into 256 (= 2⁸) gray values, so the color resolution is 256. See also *Image resolution*.

Glossary

Color temperature – See *White balance*.

COM1, COM2, COM3, COM4 – Examples of RS-232 ports. If a computer has one RS-232 port, this is referred to as COM1. If it has two, these are called COM1 and COM2, etc. See also *RS-232*.

Component view – The part of the EthoVision's interface where all experiment procedures (define arena setup, acquire data, select data, run analysis, view results) are carried out. When you click on an item in the *Experiment Explorer*, the Component view shows a dialog or view, in which you can specify the settings for that item.

Composite video signal – The standard TV video signal in which the color (*chrominance*) and brightness (*luminance*) signals have been mixed, together with the synchronization information. As a result of cross-color interference, the quality of color images is not optimal. For high-quality color display, it is recommended to use a system with separate transmission of chrominance and luminance (S-Video) or, even better, separate transmission of each primary color (RGB).

Compression – Compressing or packing data stores data (video, audio, or some combination) in a format that requires less space than usual. Data compression is also widely used in the transmission of data, backup utilities, spreadsheet applications, and database management systems. Bitmap graphics can be compressed to a small fraction of their normal size. The extent to which data is compressed is expressed as a compression ratio. Compression expansion boards compress data automatically as it is written to a disk and then decompress the data when it is retrieved. The data compression is invisible to the user but can effectively double or triple the capacity of a disk drive. Data compression can also be carried out by software encoding/decoding. See also *Decompression, MPEG, JPEG*.

Continuous variable – A variable that (at least theoretically) can assume an infinite number of values between any two fixed points. For instance, between the two measurements 1.5 and 1.6 m there is an infinite number of lengths that could be measured if one were so inclined and had a precise enough method of calibration to obtain such measurements. Any given reading of a continuous variable, for instance 1.577 m, is therefore an approximation to the exact reading, which in practice is unknowable. Examples of continuous variables are physiological signals (heart rate, blood pressure, etc.) and environmental parameters (temperature, irradiation, etc.) that must be sampled at fixed intervals.

Contrast – The degree of difference between the darkest (lowest brightness) and the clearest (highest brightness) parts of an image. A high contrast indicates that the image contains largely black and white brightness values; medium contrast indicates that the image contains a wide range of gray level values; a low contrast image contains a small range of gray level values. If you increase the gain of a

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video signal, the contrast increases. For color signals, the contrast is the range of luminance in the signal. See also *Brightness*.

C-signal – *Chrominance* component in a composite video signal. It is composed of the three primary colors red, green and blue. In a composite video signal, it is mixed with the luminance component while in S-video and RGB signals the components are transmitted separately. See also *Y-Signal*.

DAQ co-acquisition – Collecting *External data* (for example, physiological data) together with track data. DAQ co-acquisition is only available when you have selected **Live tracking** in the Experiment Settings, and you have the External data module Add-on installed on your PC.

Data AcQuisition (DAQ) system – System with which you can acquire external data. See also *External data*.

Data profile – The group of settings regarding data selection. You can use a data profile, for instance, when you need to analyze many data files regarding either treated or control subjects, but you are only interested in treated subjects. You can save a data profile and use it to perform the same analysis as you carry out more trials.

Decoder – See *Hardware decoder*, *Software decoder*.

Decompression – Decompressing data consists of decoding a compressed bitstream or file into its digital form. See also *Compression*, *Hardware decoder*.

Dependent variable – In EthoVision, a variable that quantifies movement and behavior of the tracked subjects from the raw data. For example, Distance moved or Mobility. In EthoVision 3 it was named *Parameter*.

Depth of field – The distance from a position in front of the focal point (the anterior focal boundary) to a point behind the focal point (the rear focal boundary), over which all subjects are displayed sharply. The shorter the focal length (that is, the wider the angle of view) and the smaller the aperture (that is, larger aperture number), the greater the depth of field.

Digital – A signal composed of discrete elements (digits), usually ones and zeroes. The opposite of *analog*.

Digital image – An image composed of discrete pixels of digital brightness values.

Digitization – The process of converting an analog signal into a digital value. See also *Image digitization*, *Hardware encoder*, *Compression*.

Digitizer – See *Frame grabber*.

Dongle – See *Hardware key*.

Glossary

Driver – A program that is used to control a hardware device. For instance, expansion board drivers control how information is exchanged between the PC and that board.

DV-AVI – Digital Video AVI, an uncompressed video format providing high quality video, but very large file size. Note that DV-AVI is not supported by EthoVision XT.

Encoder – See *Hardware encoder*, *Software encoder*.

Expansion board – A card that has to be placed inside the computer's system unit and that extends or enhances the possibilities of your system. For instance, a *frame grabber* allows you to store video pictures coming from a VCR or video camera in a file on disk. Expansion boards are very sensitive devices because of the integrated electronic circuits. They come in different sizes (full and half size) and are designed for a specific expansion bus.

Expansion bus – A communication channel in a personal computer to which expansion boards can be attached. There are a number of different bus architectures, e.g. ISA, EISA, MCA, and PCI. The EISA bus is downward compatible with the ISA bus. The connector attaching the expansion bus to the expansion board is called expansion slot, and forms part of the computer's motherboard.

Experiment – In EthoVision, an experiment groups all the information belonging to one experimental setup. *Trials* in an experiment may well be collected with different settings (for example, different arenas or different detection settings). However, all Trials in an experiment have the same *Independent* and *system variables* (but not necessarily the same variable values). Experiments contain not only the actual data you collect, but also all your settings saved in Profiles, *external data* (after import) and analysis results.

Experiment Explorer – The tree view part of the EthoVision's interface where you can view and manage the files and elements of your experiment. By default, it is located on the left side of the screen when you start EthoVision XT. By clicking on items included in the experiment you can view them in the Component view.

Experiment Settings – Those aspects of your experiment that remain constant during the entire course of the experiment. You can enter a description of your experiment, specify the number of arenas, the body points to be detected and the video source that you are going to use (video file or live). You can also specify what units you want to use for distance, time and rotation.

Extension ring – A ring inserted between the camera body and the lens to increase the distance between the CCD chip and the lens. This enables you to focus on subjects closer to the camera, providing greater magnification.

External data – Data that have been acquired with a separate **Data AcQuisition (DAQ)** system. This can be, for example, physiological data (e.g., heart rate, ECG, EEG, blood pressure) or environmental data (e.g., temperature, humidity). With EthoVision XT you can import any external data stored in ASCII format. You can

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synchronize tracks and associated external data and subsequently visualize, select and export these data.

Focal length – The distance from the optical center of a lens to the focus, that is, the plane where the image is projected "sharply" (100% sharpness only occurs if the original is located at far distance from the lens). The focal distance is indicated with the letter f (e.g. $f = 8 \text{ mm}$), not to be confused with the aperture number (e.g. F/8). To calculate the focal distance of the lens needed for a particular application, you need to know three dimensions: the size of the scene (height, width), the size of the sensing area of the camera (height, width), and the distance between the camera and the scene. The focal distance of a standard (usually 8 mm) lens for a video camera equals the diagonal of the plane of projection (i.e. the CCD chip).

Frame – A video image. More precisely, the total number of lines that represent an image on a display device. In two-field interlaced raster scanning, a frame covers the time interval between the vertical retrace at the start of the first field and the end of the second field. See also *Frame rate, Interlacing*.

Frame buffer – A high-speed memory designed to store one or more images and allow simultaneous video display and computer processor (CPU) access.

Frame grabber – A plug-in computer board, also called a digitizer or acquisition card, which forms the heart of an digital image processing system. A frame grabber consists of several parts: (1) an analog-to-digital converter which converts the analog input video signal into a digital image consisting of pixels; a *frame buffer*, accessible from the host computer, which is used to store the digital image, and (3) a display circuitry that transforms the digital image back into an analog signal for display on a video monitor.

Frame rate – The number of frames displayed per second. The American standard (RS-170) is 29.97 frames/second. The European standard (CCIR, PAL) is 25 frames/second.

GIF – Graphic Interchange Format: A picture format using lossless compression, developed by Compuserve. GIF is especially useful for storing bitmap images containing line drawings.

Gray value – Variations in the luminance value of "white" light, from black to white. In EthoVision XT, these values can range from 0 (black) to 255 (white). Also referred to as gray-scale value.

H.264 – H.264 AVC is a type of MPEG-4. It creates good video quality and uses previously-encoded pictures as references in a much more flexible way than in other standards, allowing the use of up to 16 reference frames. Because H.264 encoding and decoding requires significant computing power, software implementation is typically slow. With an H.264 encoder card you can create H.264 video files without slowing down your computer. EthoVision supports H.264 video files created with the Picolo U4 H.264 board.

Glossary

Hardware decoder – A device that decompresses incoming bitstream, and splits it into its audio and video components, and eventually converts those data into analog signals for playback. See also Compression, Decompression, Hardware encoder.

Hardware encoder – A device that captures and converts video images into digital information (digitizes), and compresses analog audio and video data. Also called a digitizer. In order to playback this data you have to decode it, using a decoder. See also Compression, Decompression, Hardware decoder.

HDMI – High-Definition Multimedia Interface. An interface standard for communication. It is designed for high definition audio and video signal transfer between devices such as Blue-ray or DVD players, and monitors and HD digital televisions. Many modern digital video cameras have HDMI outputs. The video signal is the same as DVI (Digital Video Interface, which is the digital output used for computer monitors), except that it also includes digital rights management (so the video cannot be copied if the source video is protected). Because the video is completely uncompressed it requires a large bandwidth (10 Gb/s) and this means that the raw signal coming from HDMI is not suitable for storage as a video file unless converted and compressed to a format such as MPEG-4.

Horizontal resolution – The number of vertical lines displayed by a camera or monitor, or the number of pixels that can be displayed separately. The larger the resolution, the higher the image quality and accuracy.

Image – A rectangular picture of a scene made by a video camera. A video camera produces a fixed number of images per second. This number is 25 for PAL cameras and 29.97 for NTSC cameras. The images are sent to a monitor or frame grabber in the form of a video signal.

Image digitization – The transformation of an analog video signal to a digital image. This process, carried out by a *frame grabber* or *encoder*, is an essential component of digital image processing. You can think of this process as follows: (1) The frame grabber places a (virtual) raster on top of each incoming image, thus dividing the picture into a large number of small rectangles called pixels. (2) For each pixel, the frame grabber quantifies the light intensity and translates this to a positive whole number.

Image resolution – The number of pixels into which the frame grabber divides a video image.

Image sensor – See *CCD*.

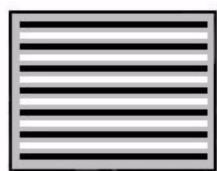
Import profile – A profile used to import external data. In the import filter you can define, for example, the sample rate or sample interval of your *external data*.

Independent variable – A variable that stays constant throughout a trial (such as temperature or the treatment you apply) and potentially determines the value of a dependent variable (such as speed of movement).

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Intensity – The amount of light at a particular point in an image. Pixels in a visible spectrum image have intensity values that are perceived as brightness by the eye. In the HSI color coding scheme, intensity (also termed *luminance* or brightness), represents the amount of light reflected by a colored surface.

Interlacing – The means by which an image is scanned, where the odd and even fields are displayed alternatively (see the figure below). Interlacing is used to reduce flicker in an image.

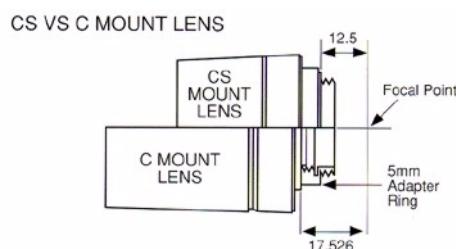


■ Field 1 □ Field 2

JPEG – JPEG stands for Joint Photographic Experts Group. It is a compression technique for color images. It can reduce file sizes to about 5% of their original size, but some detail might be lost in the compression. See also MPEG.

Lens distortion – Distortion in the image caused by a lens with an aberration. Geometric lens distortion results in straight lines in the scene appearing as curved lines in the image (curvilinear or spherical distortion). With most standard or telephoto lenses, geometrical distortion is very small. Wide-angle lenses, however, cause considerably more distortion.

Lens mount – A connection for interchangeable lenses on photo and video cameras (and some camcorders). Two types exist: the C-mount and the CS-mount (see the picture below). A CS-mount looks like a C-mount, but has a different sized flange (17.5 mm for the C-mount, 12.5 for the CS-mount). You can get a 5 mm adapter ring, so that C-lenses will fit in CS-mounts.



Light intensity – The amount of light produced by a light source, measured in *lux*.

Glossary

Lowess smoothing – Locally weighted scatterplot smoothing method. The Lowess method fits a curve to the dataset, using least square regression and a moving time window (or half size window) that contains a subset of sample points. The Lowess method in EthoVision XT uses a 2-degree (non-linear) polynomial fit. The method is weighted; sample points nearest to the point being fitted have a larger influence on the fit than sample points further away.

Luminance – The brightness component in a composite video signal. It is a single value that defines the amount of lightness or white component in an image. See also *Y-Signal, Chrominance*.

Lux – Unit of light intensity. It is equal to the number of lumen per meter. The following are a few common reference values: candle light from 20 cm distance: 10-15 lux; standard indoor room light: 100 lux; brightly illuminated office room: 400 lux; sunlight, one hour before sunset: 1000 lux; daylight, overcast: 5000 lux; daylight, bright sky: 10,000 lux; intense sunlight: 20,000 lux or more.

Macro lens – A lens with a display ratio (magnification) of at least 1:1 (that is, the size of the image is at least the size of the subject). The subject under observation is located close to the camera, so photography with a macro lens is sometimes called close-up photography. Some manufacturers use the term more loosely for lenses which give a greater magnification than normal, but not as much as a true macro lens.

Manual focus lens – A lens that manually has to be focused on an object, as opposed to an auto focus lens which focuses automatically.

Manually scoring data – Transcribing observed behavior into quantitative measurements relating to specific behaviors. In EthoVision XT this operation is done during tracking (live or from video file).

Mini DIN connector – See *S-Video connector*.

Monochrome – Any combination of colors with the same hue, but of different saturations and intensity. Generally, a monochrome image is known as black and white.

MPEG – MPEG stands for Moving Picture Experts Group. It is a file format for compressing full-motion digital video, with the extension *.mpg. EthoVision XT supports MPEG-1, MPEG-2, MPEG-4 and H.264 (a special type of MPEG-4). MPEG-4 and H.264 are only supported if the video files are recorded using the Picolo Diligent or the Picolo U4 H.264 board. Other MPEG-4 formats may not work.

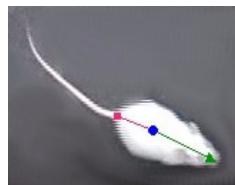
Noise – The part of a signal which is not derived from the image. It is caused by stray electromagnetic radiation inducing voltages in the cable and other equipment. The signal to noise ratio must be sufficiently high so that you can distinguish your subject from noise. You cannot do this by increasing the gain because this increases both signal and noise. It is best to define the minimum

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subject size larger than the size of the noise (usually 1 or 2 pixels). To reduce noise, use the Pixel function in the Detection Settings.

Nose point – The point on the contour of the subject considered as nose tip. It is detected by means of a complex algorithm that analyze the shape of the image detected as subject at every sample. You can have EthoVision XT detect the nose point only if you have the Multiple body points add-on installed.

Nose-tail detection – A feature of EthoVision XT that allows to track the nose tip and the base of the tail of a moving rodent besides its body center. Which points are considered as nose and tail base depend on the contour of the area detected as subject. Note that you must have the EthoVision XT's **Multiple body points** add on installed on your computer in order to use this feature. Three methods for Nose-Tail detection are available (see page 258).



NTSC – National Television Standards Committee. This is an organization that has set the American standard for color TV, based on 525 lines and 60 Hz. The NTSC standard is used in 32 countries, including the United States, Canada, Japan, and several Latin American countries.

Object – In EthoVision 3, any item tracked during a data acquisition trial. An object usually corresponds to one individual animal. Strictly speaking, it is the part of the animal that is detected. In EthoVision XT, object has been replaced by *Subject*.

Off-line – In EthoVision XT, the data acquisition is off-line if data are acquired from a previously-recorded video file.

Olfactometer – A type of maze used for measuring the effect of semiochemicals on insect behavior.



Glossary

On-line – In EthoVision XT, the data acquisition is on-line if a subject is tracked with a video camera connected to the computer by means of the frame grabber board. In that case, data are acquired from the live video footage and you must select **Live tracking** in the Experiment Settings.

On-Off signal – Signal type used to synchronize external data and EthoVision data. An On/Off signal can be used if the external data are sampled at a low rate (less than 10 Hz) and without interruption. See also *TCAP*.

Overlay text – The text appearing as an overlay on the video image (live or from a media file). You can specify what information to view during acquisition trials (see Customizing the Acquisition screen).

PAL – Phase Alternating Line. A standard color-encoding system, based on CCIR video (625 lines and 25 frames/second) and FM-modulated audio signals. It is used in all of western Europe (except France), China, Indonesia and Australia. There are several “dialects” within the PAL standard, which causes varying degrees of compatibility between countries.

Parameter – In EthoVision 3, dependent variable calculated by EthoVision on the basis of the raw data. It is now called *Dependent variable* in EthoVision XT.

Phono connector – A cylinder-shaped connector (see the figure below), also called a tulip or cinch connector or RCA jack, with a protruding pin. It is a plug and socket for a two-wire coaxial cable used to connect audio and video components.



Pixel – Picture Element. The smallest dot that the computer and monitor can display, or the representation on the screen of the value of a bit. Its gray value or color value is represented by a number.

PNG – Portable Network Graphics. A picture format using lossless compression. PNG is especially useful for storing bitmap images containing line drawings. PNG is comparable to GIF.

Preferences – A number of settings allowing you to define file paths, select in what situations you want to get warnings, and how often the program must save the data automatically.

Profile – The settings made in EthoVision XT for a specific function: track smoothing, data selection, data analysis, etc.

QuickTime – A file format for storing video and animation information with the extension *.MOV. QuickTime is built into the Macintosh operating system. PCs can also run files in QuickTime format, but they require a special QuickTime driver. EthoVision XT does not support QuickTime. See also *AVI, MPEG*.

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Raster – The area of a video display that is covered by sweeping an electron beam in the display in a series of horizontal lines from top to bottom. The beam then returns to the top during the vertical flyback interval. Raster is also used to describe any scanning pattern in the form of a grid, such as the arrangement of pixels in a bitmap.

RCA Connector – See *Phono connector*.

Real time – An operation that can be completed in one frame time is said to be performed in real-time. For PAL, a frame time is 1/25 second. For NTSC, the frame time is 1/29.97 second.

Reference image – An image of the arena EthoVision XT uses to distinguish between subjects and background when you select one of the **Subtraction** detection methods. In the Static subtraction method, the reference image is fixed (like in the corresponding method in EthoVision 3). In the dynamic subtraction method, the reference image is constantly updated using the video image of the latest samples.

RGB – Red, Green and Blue. A color coding model to represent the visible color spectrum. A specific color is split into a red, green and blue intensity value.

Running average – The mean of a set of consecutive data points, usually the last points in the data set. The number of points (called Averaging interval) remains constant, but the actual points vary. A running average is used to smooth a varying data set.

Sample rate – The sampling of a digitized video image or digital video file by an application program. The sample rate is the number of video images that is sampled per unit time. The sample rate also refers to the number of data points (e.g., from an ECG signal) that is sampled per unit time by a data acquisition system.

Saturation – A measure of how pure a color is, in relation to its brightness. For example, a fully saturated red would be a pure red. The less saturated, the more pastel it appears.

SECAM – Séquentiel Couleur à Mémoire. A color-encoding system, which originated in France, and which is almost identical to the PAL standard, except that the audio signal is AM-modulated. The video signals are compatible and interchangeable. It is used with CCIR video in 42 countries including France, eastern Europe, the Middle East and large parts of Africa.

Separated Y/C connector – See *S-Video connector*.

Settings – The settings made in EthoVision XT for a specific function: arena settings, detection, trial control. Also called *Profile*.

Glossary

Shortcut key – Keystrokes that call up a menu item or result in a specific action (e.g. **CTRL+S** = save the current experiment). They are usually the combination of a modifier key (for example the **Alt** key) and a character key. See Appendix A.

Smoothing – See *Lowess smoothing*.

Software decoder – A program that decodes a compressed video file for playback. See also *Hardware decoder, Software encoder*.

Software encoder – A program that encodes (compresses) an uncompressed digitized video feed (combined with the audio feed) to one of the available formats of the program. In contrast to hardware encoding, software encoding uses the processor (CPU) of your computer to encode the recording, so creating high-quality recordings requires a fast processor. See also *Hardware encoder*.

Speed of lens – A lens is fast if it transmits a relatively large amount of light (it has a large maximum effective aperture) and slow if it transmits less light.

SubD connector – A standard 9-pin or 25-pin connector (see the figure below), used on computers (for example, for the RS-232 port) and video cameras (e.g. color cameras with RGB output).



Subject – In EthoVision XT, any item tracked during a data acquisition trial. A subject usually corresponds to one individual animal. Strictly speaking, it is the part of the animal that is detected. In EthoVision 3, it was called *Object*.

S-Video – A video signal, also referred to as Separated Y/C signal, in which the *Chrominance* (C) and the *Luminance* (Y) components are transmitted separately. See also *Composite video signal, RGB, S-video connector*.

S-Video connector – A video connector (see the figure below), also referred to as Separated Y/C connector designed for separate transmission of *Chrominance* (C) and *Luminance* (Y) signals. This (usually 4-pin) connector is sometimes referred to as "S-VHS connector", but this is incorrect, because it is found on all sorts of high-quality video equipment, including HI-8 and S-VHS recorders, as well as high-resolution monitors.

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Surface area – The number of pixels the *Subject* consists of.

Synchronization – Changing the Start time of a data set (*Track* file, Video or *External Data* file) relative to the Start time of other data sets.

Synchronization signal – A signal from the EthoVision XT PC to an external Data AcQuisition (DAQ) system, containing time information. The synchronization signal must be recorded by the DAQ system and sent back to the EthoVision PC where it is compared with a reference copy retained in the PC. This way, *external data* and *Track* data are synchronized.

System variable – Variable that define specific characteristics of a *Trial*, *Arena* or *Subject* being tracked, and are created and updated by the system automatically. Examples of system variables include the trial's start time and duration, the track file name, the recording duration for an Arena etc.

Tail-base point – The point on the contour of the subject considered as tail base. It is detected by means of a complex algorithm that analyze the shape of the image detected as subject at every sample. You can have EthoVision XT detect the tail base only if you have the **Multiple body points** add-on installed.

TCAP (Time Code Auxiliary Parity) – Signal type used to synchronize external data and EthoVision XT data. A TCAP signal is a series of bits (called frame) that is sent repeatedly from the PC to the DAQ device. Each frame contains the current date and time and additional information. See also *On/Off*.

Template experiment – An experiment in which basic settings are pre-set, including arena definition, detection settings and parameters for analysis. There are templates for several often-used tests: Morris water maze, plus maze, 96-well plate, etc.

Track – The data from one *Subject* during one *Trial*. The data consists of the x,y coordinates of the body points (Center, Nose point and Tail base) and the surface area of the subject for each sample. Note: The Nose point and the Tail base can be recorded if you have the **Multiple body points** add-on installed on your PC.

Track file – A file containing the Track data recorded from one *Arena* during one *Trial*. The data are from a series of tracks, each track being the x,y coordinates and the surface area of one subject. Different tracks within one trial can start at different times. Note: The current version of EthoVision XT allow to record only one *Subject* per Arena, therefore the Track files always contain the data for one Subject.

Glossary

Trial – One run of experimental data. During a trial, each of the arenas being measured are recorded as a separate track file.

Trial List – The list of all the *trials* acquired up to that time in an experiment. The list also includes the variables in your experiment, which may be *independent* (user-defined) *variables*, media files, *external data* and system variables.

TV standard – Standard governing the way in which TV and video signals, in color or black/white, are transmitted. TV standards refer to the number of lines, bandwidth of the video and audio signals, the method of encoding color and brightness information, method of sound modulation etc. See also: *NTSC*, *PAL*, *SECAM*.

User-defined variable – See *Independent variable*.

Vertical resolution – The number of horizontal lines displayed by a camera or monitor. See also *Horizontal resolution*.

Video size – The size (width and height in pixels) of the frames of a digital video file. For example, an MPEG-1 file obtained for PAL video can have a video size of 352 x 288 pixels.

Video window – The window that shows the video image when you carry out a trial or adjust detection settings.

View Settings – You can access the view settings by clicking the **Show/Hide** button on the component tool bar. In the menu, you can specify which windows and objects you want to display. For instance, in the Acquisition component, via the **Show/Hide** button, you can select **Feedback** (overlay text that you can have displayed on the Video window), **Track features** (overlay body points and trails), **Arena Features** (overlay arenas and zones), **Video Source** (the current video footage) and other windows and panes.

White balance – The human eye automatically sees a piece of white card as colored white, almost no matter what the color balance of the incident light. A video camera 'sees' the card as the true color of the reflected light (that is, influenced by the color of the incident light). The white balance function of a camera automatically corrects the color balance so that white card is seen as white. This must be disabled when working with EthoVision. The spectrum of colors in a light source is its color temperature. Red is 'hot' and blue is 'cold'. Color temperature is measured in degrees Kelvin.

WMV – Windows Media Video, a compressed video file format similar to *MPEG-4*. Note: EthoVision XT does not support WMV.

Y-signal – The luminance or brightness component in a composite video signal. In a composite video signal it is mixed with the chrominance (color) component, while in S-video and RGB signals the components are transmitted separately.

Y-C Signal – See S-Video.

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Zone – Sub-division of an *Arena*. Define zones to delimit regions of significance in the experimental setup, such as a platform in a Morris water maze or the two halves of an open field. You can then analyze data relative to such zones. For example, to find the time that the animal spent in the open arms of a plus maze.

Zone Group – A series of zones within the Arena related to each other, but not overlapping. An Arena can have overlapping zones but these must be in different Zone groups. You can store several zone groups in one *Arena Settings*. In EthoVision 3, zone groups were called Zone definitions.

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