ZebraLab® v3

Automated Behavioral Analysis

for Windows XP®

USER'S MANUAL

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1 INTRODUCTION

The ZebraLab system is based on Video image analysis, and allows the user to measure animal locomotion and/or general activity.

This system includes:

* A system unit, usually based on a Pentium® or AMD ® or equivalent processor,
* A ZebraLab image processor board and its graphic board (Pinnacle PCTV),
* A CCD video camera with its appropriate lens,
* An associated software.

The Black and White images are supplied by cameras or video recorder (i.e. U Matic, VHS, SVHS or digital). According to the quality of your video recorder, you may need to use a TBC (i.e. Time Base Corrector).

The main features of this system are the following:

*Tracking of 1 or several animals (moving within separate enclosures) per camera, which allows simultaneous tracking; Number of animals detected at the same time will vary with the size of the animal observed (i.e. zebrafish, daphnia, rotifers),

* Areas of interest for each enclosure may be defined by the user,

* Graphic board to provide overlay on the computer screen.

In its "movement tracking" version, this system allows you to easily automate most tests based on locomotion activity:

* Open field
* Mazes (radial, x-shaped, 8-arm maze, etc.)
* Other applications (i.e. Fish locomotion)

In its "movement quantization" version, tests involving general activity can be automated as well:

* Freezing test
* Ecotoxicology experiments, etc.

By using cameras sensitive to infrared lighting, the investigator may use this type of technology when considering other applications such as chronobiology or general activity measurements in different conditions of light (i.e. pseudo-darkness alternation over long periods of time).

Also, the investigator will observe that status panels and pull-down menus from the ZebraLab system are very user-friendly.

CAUTION

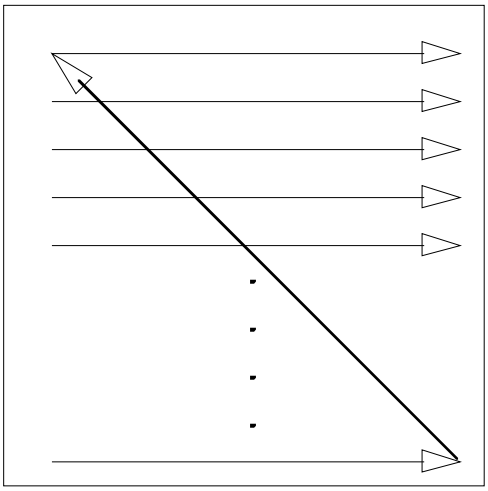
This user's manual has been designed for all types of ZebraLab systems. Some possibilities and functions are available as options. For instance, additional cameras may be added on this type of technology depending on the set-up of your experiment.

2 OPERATING PRINCIPLE

The camera connected to the ZebraLab system, supplies an analogue voltage that varies over time.

The camera connected to ZebraLab analyses the image by dividing it into 600 lines (with the CCIR standard and 480 lines in NTSC), each line being scanned within a few microseconds.

This rapid scanning produces an image made up of a large number of points (800*600).



A complete image is scanned in 40 milliseconds, i.e. at the rate of 25 images per second (according to CCIR standards, 30 images/second according to NTSC standard). ZebraLab is therefore unable to analyse motions lasting less than 40 milliseconds (30 milliseconds in NTSC).

The signal transmitted to ZebraLab is therefore composed of the measurements of the luminosity of each point, transmitted on a point-by-point, line-after-line basis. This signal's voltage represents the level of luminosity of the point currently being scanned. Synchronisation signals mark the beginning of scanning of a line, as well as the beginning of transmission of an image.

The analogue signal supplied by the camera (line after line, image after image) is digitised on by ZebraLab on 8 bits by digital analogue conversion.

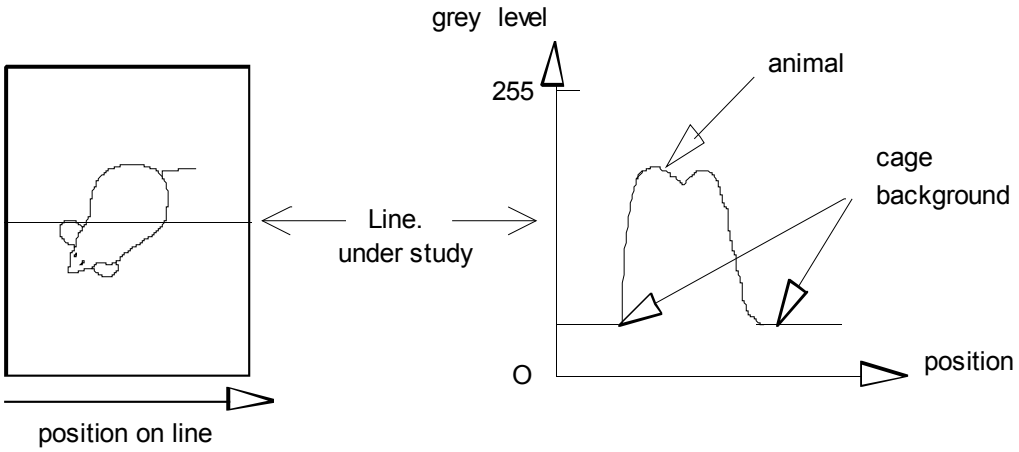
With 8 bits, you can code 256 different luminosity values (from 0 to 255). This makes up the input dynamics of the digital-to-analogue converter.

This converter automatically assigns a digital value to each pixel according to its luminosity as supplied by the CCD (Coupled Charge Device) captor of the camera:

* 0 corresponds to pure black
* 255 corresponds to pure white

A black and white image which is well contrasted has its pixels ranging between 0 and 255 (mini-maxi).

An image which is too dark (insufficient lighting, aperture of objective diaphragm too small, etc.) only supplies values in the lower range (from 0 to 100, for instance), whereas an image which is excessively lit (too much light, diaphragm aperture too big) supplies an image saturated with pixel values in the upper range (e.g., from 150 to 255).
In the second case, the darkest point of the image corresponds to 150 and the most lighted point cannot exceed 255, which may considerably reduce contrast in the scene being filmed.



White animal on a dark background

For good use of ZebraLab, use the following elements to properly adjust its input dynamics:

- lighting (indirectly if possible so as to avoid shadowing)
- aperture of your objective

With its functions of image overlay, and detection threshold adjustment, status panel helps you easily evaluate your input dynamics and guides you to perform all necessary corrections.

The main purpose of ZebraLab is to track the movements of a laboratory animal.
This obviously implies that the animal must be properly contrasted with respect to the background:

- either the animal is white on a black or grey background
- or the animal is black on a white or light background.

The video image (from 0 to 255) containing all intermediate grey levels is converted to a binary image: each pixel is black (0) or white (1).

This operation, called detection, allows you to distinguish the animal from the background, thereby supplying ZebraLab with the information it requires.

You must therefore set up a threshold in the grey level scale in order to make this distinction.

3 GENERAL REMARKS

3.1 WINDOWS XP

Users are supposed to be familiar with the use of Windows XP®.

ZebraLab must be used with the Administrator rights on a standard Windows XP® set-up.

If you need to work without the administrator rights, please be sure to follow these guidelines:

1-create a group of user (i.e. ZebraLab),
2-go to the Policies/user rights menu,
3-check Show Advanced User Rights,
4-Do the same with “increase scheduling priority” right,
5-Now each time you create a new user on the system, you will need to set him as a member of ZebraLab group and a member of User group.

If such operation fails, you will get an error message while starting the ZebraLab, which will state: “No memory rights for the current user”.

3.2 ANTIVIRUS / BACK-UP / POWER SAVING

As ZebraLab is generally connected to a network, it is strongly advised to install an antivirus software. Nevertheless, some conflicts may occur if antivirus software is scanning the system while experiment is running. So we recommend to schedule scans at a time no experiment is in progress.

The same caution should be taken to manage back-ups and prevent from simultaneous access to hard disk.

On Windows XP check the following parameters to prevent the computer to go to sleeping mode during acquisition

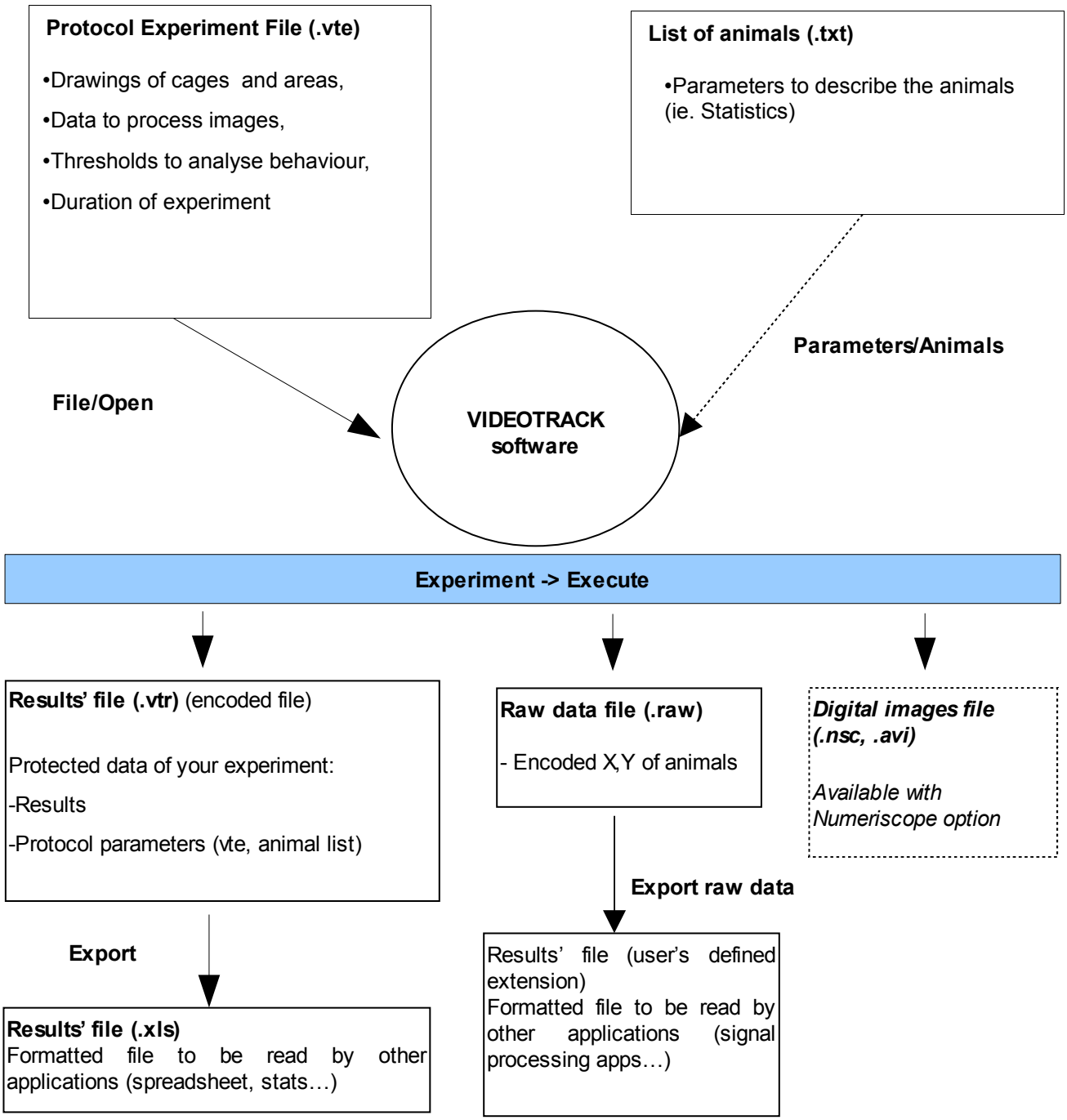
To change power parameters
* right click on the desktop,
* choose Screen Saver and set it to None,
* Click on power,
* in power schemes select always on
* all list box should be set to "never"
* select Hibernate and uncheck Enable hibernate support.

4 OPERATING ZebraLab

4 steps are necessary in order to run an experiment:

-create a complete protocol,
-if necessary create a list of animals to be analysed in the experiment,
-execute the experiment,
-export and process the results.

Files organisation is as following :



4.1 - CREATING A PROTOCOL

First you need to start the ZebraLab software with a double click on the ZebraLab icon on Windows desktop. Then go to File/new

4.1.1 Protocol parameters

ZebraLab software displays the Protocol parameters window in which you will fill in the values for the new experiment you are creating.

Several tags indicate the type of values to be defined

Animal number: In this field you will get the number of animals that will be analyzed at the same time. It is the total number of cages that will be placed under the cameras. This field can not be edited and will be updated automatically depending on the number of locations selected for this experiment.

Location count: This field displays the number of locations (number of cages) that will be analyzed at the same time.

Experiment duration: The duration is the total time of the experiment. At the end of this time the experiment will stop automatically for the animals in the cage(s). The duration is specified in days(D), hours(H), minutes(M), seconds(S).

Integration period: The user will have a result for each integration period, which is a time frame in seconds defined in the protocol. For instance, with an experiment duration of 10 minutes and an integration period of 10 seconds the animal is analyzed during 10 minutes, and data is integrated each 10 seconds (Secs).

Then click OK to validate the new parameters.

4.1.2 Parameters for each location

Depending on the number of animals, you will have to define the parameters to be able to process the test correctly.

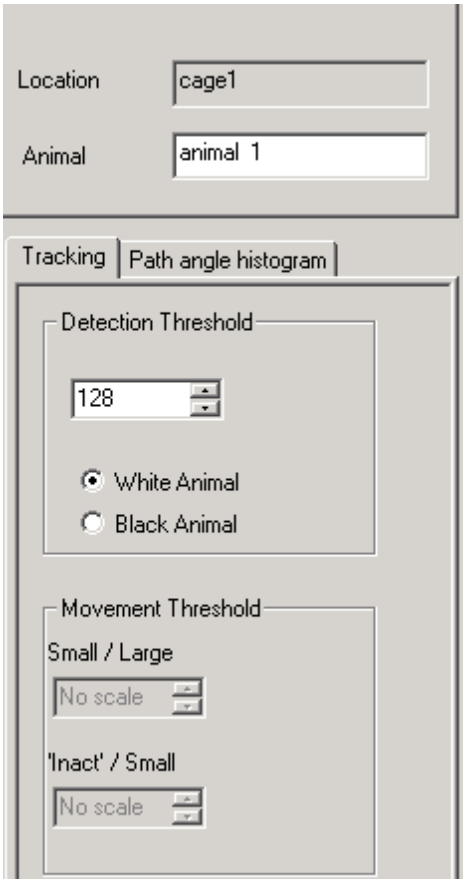
The tile screen displays several windows, and each will be assigned to a cage (location).

A double click on one of the windows, will display the full screen window.

4.1.2.1 Parameters for Tracking version

Animal name: This field is used to give a name to the animal that will be placed in the cage. An additional session number will be added to identify the animal in the results' file. You will see later on that if several animals have to be placed in the cage successively, it is also possible to define a list of animals that will overload the default name.

Location: As the system can be used with several cameras and each camera can see several cages (depending on the version used), we can define several locations that are possible to place the animals. For example, if you have 2 cameras in 2 different rooms, and each camera monitors 2 cages, you will have 4 locations:
Room1-Cage 1
Room1-Cage 2
Room2-Cage 1
Room2-Cage 2



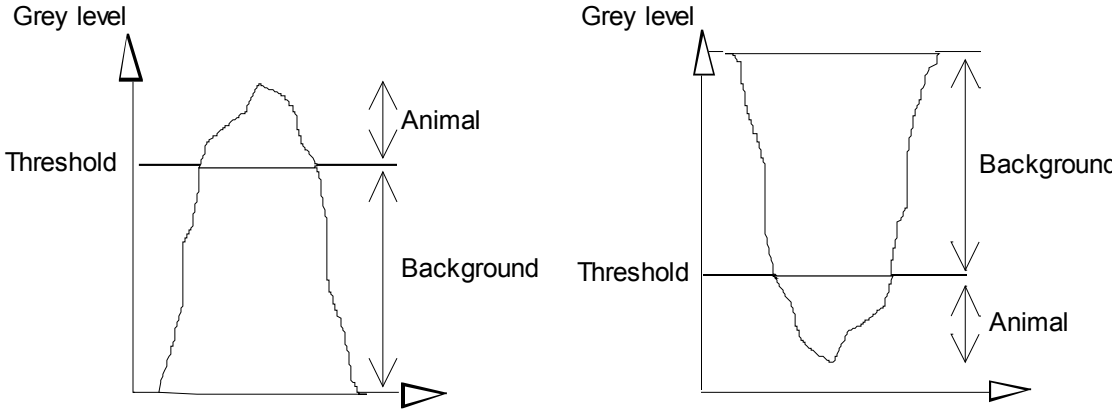
Animal color

Use this button to select the color of the animal (black or white or transparent).

This button is important to operate ZebraLab according to the detection threshold you have selected:

- If the animal is white, ZebraLab will only track pixels whose luminosity is greater than the detection threshold. It implicitly considers that the background is dark, therefore of lower luminosity than the detection threshold.

- If the animal is black, ZebraLab only tracks pixels whose luminosity is lower than the detection threshold, considering that the background, being light or white, is higher than this threshold.



WHITE ANIMAL BLACK ANIMAL

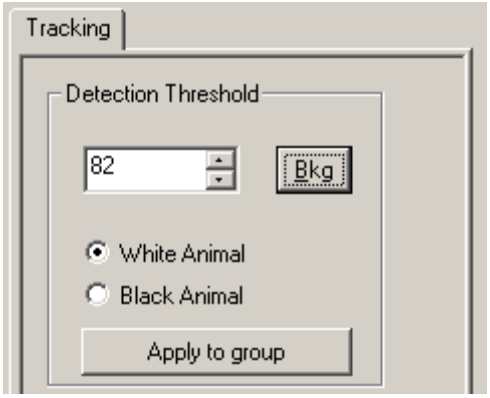
- **Detection threshold / background subtraction**

The detection threshold allows the ZebraLab to distinguish between the image background and the animal. You must therefore use extreme caution when adjusting the detection threshold in order to obtain correct results.

This may be a problem if the intensity of the lighting in the room varies since the contrast remains the same while the gray level of each point in the image changes.
The part of the image detected as the animal is then significantly altered.

You may modified the threshold, and using the overlay, visually check which are the parts of the image detected as the animal and which are detected as the background. The focus on this value switches automatically the red overlay.

As an option, ZebraLab is able to work with a background subtraction. In this case, the system is less sensitive to differences in the quality of the lighting or colors of the background. To set-up the detection threshold, the user needs first to take an image of the background with a click on the “Bkg” button



This has to be done when the cage is empty. Then place the animal in the cage and adjust threshold for a good detection. When running the experiment, the user will be asked to store the background between each session when the cage(s) is (are) empty and then place the animal(s) in the cage(s).

4.1.2.2 Parameters for movement quantization version

Animal name: This field is used to give a name to the animal that will be placed in the cage. An additional session number will be added to identify the animal in the results' file. You will see later on that if several animals have to be placed in the cage successively, it is also possible to define a list of animals that will overload the default name.

Location: As the system can be used with several cameras and each camera can see several cages (depending on the version used), we can define several locations that are possible to place the animals. For example, if you have 2 cameras in 2 different rooms, and each camera monitors 2 cages, you will have 4 locations:
Room1-Cage 1
Room1-Cage 2
Room2-Cage 1
Room2-Cage 2

Whereas, it is possible to define several areas of interest for movement quantization version, results will be given for the union of all areas (area 0) only.

Detection sensitivity

This threshold makes it possible to remove noise in the image which might generates inaccurate activity detection. Indeed, lighting might be flickering and camera can supply slightly different images even if nothing is moving.

The lower the threshold the highest the sensitivity. Usually a value of 20 is used. While adjusting this threshold, detected movement are shown as a red overlay on the actual image

Activity burst threshold

When the value of the moving surface is above this threshold, the activity curve is displayed in red and this represents burst activity.

Freezing threshold

When the value of the moving surface is lower than this threshold, the activity curve is displayed in white and this represents the freezing state.
Between the two thresholds values, the curve is displayed in green and animal is in normal activity.
To adjust these thresholds, the user needs to place one animal in the cage and adjust the values so that the colour of the curve matches what he would score manually.

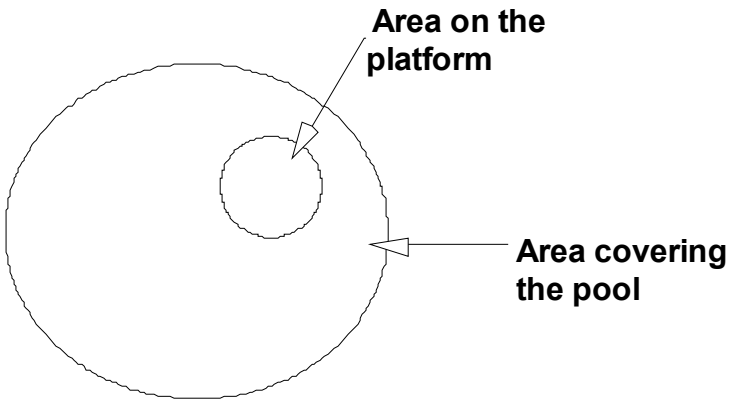
Scale

This parameter defines the maximum value of the activity that can be displayed in the curve window. So the vertical axis will display a curve whose values will be between 0 (at the bottom) to [Scale] at the top. Even if the values above will not be seen, the actual value will be used to generate the results.

4.1.3 Areas of interest

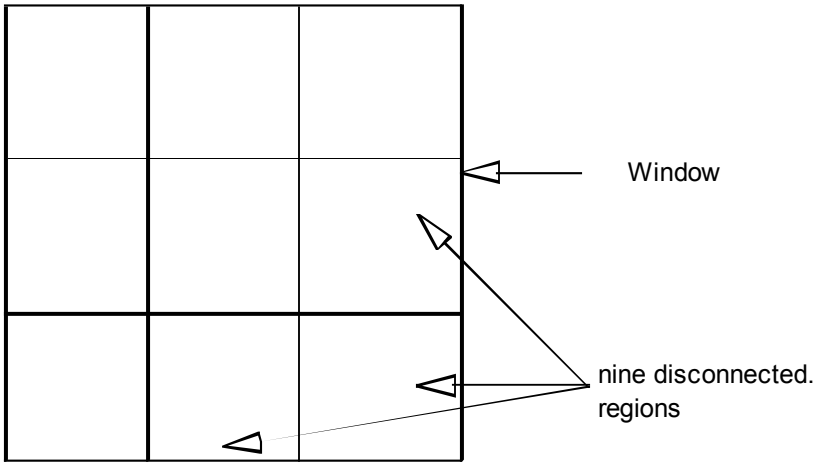
The user may subdivide the cage into several areas of interest (for instance, the center, the corners, and the platform in the watermaze test, etc.), and for each area of interest he will have the numerical results (i.e. number of entries into the field, time spent, distance covered, etc.)

Using the mouse, the user simply draws the areas within the cage he is interested in.



Example 1 : Watermaze

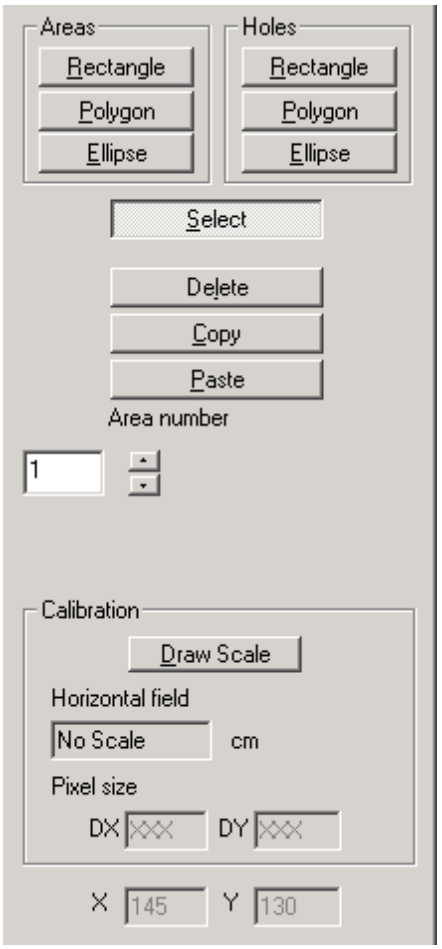
To avoid the detection of objects that are presented outside of the pool (white area), ZebraLab processes only part of the image that is included within one area.



Example 2 : Openfield

In order to draw the areas, the user will need to have the display of a location in a full screen. Then, he will place the cursor on the video window and click RIGHT button on the mouse, thus opening the contextual menu. Choose Edit

4.1.3.1 - Area number indicator



This edit area defines a specific area of interest. The two arrows let you increase or decrease the area number.

4.1.3.2 - Area shape buttons

Use these buttons to select the shape of your area of interest. You can choose between the following geometrical figures:
-Rectangle
-Ellipse
-Polygon

The selected shape (a rectangle, by default) is displayed as highlighted.

The user can choose between drawing an area of interest or a hole (excluded area). A hole is a part of the image that will not be processed. It makes it possible to remove a part of a cage with the same color than the animal.

Rectangle
To draw a rectangle, select Draw Rectangle. Select the Area Number you want to assign to this rectangle. Move the mouse to a point where you want to position a corner of the rectangle. Click on this point and keep pushing until you reach the opposite corner, then release it.

Ellipse
To draw an ellipse, select Draw Ellipse. Select the Area Number you want to assign to this ellipse. Move the mouse to the point where you want to position the centre of the ellipse. Click on this point and keep pushing until you have the correct size.

Polygon
To draw a polygon, select Draw Polygon. Select the Area Number you want to assign to this polygon. Position the mouse on the first side of the polygon you want to draw. Click and keep the mouse button pressed. As the mouse moves, the first side of the polygon is drawn. Release the button define the second corner of the polygon

Click once again each time you want to add a corner.

The current point, corresponding to the position of the mouse, is always connected to the starting point of the drawing. In other words, the drawing of the last side of the polygon is automatic.

Validate the polygon once the next-to-last side has been fully drawn, by double-clicking on the mouse (press the validate key twice very quickly).

4.1.3.3 - Select button

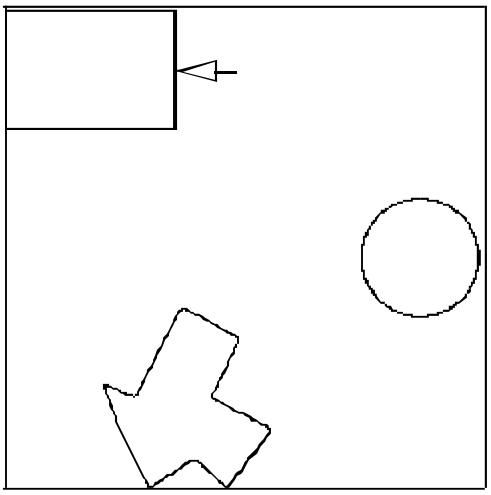
It may happen that the drawing you have performed with the mouse does not exactly match:

- either the animal's enclosure
- or the desired area.

This "modify" button gives you the opportunity to make the corrections on the drawings. Use the indicators to select the pattern that you will modify the area of interest. This pattern is displayed in a different colour from the

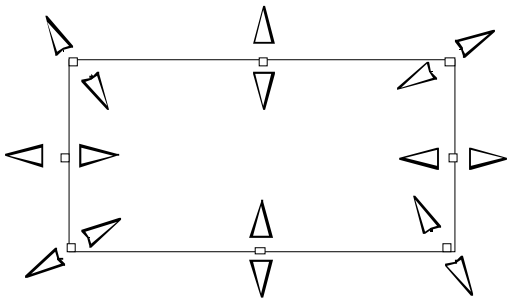
other areas.

Bring the mouse to one of the sides of the pattern then click.

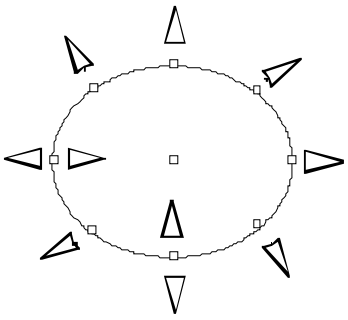


Click on the figure to be modified

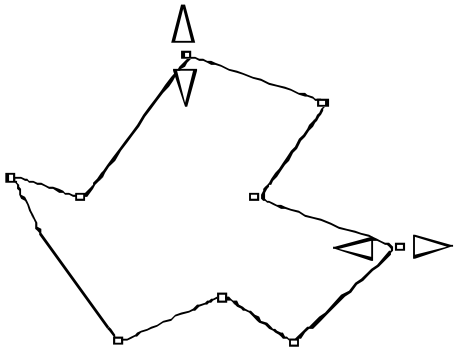
Handles appear at various points of the outline, depending of the type of outline



Rectangle



(center handle : increase/ decrease in diameter)



Use handles to move each point

The handles allow the figure to be stretched in a particular direction. The figure can be moved by clicking on its lines, handles excluded. The new shape in position of a figure is validated by the validation key. Escape cancels any pending modification.
Use the double-click to validate a polygon.

4.1.3.4 - "Delete Selection" button

This button lets you select the option to delete areas.

Select area to delete by area number.

4.1.3.5 - Copy button

This button stores the selected area to the ZebraLab's clipboard in order to reuse it later on.

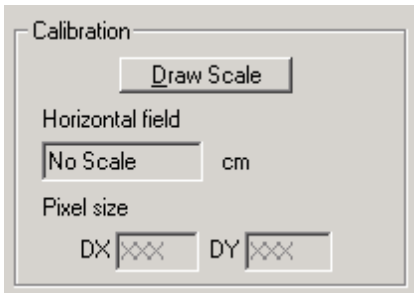
4.1.3.6 - Paste button

Each time this button is used, ZebraLab creates a new area with the current area number and whose shapes is the one that has been stored previously in the ZebraLab's clipboard. The new area is slightly shifted from the previous one.

To create new areas with the same size than an existing one, just follow the steps hereafter:
-select the area which will be duplicated and hit Copy button,
-change the area number to the one you want to assign to the new area,
-hit Paste button,
-Move the new area to its position,

When copying areas from a given location the another location, the areas' number will remain the same.

4.1.3.7 - Calibration button

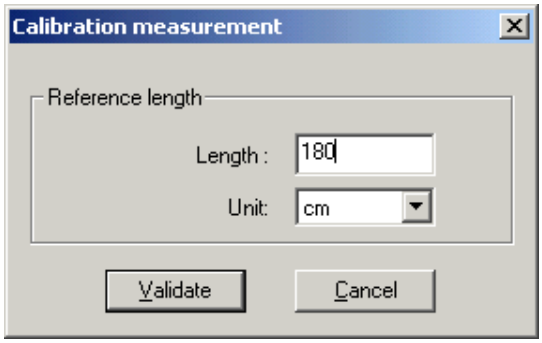


Once the drawing of areas is completed, the video environment must be calibrated.

The camera's field is 800*600 pixels, and the focal distance of your objective determines a specific surface, therefore a specific distance for its side. All ZebraLab's computations are done in "pixels", which must be converted into the corresponding units (cm, mm, µm., cm per second, mm per second...). The "Calibration" section lets you perform this conversion easily.

Click on the " Draw Scale" button which becomes validated.

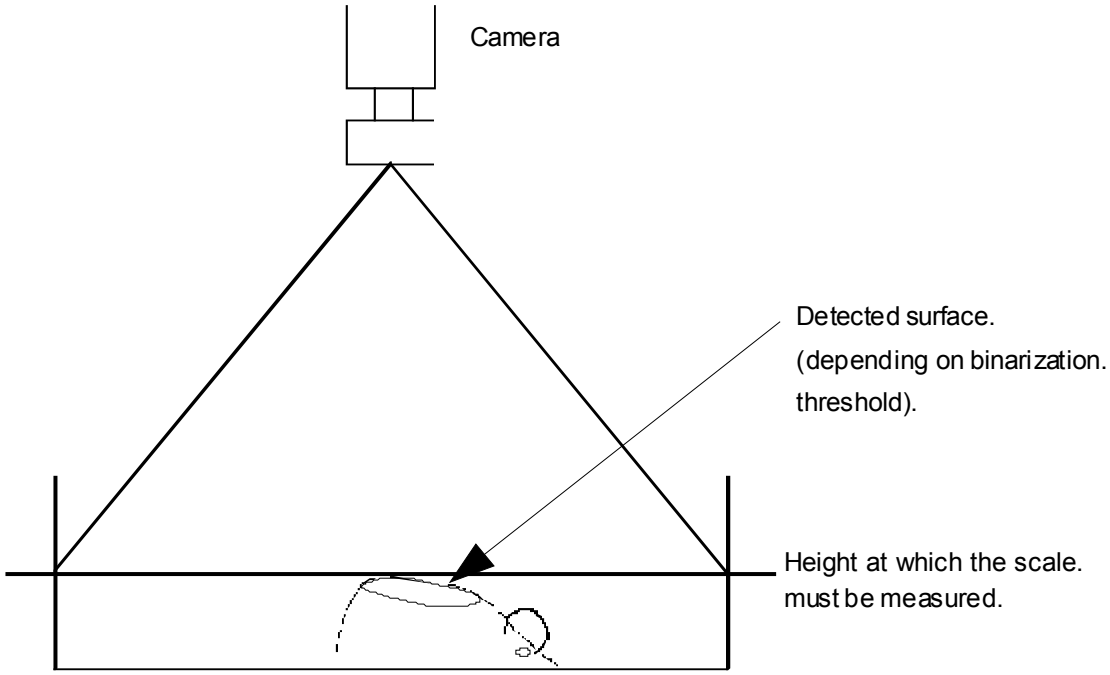
Click on a marked point of the video environment, and then move the mouse to another marked point (horizontal or vertical) of the video environment. Once you draw this line, the ZebraLab asks you to enter the length and the unit of the line in order to validate it. If you make a mistake, you can repeat the operation. The scale will be stored with the video environment of the corresponding camera.



After the calibration is done, the horizontal field of view is displayed as well as the horizontal and vertical size of the pixel.

Note : The geometric distortion of the camera's objective can cause an error in scale measurement. It is important to measure this scale at the level of the detected surface.

While using more than one location, it is possible to set a scale per location even if these locations are coming from an image of the same camera. This allows you to track the animals in the cages which are not at the same level of the floor.



4.1.4 Movement calibration

4.1.4.1 - Principles and algorithms

Once the adjustment of the video and spatial environments are completed.
You may now place your animal into the cage device to measure its locomotion activity.

Within a cage, the ZebraLab binaries the image, computes the co-ordinates of the center of gravity of the binary object, and transmits the data to the processing software.

The software uses the data to compute a number of useful parameters and possibly to initiate some actions. One extremely common application of movement tracking consists in determining the time spent (and distance covered, etc.) in three types of locomotion activity :

- ambulatory movements
- small movements (including stereotypes)
- inactivity (or very small units depending on the thresholds)

This distinction into three types of movement, while quite natural by visual observation alone, is not that obvious to the software which only "sees" one center of gravity every 40 milliseconds.

As the co-ordinates of this center of gravity are supplied in pixels, the software needs a certain amount of information in order to make the proper classification.

Numerous algorithms can be considered for this classification, using all the temporal successions of the co-ordinates of the center of gravity (every 40 milliseconds in this particular instance).

The algorithm used by ZebraLab is largely based on the subjective notion of movement (with respect to spatial environment and video calibration).

Briefly this is how it works :

- the co-ordinates of the object's (animal's) center of gravity are permanently stored for the last 5 images.

- ambulatory movement is considered to have occurred (requiring locomotion in a given direction) if the distance covered between positions image 1-to-image 5 is greater than a given threshold.
In fact, one can consider that if ambulatory, the movement is directed toward a goal and that the vectorial distance covered (image 1 - image 5) is slightly less than, or at most equal to (if the trajectory is a straight line) the distance actually covered.

The movement thresholds therefore allows you to distinguish between ambulatory movement and other types of movement.

- If the movement is not ambulatory, although there is movement and therefore the center of gravity is displaced: this movement is not directed to any particular point in space and the vectorial distance covered between image 1 and image 5 is generally fairly low, less than the previous threshold.

- On the other hand, if the animal is inactive (or immobile) during this "sliding" period of 5 images, its centre of gravity will move little, and the distance will most probably be less than another threshold (lower than the first) corresponding to the average distance covered by the centre of gravity of an animal not immobile, but awake (washing, feeding etc.)

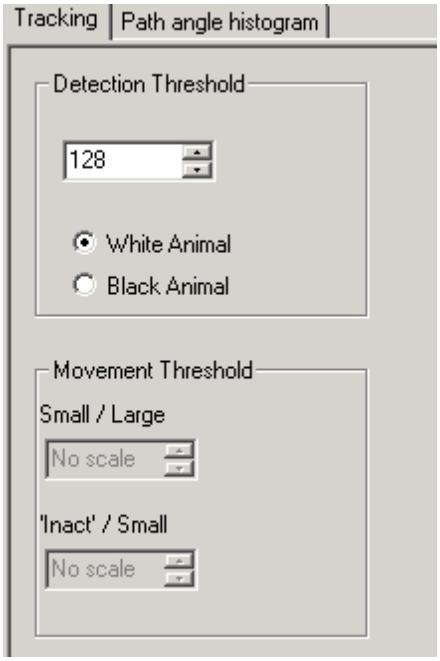
These two movement thresholds are highly dependent on the animal considered and its video and spatial

environment.

While setting up the movement thresholds, the colour of the centre of gravity change depending on the speed of the animal.

This step allows you to make sure that the detection thresholds are properly suited to your lighting or to the aperture of your objective, and that there are no abnormal foreign spots in the animal's path.

Some abnormal spots may be cause by a synchronisation error in the video recorder. On some video-recorders you are advised to use a "Time Base Corrector" for perfect image synchronisation.



Adjusting the small/large movement threshold

The corresponding threshold (in cm/s) is displayed in the appropriate indicator to the right of the status panel.

If you click on the selection button the threshold shown in the appropriate indicator corresponds to the small/ambulatory movement threshold. With increasing of this threshold (starting with a 0 default value) using the up arrow button, you can see the colour of the points or the lines between the points sometimes change from red to green and vice-versa.

According to the threshold displayed (small/large) the software displays on-line :

- the colour green small movements
- the colour red for large or ambulatory movements (greater than the threshold).

The trajectory must be red only when you consider the animal is moving.

Small movements / inactivity movement threshold

This value is for adjusting the threshold differentiating between small movements and inactivity (inactivity/small) as described earlier.

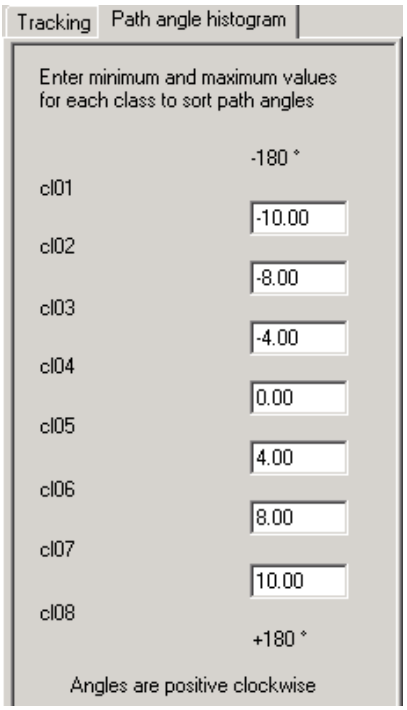
- Any movement less than the "small/large" threshold is viewed in green.

- Except if this movement is less than the inactivity/small threshold, in which case it is viewed in white.

As a rule, this threshold is set fairly low and should correspond to periods of sheer inactivity (sleep, attentive waking).
However, these two thresholds can be used for convenience, for instance to distinguish :

- Inactivity or small/large/very large movements
- Inactivity/very small/moderate or large movements

4.1.5 Rotation analysis



4.1.5.1 - principles and algorithms

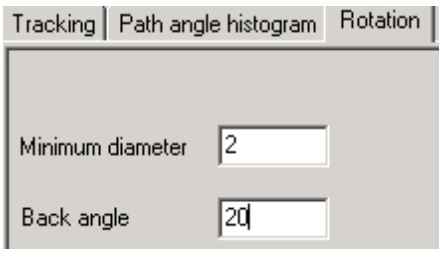
All calculations for rotation and angles histogram are base on the angle measured on the path of the animal. Each time we have 3 successive centres of gravity we can calculate the angle. When the animal is going straight forward, the angle is 0; if the animal is going slightly to the right the angle is positive; if the animal is going slightly to the left the angle is negative; if the animal is going backward the angle is -180 or +180.

To generate the histogram, we sort the value of the angle depending on the limits defined by the user. For the rotation analysis, we make the sum of the angle. When it reaches +360 it means the animal has made a complete clockwise rotation while -360° indicates a counter clockwise rotation.

4.1.5.2 - Rotation parameters

For the rotation, two parameters have to be set:

- the minimum diameter which makes it possible to disregard rotations below a given diameter,
- the back angle: this feature is designed to keep some “memory” of what the animal has done. If the animal has started to rotate in a given way and turn back to rotate the other way, the algorithm will keep the memory of the previous rotation angle until the animal has reached the value of the back angle.



4.1.5.3 - Histogram parameters

The user has the ability to define the limits of 8 classes to sort out the path angles.

In the example here below, the first class (cl01) will be for angles between -180° to -10°, second class (cl02) for angles between -10° to -8°, etc.

Each time an angle is calculated, it is compared with the values of the range of each class and 1 is added to the appropriate class (the one that contains the angle value).
Thus, at the end of the experiment, the user will get the number of times the angles has been within each class.

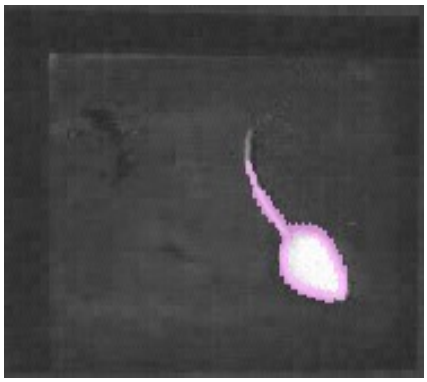
4.1.6 Nose detection

4.1.6.1 - Principles and algorithms

Nose detection is shape-based analysis so a very good lighting is required. Detection threshold has to be set to see correctly the shape of the animal including the tail. To ease this operation, we strongly recommend to use our infrared floor and/or background subtraction feature.

Settings are the same than the ones for the tracking algorithm.

4.1.6.2 - Set-up detection threshold



As mentioned before, shape of the animal must be accurately detected. When adjusting the detection threshold, the display shows the outline of the animal as shown here below.

If the animal is equipped for microdialysis or electrophysiology, the canula or wires are likely to mask part of the animal and generate artefacts with the animal shape detection.

4.1.7 Group definition / Copy to Group

For a faster definition of the parameters, it is possible to define a group of locations and to copy values from a given location to all the locations included in this group.

To define a group, go to tile window display.

Each location can be selected by clicking once on it and to add a selected location, just keep Ctrl key pressed while clicking. Each selected location is then displayed with a green title bar.
To select several locations at the same time, it is also possble to draw a rectangle with the mouse. Each window partially in that rectangle will be selected. Pressing Ctrl while drawing the rectangle makes it possible to add several locations to an existing group.

To cancel the group definition simply click outside of all window location.

By clicking on the button “Apply to Group” available in different control panels, the associated value(s) are copied to all the locations selected for the group.
Values that can be copied this way are:

- detection threshold
- sensitivity threshold,
- scale,
- movement thresholds,
- quantization thresholds,

4.1.8 Save experiment

Once all values have been defined, the complete set up of the experiment can be stored in a file with the extension VTE (ZebraLab Experiment). You can use Save or Save As from File menu.

Experiment can be recalled using Open and all parameters are restored to allow the execution of the experiment.

4.1.9 Export experiment parameters

To have access to the parameters that define the experiment, the user is able to export the .VTE file and get data to import into a spreadsheet for example. Each location used in the protocol is exported with the associated parameters.

An example is given below

location	expdur	period	ldetthr	hdetthr	anicolor	inasmlthr	smllarthr	scalex	scaley	horfield	verfield
cage 1	00 / 00:05:00	30	128	128	White	8.333	7.700	0.111	0.130	85.333	63.268
cage 2	00 / 00:05:00	30	37	37	Black	2.432	3.300	0.048	0.057	37.352	27.693

For the meaning of each column see Appendix Result Files Caption.

4.2 ASSIGN ANIMALS TO LOCATIONS

The experiment contains all the data to run the test but it can be of use to add parameters defining the animals.

This is done by Parameters/Animals where you can import the file named ANIMALS.TXT of the current directory (the one in which the current vte file is stored).

4.2.1 Animal list file Format

The format of this file is as follows:
First line is a header line the 2 first items MUST be Location and Animal

Location value has to be the same of one of the location used for the .vte file
Animal value will usually represent the Identifier of the animal (animal number)
Param1, Param2 have to be replaced by the meaning of the column (i.e. SEX, WEIGHT, DOSE ….)

Location <TAB> Animal<TAB>Param1<TAB>Param2….
CAGE1<TAB>1<TAB>val1_1<TAB>value1_2<TAB>…
CAGE2<TAB>2<TAB>val2_1<TAB>value2_2<TAB>…

For example 2 animals in 2 different cages

Location <TAB> Animal<TAB>SEX<TAB>DOSE….
CAGE1<TAB>1<TAB>M<TAB>0.2<TAB>…
CAGE2<TAB>2<TAB>F<TAB>0.1<TAB>…

Or 4 animals in 1 cage

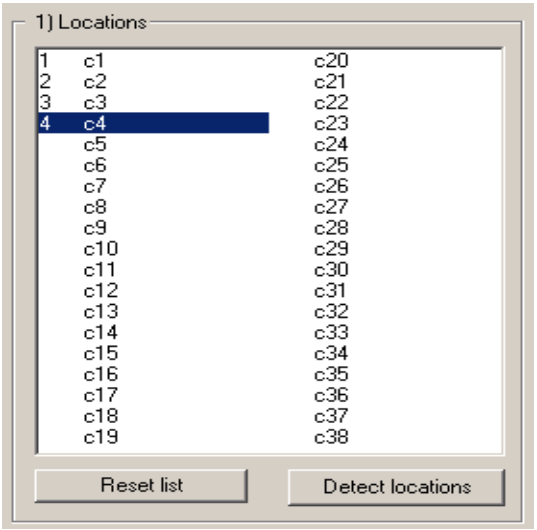
Location <TAB> Animal<TAB>SEX<TAB>DOSE….
CAGE1<TAB>1<TAB>M<TAB>0.2<TAB>…
CAGE1<TAB>2<TAB>F<TAB>0.1<TAB>…
CAGE1<TAB>3<TAB>M<TAB>0.15<TAB>…
CAGE1<TAB>4<TAB>F<TAB>0-<TAB>…

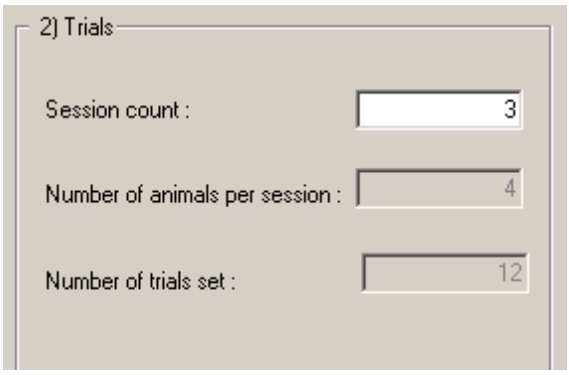
<TAB> means that each field is TAB separated or that you save your file as a Text Tab separated file using Excel for example.

4.2.2 Animal list creation wizard

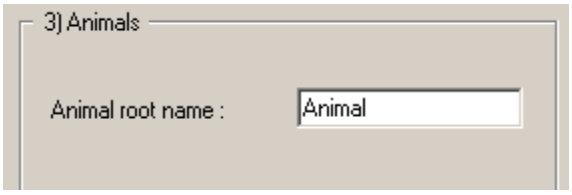
To ease the creation of the animal list file a wizard is available in the ZebraLab software. Go to Edit/Animal list creation wizard. You will need to select the list of locations that are used in the experiment you are creating the animal file for.

The “detect locations” button will analyze the protocol (.vte file) currently loaded to define automatically the locations used.



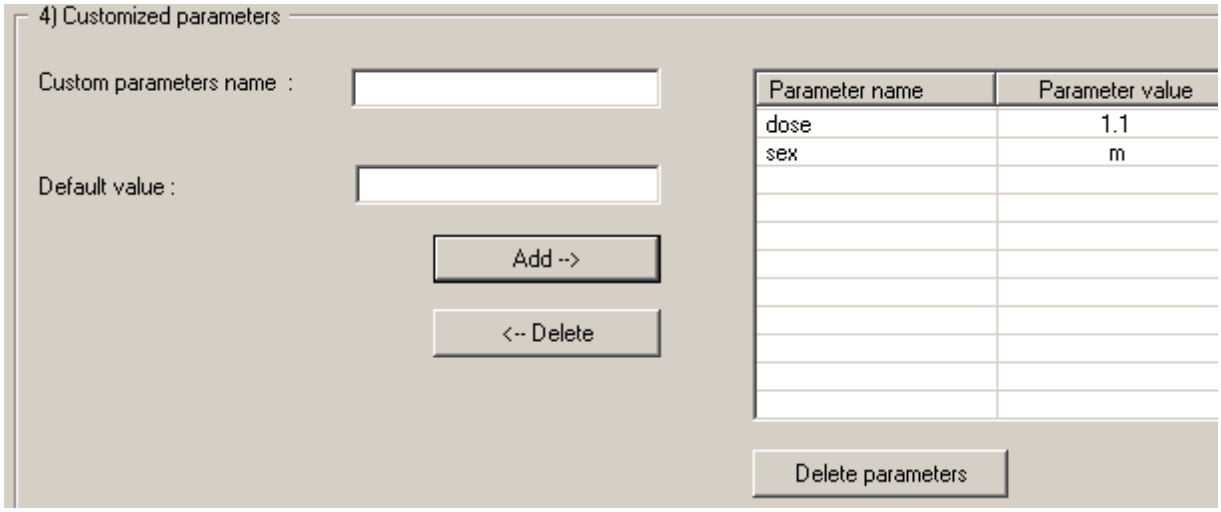


The number of animals per session is automatically updated. For example, an experiment with 4 locations will display 4 animals per session. If your experiment needs 12 animals then 3 sessions will be necessary to complete it. Just enter “3” in the session number field.



The animal root name will define the name of the animals listed in the file to which will be appended a number

The user can then define the list of parameters and their default value in the custom parameters section. The parameter name will be used as a header in the results file to identify the column containing that parameter. The default value is set for all the animals in the list.



Once the list of all parameters has been defined, click “Create List” and give a name to the file to create.

In the example above, the file created will be

Location	Animal	dose	sex
c1	Animal01	1.1	m
c2	Animal02	1.1	m
c3	Animal03	1.1	m
c4	Animal04	1.1	m
c1	Animal05	1.1	m
c2	Animal06	1.1	m
c3	Animal07	1.1	m
c4	Animal08	1.1	m
c1	Animal09	1.1	m
c2	Animal10	1.1	m
c3	Animal11	1.1	m
c4	Animal12	1.1	m

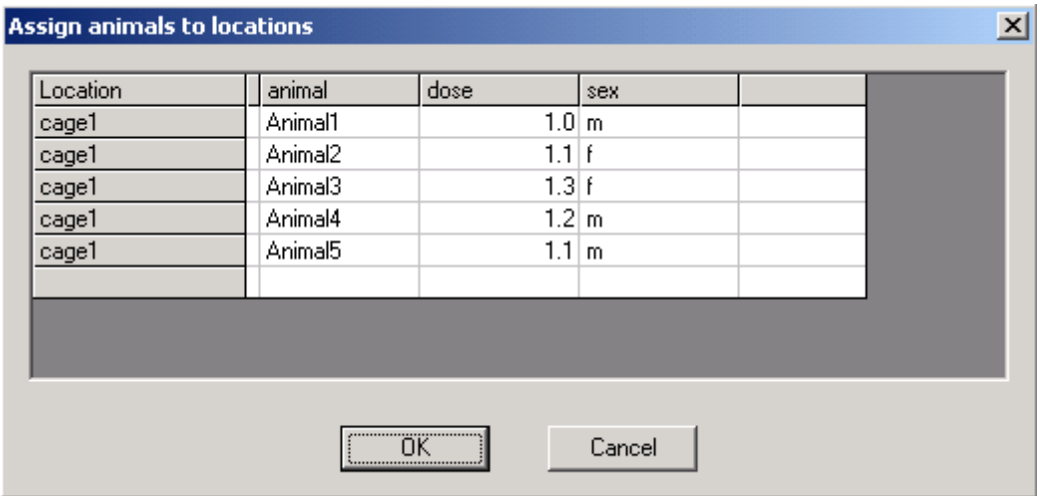
It is possible to edit this file with a spreadsheet to modify the value for each animal.

4.2.3 Import Animals' file (Parameters/Animals)

Once the list has been created in can be imported before running the experiment to define the animals that will be used for the experiment.

To do that go to the Parameters/Import Animals List menu then select the appropriate file containing data for the animal's you want to use in the experiment and click OK..

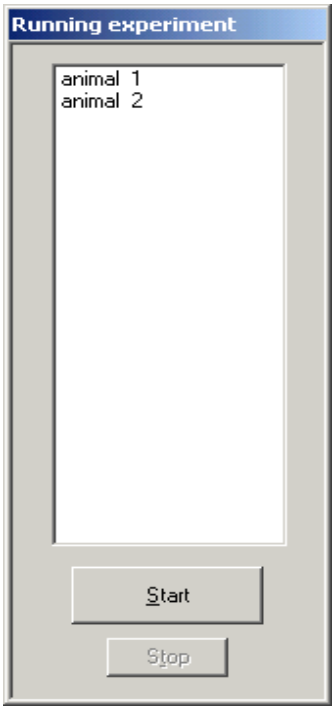
The following window is then displayed for you to check the parameters that will be used. If the file is not the good one, you can redo the same operation to select a new file.



4.3 LAUNCHING AN EXPERIMENT

First you have to load the protocol describing the parameters for the experiment using File/Open. Choose the one you want to use among the different protocols you have defined.

Then you may choose Experiment/Execute. ZebraLab asks for a result file name



The acquisition is used to produce results. These results are calculated on-line, and go directly into a results file with the extension .VTR (ZebraLab Results).

In its standard version, the software does not control how much room is available on your hard disk to store results files. You should make your own estimate (using Disk Manager or Explorer) prior to acquisition.

For your information, approximately 100 bytes are required per animal, per area and per "period" of the temporal protocol.

At the same time ZebraLab stores a raw data file (extension .RAW) which can be of some advantage, during an adjustment stage, to replay acquisition with new movement thresholds or new experiment parameters for example.

The raw data storage facility allows the information (co-ordinates of the centre of gravity) calculated by ZebraLab to be recorded. By raw data replay, various movement threshold values can be tested on this set of data.

The animal (name or identifier as defined in the corresponding column of the animals.txt file) is displayed in the current animal window. Then you can place this animal in the cage location. A click on the start button will start the experiment. You can either wait for the normal end of the experiment (Duration defined in the Experiment Duration) or stop the acquisition with the Stop button. The next animal if any is then displayed and may be placed in the cage. The Start button will give you the possibility to analyse this animal.

While using the remote control, green button has the same effect than “Start” and red button has the same effect than “Stop”.

When more than one location is used, a list of the animals which are in different locations will be displayed in the window.

Also, it is available on the screen a large digit timer which displays the elapsed time of the experiment. This time is displayed in red when the experiment is running (the user is supposed not to enter the field of view), and in green when the experiment has stopped.

4.4 MANAGING RESULTS OF THE EXPERIMENT

4.4.1 Export results

Use the Result/Export to convert the result files from the encoded format .VTR in their sequence of acquisition in the form of text files (or ASCII files) which can be used by various software packages, such as spreadsheets for instance, to conduct further statistical analyses on these results. These text files are stored on hard disk. Again it is for the user to check how much room he has available for them. The user should also make sure these files are properly duplicated onto other archive media (tape-streamers, diskettes, CD-ROM etc…).

The files created are given the extension ".XLS" (can be changed in Options/customize/Export Set-up).

For a complete description of the exported columns, see Appendix "results file caption"

4.4.2 Export and merge results

Use the Result/Export and Merge to convert the result files from the encoded format .VTR in their sequence of acquisition in the form of text files (or ASCII files) which can be used by various software packages, such as spreadsheets for instance, to conduct further statistical analyses on these results.
With the merge capability, the user is able to select multiple files and the output will be a single file to containing data from all the .VTR files selected. An additional column "vtr" makes it possible to identify the original .vtr where the data are coming from.

The files created are given the extension ".XLS" (can be changed in Options/customize/Export Set-up). The exported file will be given the name of the first selected file.

For a complete description of the exported columns, see Appendix "results file caption"

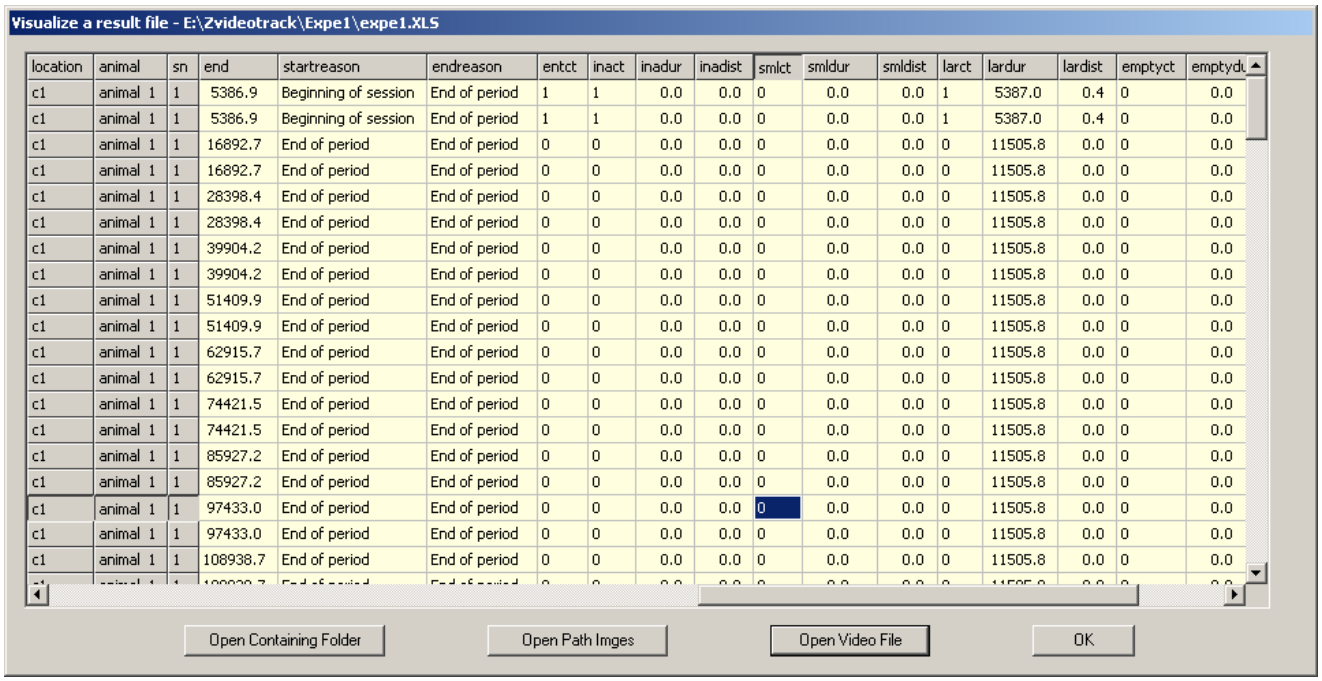
4.4.3 Display exported files

To be able to check quickly what has been exported, go to Result/Display an exported file

You can select the file (default settings only list files with .xls or .csv extension) you want to view and display it in a read only spreadsheet.

From this spreadsheet it is also possible to access additional informations regarding the experiment:
- open the list of path images stored at the end of each session,
- open video files of the experiment if Numeriscope option was used during the actual experiment,
- access the containing folder where e results were stored,

To do that click on the appropriate button.



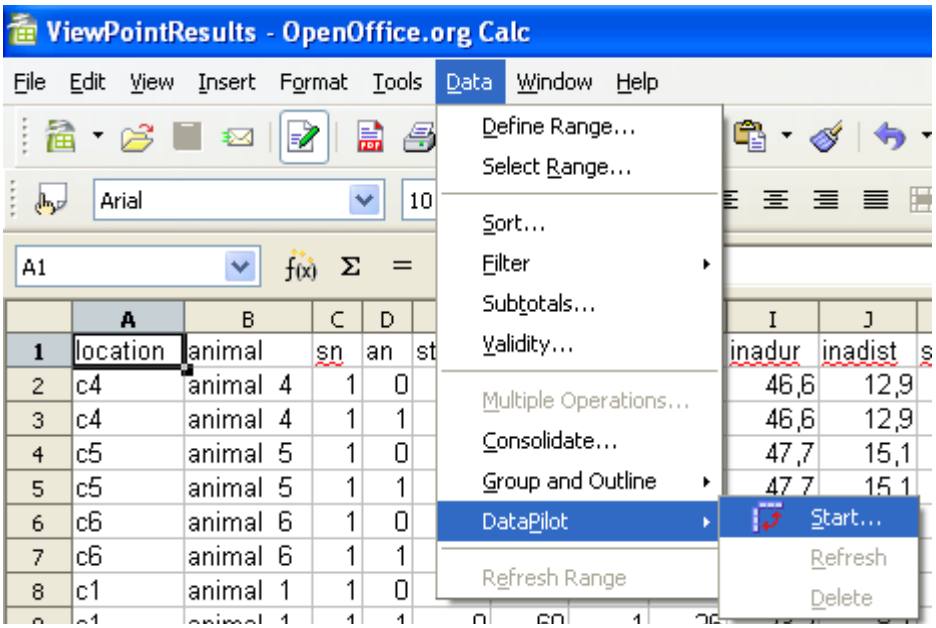
4.4.4 Results Layout for statistical softwares / *pivot table*

Results may be transposed and modified to be compatible with certain layout statistical softwares .

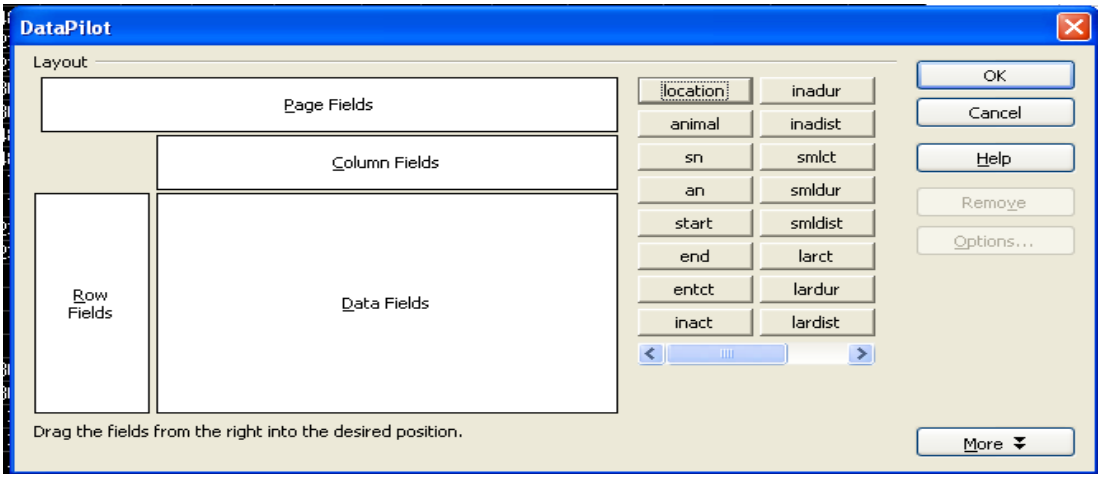
Standard spreadsheets such as Excel and OpenOffice, offer the *pivot table* option to transpose ZebraLab results.

This short tutorial will help you through OpenOffice procedure. The Excel process varies slightly.

Open your ZebraLab result file. Then launch “DataPilot” in OpenOffice or “PivotTable wizard” in excel available in the Data menu.

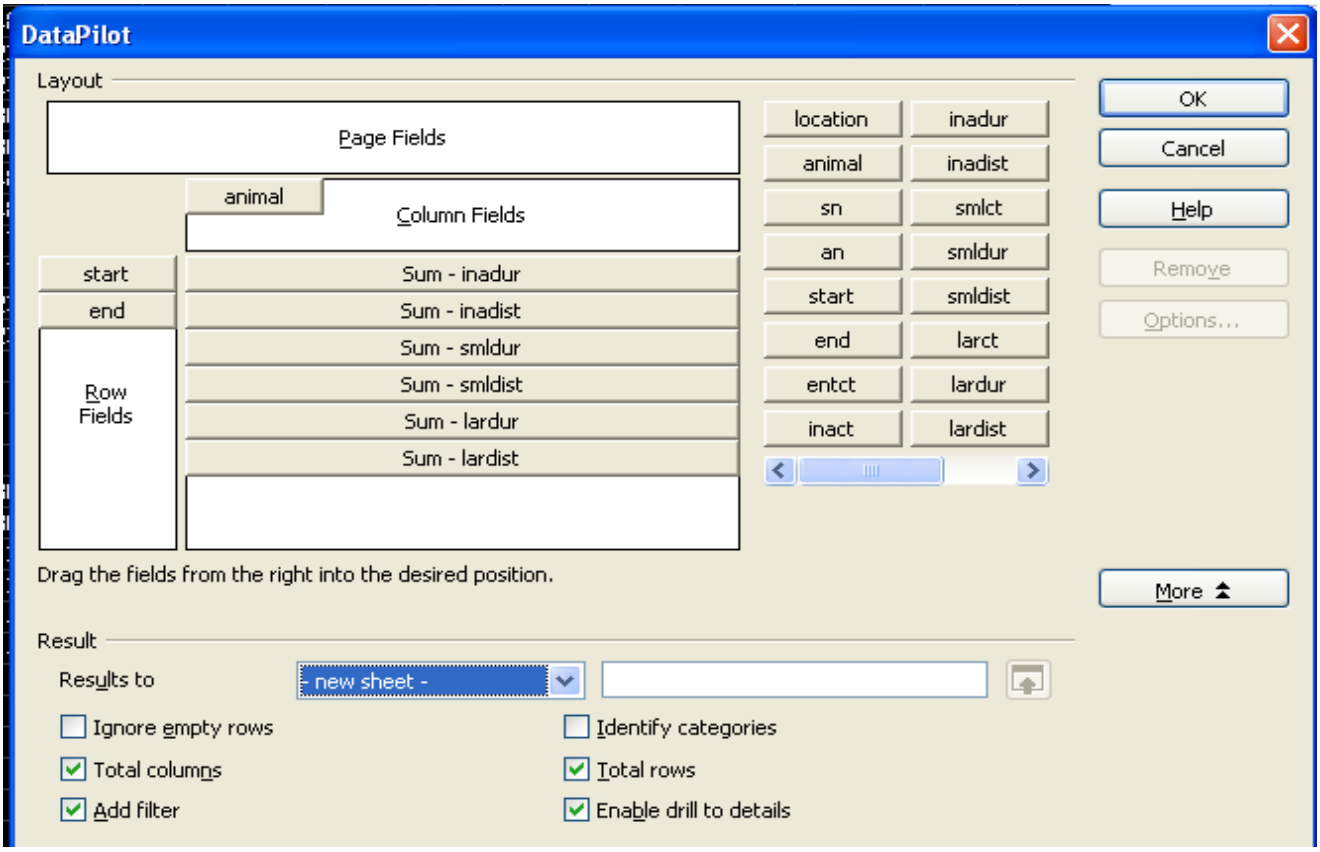


This window will pop-up:



Drag and drop the informations you need from the data on the right to the lay out on the left.

Select “New Sheet” option in the “More” table at the bottom of the screen.



The results will appear in a new spread sheet like this :

	A	B	C	D	E	F	G
1	Filter						
2							
3				animal			
4	start	end	Data	animal 1	animal 2	animal 3	animal 4
5	0	60	Sum - inadur	46,4	73,6	96,4	93,2
6			Sum - inadist	16,2	94,2	15	25,8
7			Sum - smldur	35,2	19,6	9	5,8
8			Sum - smldis	409	165	107,2	138,8
9			Sum - lardur	8,2	0,8	1,6	3,6
10			Sum - lardist	103,2	15,8	66	82,2
11	60	120	Sum - inadur	3	73,4	27,4	35,8
12			Sum - inadist	3	111,2	69,6	129
13			Sum - smldur	55,2	28,6	0,4	37,8
14			Sum - smldis	678	358,6	6	415,6
15			Sum - lardur	2,4	17,4	0	3,2
16			Sum - lardist	129,2	308,8	5	236

4.5 VIRTUAL EXPERIMENT AND RAW DATA

Raw data are stored during the experiment and contains informations about time and results of image processing. For example with a tracking version, x,y co-ordinates of the animal(s) are stored for each image analysed.

4.5.1 Export Rawdata

For advanced users, the possibility to export raw data is a way to develop customized algorithms for special analysis. In such a case the output file will include the data calculated from each acquired frames.

For example with the tracking version, the output will be as follows

abstime	time	location	type	data1	data2
7880.00	0.00	c1	71		
7880.03	0.03	c1	101	225	452
7880.06	0.06	c1	101	229	462
...					

For the meaning of these columns refer to the Appendix “results file caption”

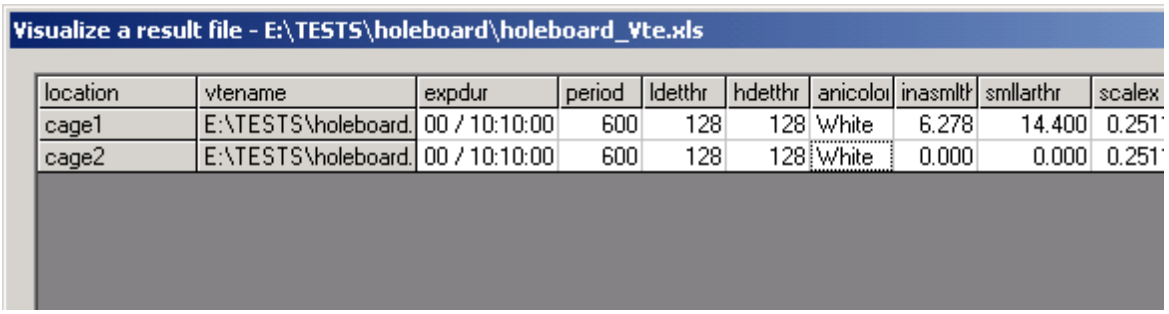
4.5.2 Wintrack export

We have included in the software the possibility to export the rawdata in a file that can be processed by wintrack software.

Go to the Rawdata/Wintrack Export and select the raw data file you need to export. And click OK. It will generate a text file (.txt) in the same folder than the raw data file with a name as follows

CCCC_NNN_RRR_raw.txt where

CCCC is the name of the location(s) that were used in the experiment to generate the raw data (one file per location,
NNN is an index if the file is too big
RRR is the name of the raw data file exported.



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4.5.3 Replay rawdata

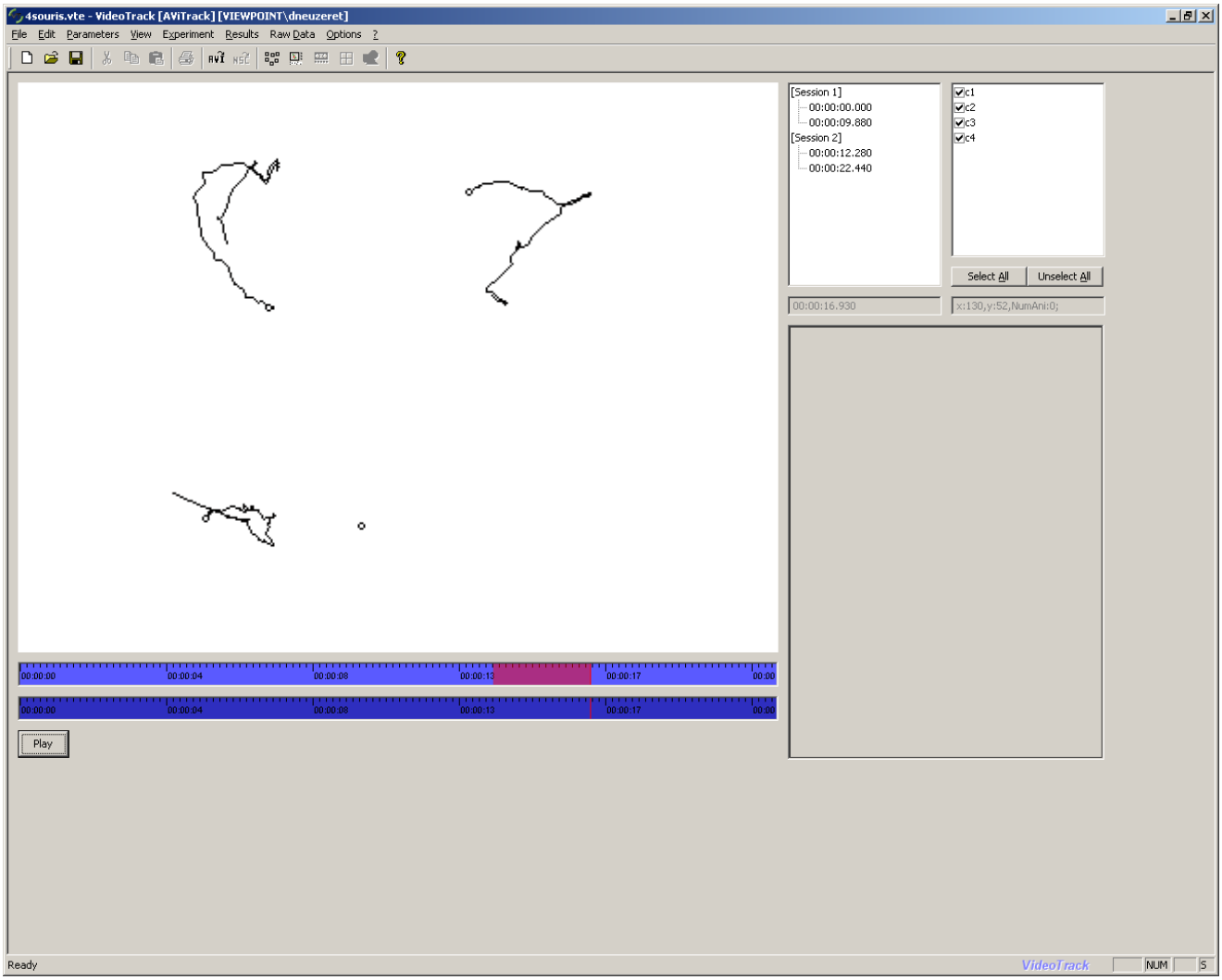
Replay raw data is a way to generate new results for an experiment without the need to use new animals.
To replay the raw data proceed as follows:

- open the protocol file (.vte) originally used to run the experiment,
- modify the parameters. This can be
  - movement thresholds,
  - positions, shapes of the areas,
  - add/remove areas,
  - change duration (decrease only),
  - change period,
- modify the parameters. This can be
- save the protocol with a new name,
- go to the RawData/Replay Raw Data menu
- select the file you want to re-analyse
- give the name for the new result file (default value is PreviousName_Rep.xls
- click on save button
- then start Replay
- At this time the replay is done at full speed so for short experiments, you might not have the time to see what is displayed
- Then go to Results/Export and select the result file that has just been generated to convert it into an ascii file
- You are now able to display the result file in Result/Display or to import it into a spreadsheet or statistical software,

4.5.4 Play rawdata file

To have a look at raw data file, you can use the Raw Data/Player menu.
Select the file you wish to play.

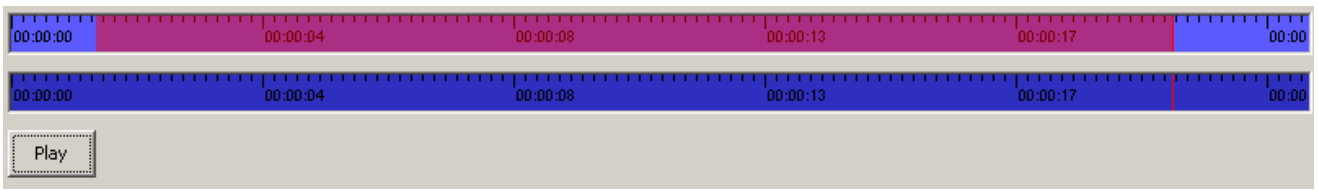
Then you will have the following screen with different parts:
On top left of the window you have the stands the trajectory display where you will be able to see the path of the animal(s).
On the top right stands the information area where you will know how many session are stored in the raw data file and what are the locations



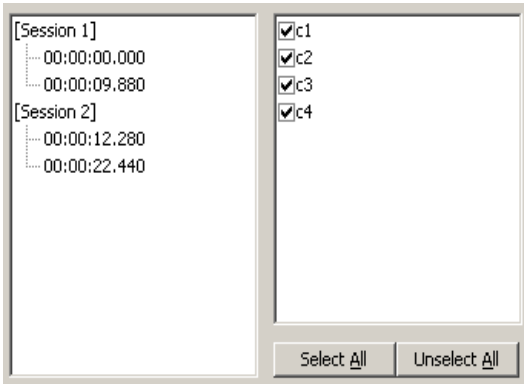
- On top left of the window you have the stands the trajectory display where you will be able to see the path of the animal(s).

- On the top right stands the information area where you will know how many session are stored in the raw data file and what are the locations

- On the bottom left you have the control area that will be used to move in the raw data to display a particular period of it.



- On the bottom right, you have the display for raw data coming from movement quantization version



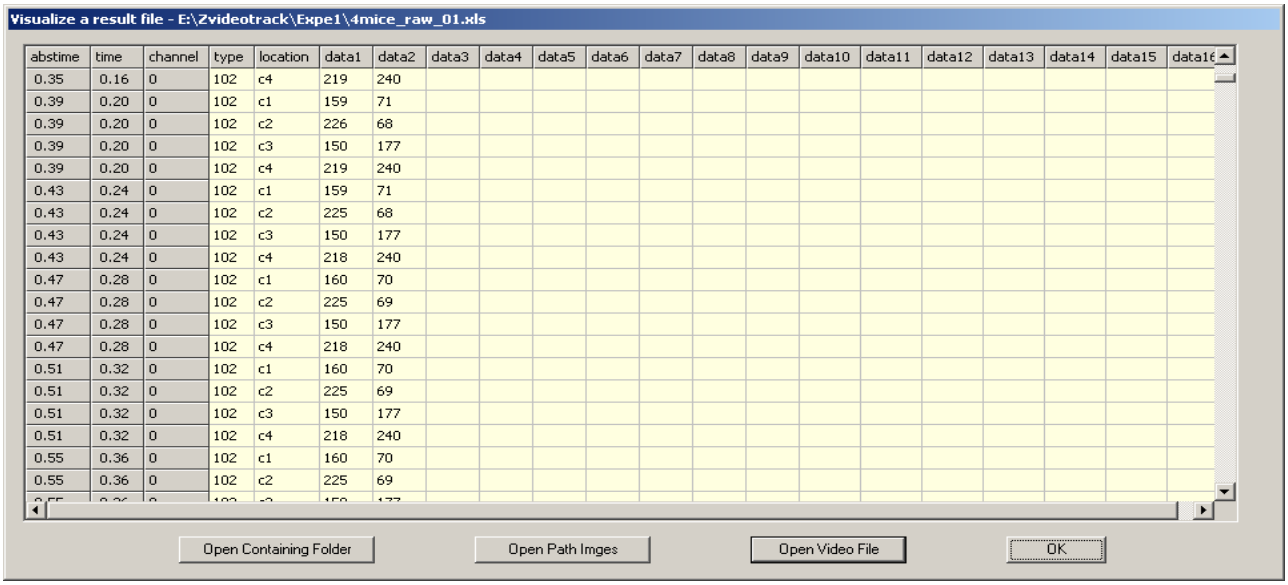
First, you need to select the locations you want to display by checking their names from the list in the information area.
Then click play to have the display of the path on the screen. The pink area on the above time line represents the time during which the path is currently displayed.
It is possible to change the duration of this period by putting the cursor at the beginning of the pink area and click and keep press the mouse button to move the pink area.
Using the mouse click at the same time your holding Ctrl or Shift keys has additional functions:

- with Ctrl
  - a click in the time line, the display is instantaneously set to the time selected
  - a click and hold with a move scrolls the time,
- with Shift
  - it is possible to zoom in zoom out in the time to be more accurate in the display or to see a larger period of time.

The dark blue period on the bottom time line represents the total range of time in the top time line.

4.5.5 Display rawdata file

The display of the raw data makes it possible to see what is in a raw data filer that has been exported. It is necessary to first use Raw Data/Export and then go to Raw Data/ Display and select the file you want to display.



abstime	time	channel	type	location	data1	data2	data3	data4	data5	data6	data7	data8	data9	data10	data11	data12	data13	data14	data15	data16
0.35	0.16	0	102	c4	219	240														
0.39	0.20	0	102	c1	159	71														
0.39	0.20	0	102	c2	226	68														
0.39	0.20	0	102	c3	150	177														
0.39	0.20	0	102	c4	219	240														
0.43	0.24	0	102	c1	159	71														
0.43	0.24	0	102	c2	225	68														
0.43	0.24	0	102	c3	150	177														
0.43	0.24	0	102	c4	218	240														
0.47	0.28	0	102	c1	160	70														
0.47	0.28	0	102	c2	225	69														
0.47	0.28	0	102	c3	150	177														
0.47	0.28	0	102	c4	218	240														
0.51	0.32	0	102	c1	160	70														
0.51	0.32	0	102	c2	225	69														
0.51	0.32	0	102	c3	150	177														
0.51	0.32	0	102	c4	218	240														
0.55	0.36	0	102	c1	160	70														
0.55	0.36	0	102	c2	225	69														

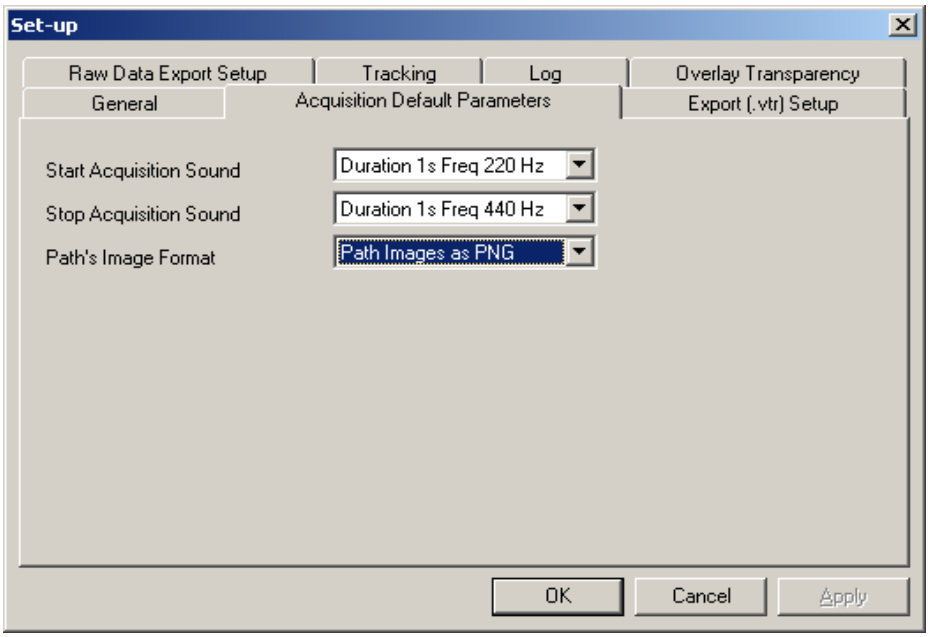
For the meaning of each columns, please refer to the Appendix “RESULTS FILE CAPTION”

5 ZebraLab'S PREFERENCES

5.1 Acquisition Default Parameters

The user can define Acquisition Default Parameters as well.

Go to Options/Customise menu and select Acquisition Default Parameters.



The following parameters can be set:

* Start Acquisition Sound: each time the acquisition on a given location is started a sound with the characteristics defined in this filed is played.
The choices are:

No sound
Duration 1s Frequency 440 Hz
Duration 1s Frequency 220 Hz
Duration 1s Frequency 110 Hz
Duration 1s Frequency 47 Hz

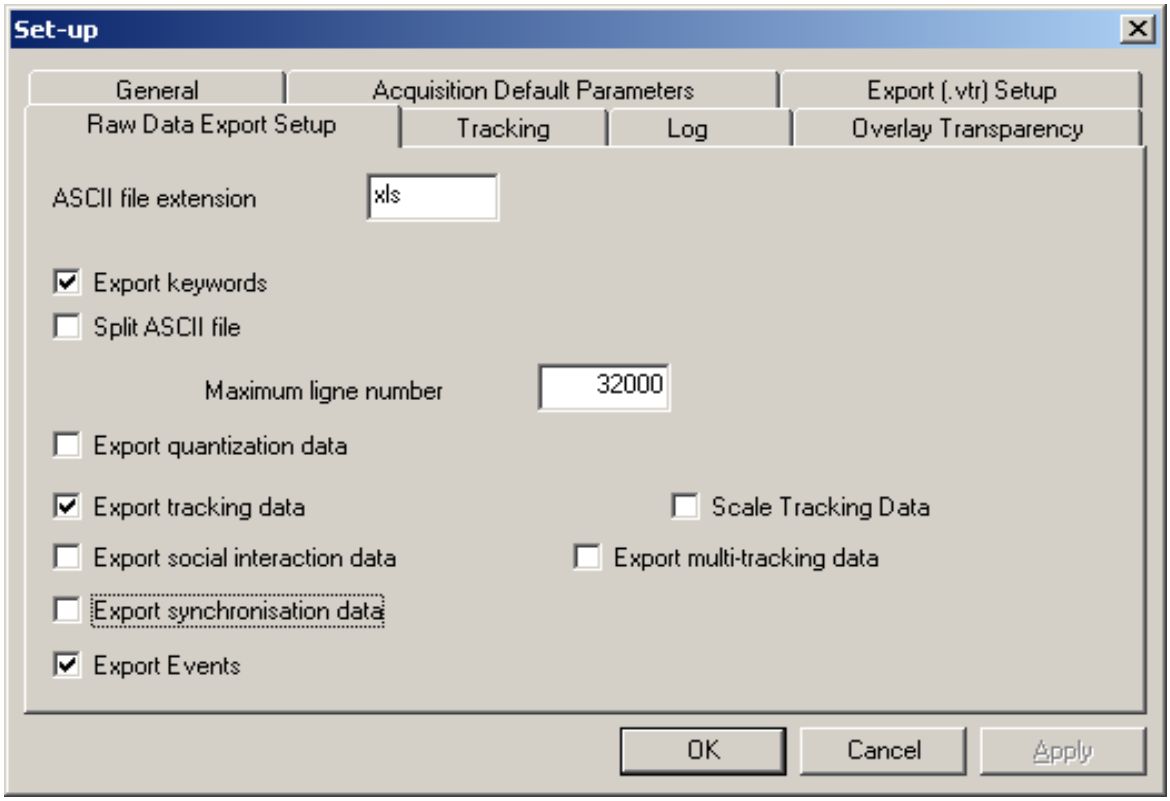
* Stop Acquisition Sound: same but for the end of the acquisition at a given location and for a given animal.

Path's Image Format
While running the experiment, the user will generate an image of the animal's path just after the acquisition of a given animal has been stopped. Three choices for the output formats are available:

No Images
Bitmap file (about 800kBytes per image),
PNG file (compressed file about 20kBytes),
JPEG, GIF and TIFF are also available

5.2 Raw Data Export parameters

With this option it is possible to adjust the settings for raw data export.



Save raw data during Experiment: If not checked not raw data will be written during experiment and it will not be possible to export the curve of activity as a text file.

File extension: when export is performed, the extension defined in this field is added to the name of the file.

Export header line: if checked, the first line of the exported file includes the header so as to define the meaning of each column.

Split raw data file when max line number is reached / Max line number: if checked, the exported file is split when the max line number is reached. Indeed, raw data file can be very large and for example Excel is not able to accept more than 65000 line per sheet.

Split raw data by period: if checked, the exported file is split when the duration of the data period in the file exceeds the value specified in the Period field.

Export Raw data (quantization): if checked, the exported file will contain the quantization data.

Export Raw data (trajectory): if checked, the exported file will contain the X,Y of the path of the animal if available data.

Export synchronization data: if checked, the exported file will contains synchronization data (internally used by Vigie Primates).

Export Events: if checked, events data (external data input to the Vigie Primates) are exported.

6 ACTIVATING OPTIONS

6.1 Stop On Area

Principle

This possibility has been designed to ease tests in which particular position of the animal in the cage leads to stop the session.
For example, in watermaze test, it is not necessary to record the activity of the animal when it reaches the platform.

As soon as the animal enters the defined area, the experiment will stop automatically.

The user will need to add one column named "stoparea" in which will be defined the area number that will stop the experiment for this animal in this location.

Example:

Location	Animal	stoparea
Cage 1	1	1
Cage 1	2	2
Cage 1	3	3
Cage 1	4	4
Cage 1	5	5
Cage 1	6	6
Cage 1	7	7
Cage 1	8	8
Cage 1	9	9

Animal "1" will stop the experiment as soon as it will enter area number 1, Animal "2" will stop the experiment as soon as it will enter area number 2… .

If you do not wish to have a stop area for a given animal just enter "-1" in the stopa rea column for this animal.
If the stop area column is not implemented, the software will behave normally.

6.2 Relay triggering

Principle:
The user has to define several areas in the location where the animal will be placed. Each time the animal will enter a given area, the associated relay will be switched on. As soon as the animal will exist from this area, the relay will be switched off.

The PIA board used has 16 relays (number 1 to 16). The user will associate a list of relays number that will be triggered by area 1..n.
The first relay of the list (firstrelay) will be triggered by area number 1 of the given location used with a given animal. The second relay of the list (firstrelay+1) will be triggered by area number 2 of the given location used with a given animal…

To define the association between relays and areas, the user has to add 2 columns in the ASCII file used to assign animals to location. The first column is named "firstrelay" which will be triggered by area 1 and the second column is named "lastrelay".

Example:

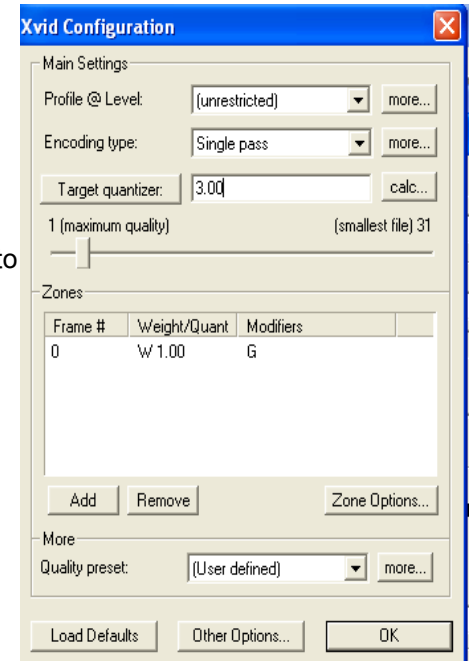
Location	Animal	firstrelay	lastrelay
Cage 1	1	1	4
Cage 1	2	5	8
Cage 1	3	9	12
Cage 1	4	13	16
Cage 1	5	1	16
Cage 1	6	1	16
Cage 1	7	1	16
Cage 1	8	1	16
Cage 1	9	1	16

Animal 1 in location cage 1 will trigger relay 1 with area 1, relay 2 with area 2, relay 3 with area 3, relay 4 with area 4,
Animal 2 in location cage 1 will trigger relay 5 with area 1, relay 6 with area 2, relay 7 with area 3, relay 8 with area 4,

6.3 Labwatcher

This option will let the user quote manually stats and event while an experiment is running. Thus enabling to enrich the automated analysis with your own data. Please refer to the LabWatcher manual for additional informations.

6.4 Numeriscope Digital recorder



This option will record images while the experiment is running.

If the option is activated; at the end of the experiment, in the same directory than the result file will appear 2 types of files related to the images: ".AVI" files in an equal number of sessions executed during the experiment and one ".NSC" file which is used by ZebraLab software to store data about AVIs. The AVIs have the same name than the result file to which is added a "_xxxx" that represent the session number.

To read AVI files on another computer simply double click on it. If the reader is not able to read it, install the XVID codec.

Tunning the video quality

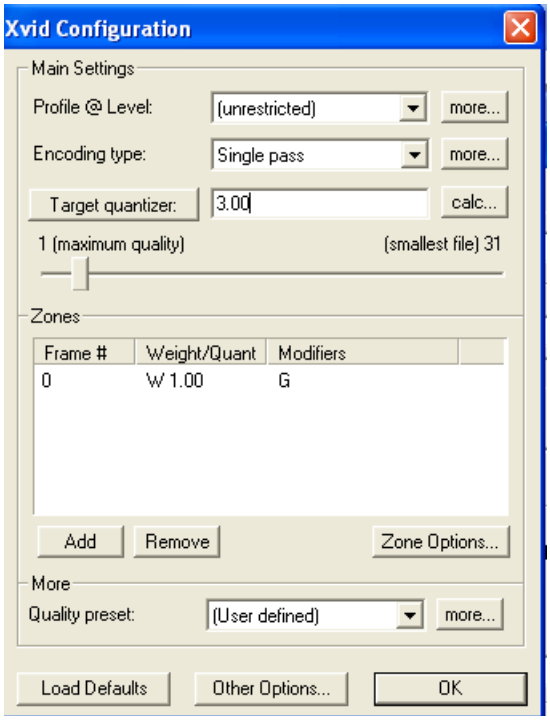
IMPORTANT NOTICE :
Video recording has an important impact on overall performance.
Parameters are set at installation by ViewPoint to match the computer performance.
Miss use of those parameters may results in slow down or crash of the system.
Please contact ViewPoint support team if you have questions concerning those parameters.

Target Quantization is the main parameter you want to modify to change video quality:
Select "Target quantizer" value by going in " All Programs" and select XVID → "Configure Encoder"
For a good video encoding set this value between 2 and 3. For smaller files set it to 3 to 4.

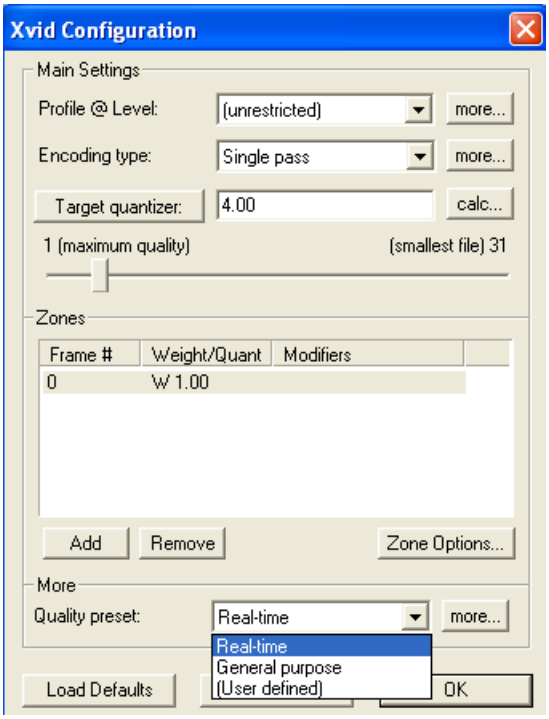
Resetting default parameters is always possible by following the reset procedure. This reset procedure applies for computers after 2000. (Older computer will need to have "Motion search precision" to 1)

To reset the default parameters of the XVID compressor follow those steps.

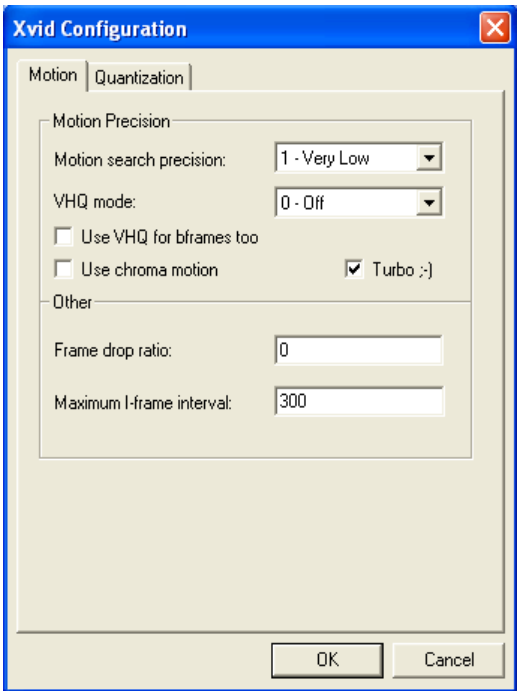
1- Go in " All Programs" and select XVID → "Configure Encoder"



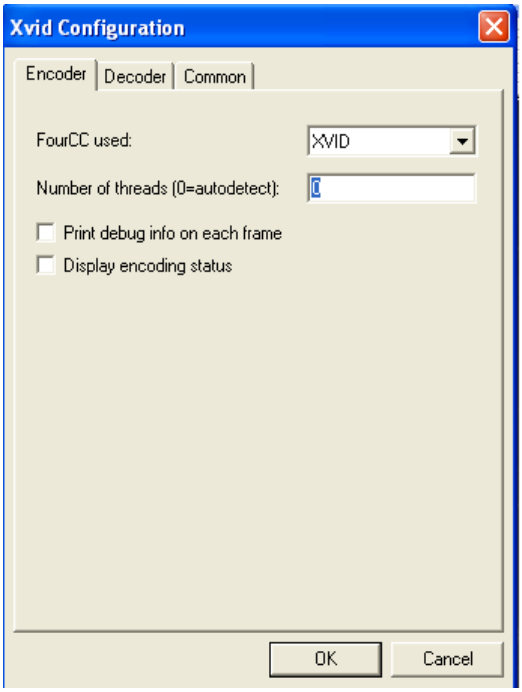
2-Select real time and click more then "Yes"



3- Set "Motion search" to 3-Medium (May be set to 4 if only one camera is connected)
Set "VHQ mode" to 0 (Higher settings will slow down the computer.)



4-Go back to step one and select "Other option" then be sure that
"Display encoding status" is unchecked.



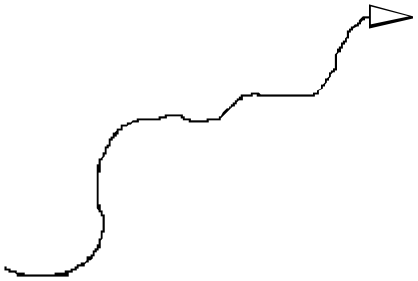
APPENDIX

7 APPENDIX – MOVEMENT ANALYSIS

7.1 DISTINCTION BETWEEN LARGE AND SMALL MOVEMENTS

7.1.1 - Principles

The purpose of differentiating types of movements is to detect the animal's ambulatory movement (i.e. the movements of an animal in a particular direction). The algorithm for large movements detection identifies those movements of the centre of gravity whose long-term resultant exceeds a threshold distance.

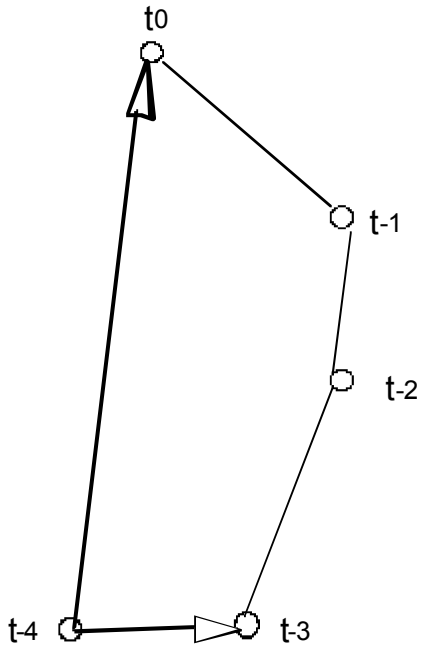


ambulatory movement

non ambulatory movement

The duration of the long-term has been empirically set to five images, or 160 milliseconds ; this period is short enough to take into account any abrupt change in direction of the animal, and is long enough to allow integration of several images.

To determine what is type of the animal's motion at the current time point (t0), the distance is calculated between the animal's positions at t0 t -4 (i.e. five images before).
If this distance exceeds a threshold defined by the user (set a channel calibration), the animal is considered to be in large movement at t0.



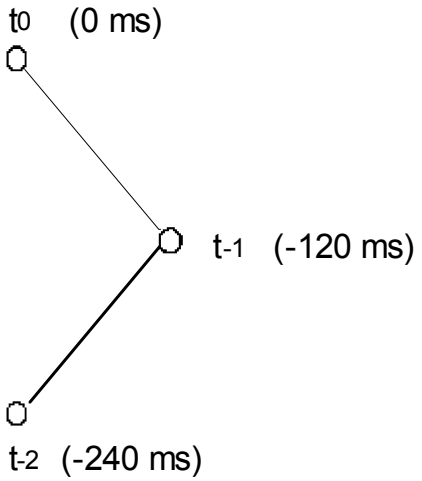
If this distance is less than the threshold, the animal is considered not to be in large movement (the small movement/inactivity separation algorithm is then initiated).

7.1.2 - Notes

Note 1: Detecting a large movement is possible only 160 milliseconds after acquisition has started. Acquisition is locked until the points required by the algorithms have all been calculated. When large movements are used, acquisition thus starts after a 160-millisecond time lapse once the F1 key has been pressed.

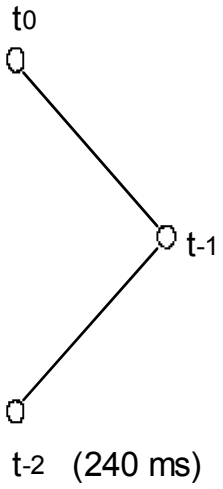
Note 2 : When several cameras are in use, the 160 millisecond time lapse may no longer be observed. For instance, when three cameras are being used, each camera is analysed every 120 milliseconds.

To make sure that the results are consistent between acquisition performed using three cameras, the distance is calculated between the current image and the first image located more than 160 milliseconds before the current image.



The distance between two images is then referred back to 160 ms and is compared to the threshold.

$$d(t_{-2} - t_0) \times \frac{160}{240}$$



This approximation allows similar distance thresholds to be retained between acquisitions performed using different numbers of cameras, but it may alter the animal's movement between two acquisitions. A comparative study of the animal's movement profiles may thus be conducted only in experiments using the same number of cameras.

Note 3 : In the event of an error in the animal's detection (points in excess in the image, exit from the window, loss of inlay), acquisition is stopped, but the time count continues.

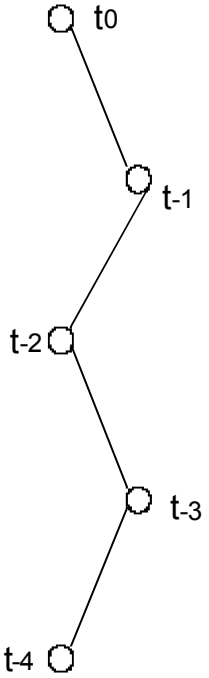
If the error is less than 64 images in length, the algorithm relates the distance covered during the error to 160 ms, and is applied normally (the animal's path is assimilated to a straight line connecting its point of disappearance to its point of reappearance).

If the error is more than 64 images in length, the time count becomes false by default. The animal's speed is then over-estimated, and large movements are more often detected.

7.2 DETECTION SMALL MOVEMENT/INACTIVITY

7.2.1 - Principles

Small motions are detected when the animal's centre of gravity moves over a long enough distance between two images (if the animal is not in large movement).
To correct any artificial movement of the centre of gravity related to variations in surface detection with animals of shaded colours (when using white animals, the progressive darkening of the animal seen from above at flanks level may cause video thresholding imprecision), the detection of small movements is performed over the average distance covered since the last images.



$$\frac{d(t_{-1} - t_0) + d(t_{-2} - t_{-1}) + d(t_{-3} - t_{-2}) + d(t_{-4} - t_{-3})}{4} = Davg_0$$

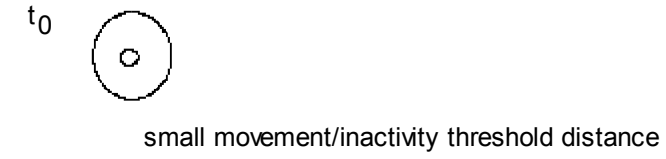
Small movement at t 0 if Davg 0 > threshold

7.2.2 - Notes

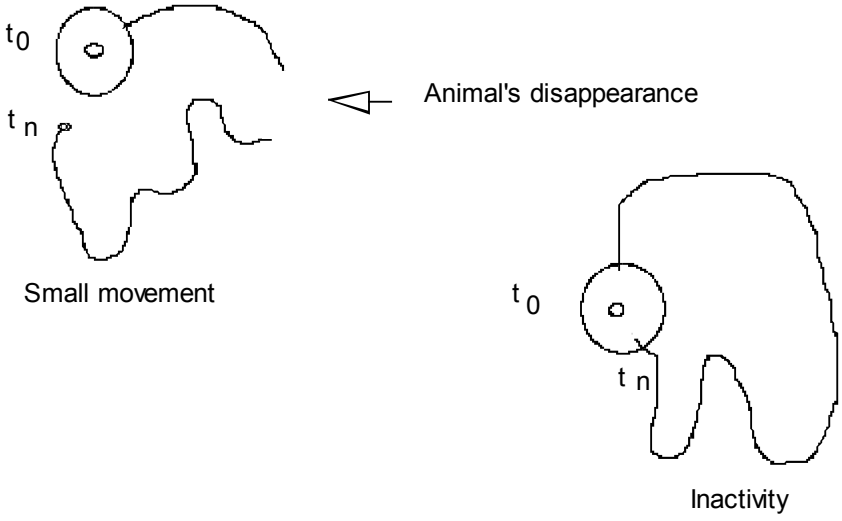
Note 1 : The small movement/inactivity separation algorithm causes acquisition to start 160 milliseconds later (for one camera).

Note 2 : When several cameras are in use, the detection of small movements concerns more than one image. No sampling correction is performed. Therefore, the results obtained with acquisition using four cameras will be quite different from those obtained using a single camera.

Note 3 : In the event of an error, the distance is calculated between the point of disappearance and the point of reappearance of the animal.



small movement/inactivity threshold distance



Note 4 : The separation between small movements and inactivity is based on a movement threshold. By increasing this threshold it is therefore possible to count certain movements (slow movements) as inactivity. The results hence produce the "distance covered in inactivity".

7.3 GENERAL

7.3.1 - Threshold values

To avoid the user having to take into account the number of cameras, all movement thresholds are expressed in cm/s, i.e. they are referred back to the time lapse between two images of the same channel. But the algorithm applies :

- over a distance of 4 images in length for large movements

- over an average distance for small movements.

7.3.2 - Measurement errors

The distances measured are based on a scale defined by the user. Any error in measurement is therefore due to :

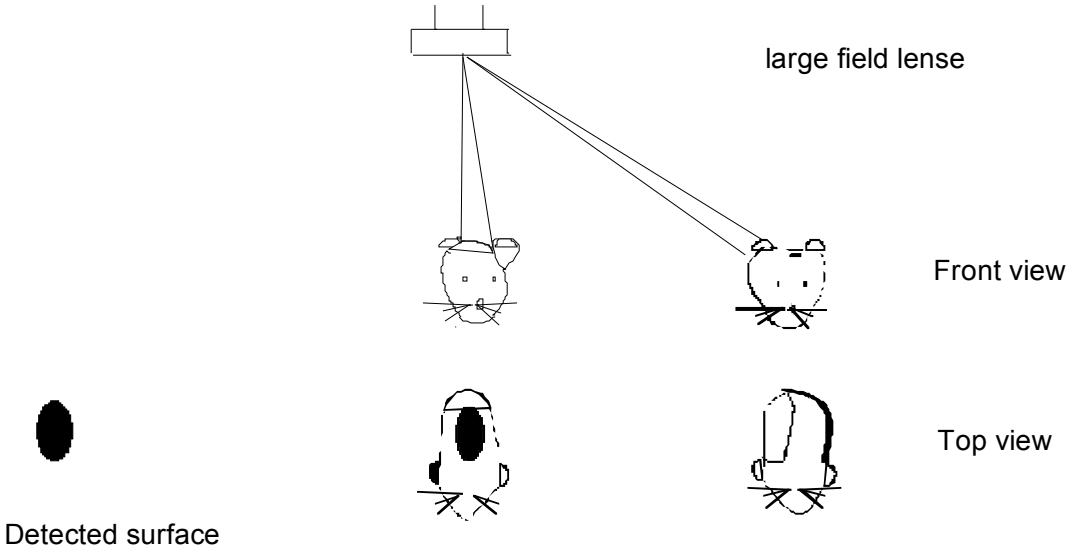
- lighting conditions (most precise measurements are performed on a well-contrasted animal)

- the precision in the measurement of the scale.

7.3.3 - Image distortion

The animal's speeds estimated at the periphery of the camera's field are lower than those measured at the centre because of the distortion of distance which is itself related to the angle of the shooting field. The use of large-angle objectives (<8,5 mm focal distance) is therefore not advised.

This error is even more noticeable since the angle of incidence of the rays at the periphery of the field is larger, and the surface area of the animal "seen" by the camera is not the same.



7.3.4 - Artefacts

While using a video recorder with tapes of bad quality, distance measured may be wrong due to some problems of synchronisation or detection of the animals.
In such a case, it is better to use a Time Base Corrector connected between ZebraLab and video recorder.

8 APPENDIX – RESULTS FILE CAPTION

8.1 Main results

In any result file you will find the following rows :

location:
Arena's identification. Third column is giving the name of the location where the results are coming from.

animal:
This column contains the default name given to the animal (animal 1, animal 2...) or the one that has been set by the user or imported from a list of animals.

sn:
This field is the session number. When a location is used successively to run several animal without importing a list of animals, this field starts at 1 and is incremented each time e new animal is placed in the location and started

start :
This is the starting time for the set of results.

end :
This is the ending time for the set of results.
E.g. if you set a period of 10 s and duration of 30 s, you can check that starting time and ending time are :

0 -10	first period
10-20	second period
20-30	third period

Remark: Depending on the number of cameras and on the frame period these values might not be exactly equal to the calculated ones (e.g. 20,04 s instead of 20 s) which is due to the fact that ZebraLab is not calculating data at this time

an :
This is the area identification as set when drawing the areas. All results on a given line are for the area specified in that column. ZebraLab adds an area “0” which covers all the areas that have been drawn in the location.

stdate:
Date at which the experiment has been started (Optional)

sttime:
Time at which the experiment has been started (Optional)

8.2 Tracking mode

When ZebraLab is set in tracking mode you will find the following rows.

entct :
Entries count : each time the animal enters an area, the entries counter for this area is incremented by one. If a problem with detection occurs (e.g. no animal detected within a window), this counter is not incremented if the animal reappears in the same area.

larct :
Large movement count : each time the animal speed goes above the small/large movement threshold, this counter is incremented.

lardist :
This is the total distance (in cm) covered by the animal in large movements.

lardur :
This is the total duration (in s) spent by the animal in large movements

smlct :
Small movement count : each time the animal goes below the small/large movement threshold or above the inactivity/small movement threshold this counter is incremented.

smldist :
This is the total distance covered by the animal in small movement.

smldur :
This is the total duration spent by the animal in small movement.

inact :
inactivity count : each time that animal goes below the inactivity/small movement threshold this counter is incremented.

inadist :
This is the total distance covered by the animal in inactivity. This distance is available because the inactivity/small threshold is set by the user. If this threshold is too high, the animal can move while being in inactivity. That's why this distance is provided.

inadur :
This is the total duration spent in inactivity.

emptydur:
Time during which no object/animal is detected in the location (no way to calculate center of gravity).

emptyct:
Number of times during which no object/animal is detected in the location.

react :
rearings count : each time the animal starts a rearing this counter is incremented.

readur :
This is the total duration spent in rearings by the animal.

8.3 Rotation and angle results

cl1,cl2,cl3, cl4, cl5, cl6, cl7, cl8:

(only available with rotation option) :
That fields give the number of time the angle on the path of the animal is within the values of the class:

Each column will display the value of number of times the path angle was between the limits defined by the user. See limit0..limit08 for further details.

cw :
(only available with rotation option) :
Number of clockwise rotations

ccw :
(only available with rotation option) :
Number of counter clockwise rotations

8.4 Nose detection results

noscnt: number of time the nose entered the specified area,

nosdur: duration spent by the nose in the specified area,

nosedist:
Absolute distance moved by the nose minus distance moved by the center of gravity

8.5 Social contact

contct: number of time the 2 animals are close to each another (below the defined distance)

contdur: duration of those contacts within the periode

8.6 Animal versus Area

avgdist: average distance between animal and border of the specified area

8.7 Quantization mode

When ZebraLab is set in quantization (eg: porsolt test, freezing behavior...) mode you will find the following rows.

frect (only available with movement quantization software) :
Freezing count : each time the animal is below the freezing threshold this counter is incremented.

fredur (only available with movement quantization software) :
This is the total duration spent in freezing.

midct (only available with movement quantization software) :
medium activity count : each time the animal is below the freezing threshold this counter is incremented.

middur (only available with movement quantization software) :
This is the total duration spent in medium activity.

burct : (only available with movement quantization software) :
Burst count : each time the animal activity is above burst threshold this counter is incremented.

burdur : (only available with movement quantization software) :
This is total duration spent in activity burst.

Actinteg: area under the curve of activity = number of pixels with activity

actc : number of activity periods detected (blue curve)

actdur : total duration of activity periods detected (blue curve)

actlatin : Latency of first occurrence of activity periods

actlatout : Latency of end of the first occurrence of activity periods

zerdur : This is the total duration spent with no activity. (number of pixels with activity =0)

zerct : each time the animal activity comes to 0, this counter is incremented.

8.8 Animal versus Area

avgdist: average distance between animal and border of the specified area

8.9 Additional parameters - Protocol parameters

vtename: the name of the file used to store the protocol,

expdur: duration of the experiment in second as defined in the protocol,

ldetthr: period (time bin) as defined in the protocol,

ldetthr: low value of range detection threshold to make difference between object/animal and background

hdetthr: high value of range detection threshold to make difference between object/animal and background

inasmlthr: Movement threshold to make difference between inactivity and small movements

smllarthr: Movement threshold to make difference between small and large movements

sensthr: Value of the sensitivity threshold

freezthr: Value of the freezing threshold

burstthr: value of the burst activity threshold

limit0..limit08: values of the limits for each class on the path angle. The range from limit0 to limit01 will define the class cl1 in the results.

act: number of areas defined (including area 0)

acogxx: coordinates of center of gravity of area xx

asurfxx: surface of area xx

ashapexx: shape of the area which id is xx

anamexx: name of area xx

scalex: size of one pixel horizontally

scaley: size of one pixel vertically

horfield: horizontal field seen by the camera

verfield: vertical field seen by the camera

unit: unit in which distances are measured (µm, mm, cm, m, km)

starttyp: Detailed the way locations are started when running experiment

comment: Alphanumerical field to enter comment about the protocol (vte)

Raw data parameters:

abtime: Absolute time from entering in Experiment/Execute menu. This value is mainly exported for sorting purposes when several sessions are included in the same file.

Time: relative time from start of session.

Type: This field makes it possible to identify the meaning of data in the following columns.
Type = 71: start of session for the location defined in the location column,
Type = 72: stop of session for the location defined in the location column,
Type = 99: error; data1=1: no animal detected; data1=2: camera in sleep mode;
Type = 101: quantization data; data1=quantity of movement. Number of pixel moving for this frame.
Type = 102: tracking data; data1=X, data2=Y . X and Y are in pixels (0,0) is the top left corner.

Data1,data2, data3...: see above

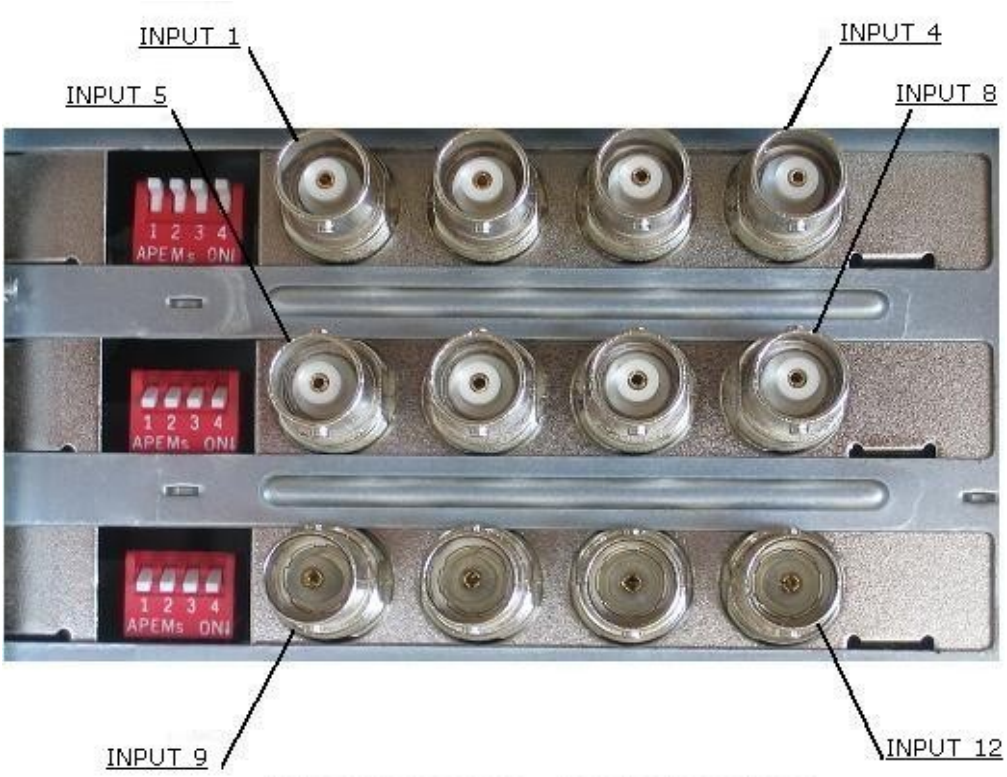
9 APPENDIX – HARDWARE and SOFTWARE INSTALLATION

HARDWARE

We recommend Dell computer model Optiplex 740 with 1GB memory and dual Core processor at 2 GHz.

Graphic board able to work in 32 bits colours an resolution of 1280*1024 is recommended.

Depending on your set-up you can have from 1 to 16 BNC connectors at the back of the system. Connect camera 1 on connector labelled 1, camera 2 to connector labelled 2...



SOFTWARE

To perform software installation, you will need to log on with administrator rights.

Default installation uses Administrator account. If this is not acceptable in your lab, contact your system administrator to create a new user group that has the same rights than normal user and an advanced user right named “increase scheduling priority”.

If such operation fails, you will get an error message while starting ZebraLab stating “No memory rights for the current user”.

From installation CD

Insert the installation CD and follow the instructions on the screen.

10 TROUBLE SHOOTING

Q: I have no video in ZebraLab **Q: The software crashes/ freezes**	

Answer = You may have a hardware failure.

1. Check the power supply is connected to the camera
2. Check the video cable is connected to the camera
3. Check the video cable is going to the correct plug on the video board (Appendix 9)
4. Launch the Viewing software :
   EURESYS Board : Launch Euregrab or Evision Evaluator on the desktop
   Do a new camera.
   Select NSTC or PAL.
   Select VID1.
   Click the running mode for that camera.

IF the message « No signal» appears on the screen.

THEN please check back the camera connection.

IF the cables are good THEN
Shutdown the computer
Open it to access the Euresys video card
Unplug the Euresys card
RE-Plug the Euresys card in its slot

=>WHEN the picture is back :
Close the program and launch back ZebraLab.
=>IF the picture doesn't come back contact your ViewPoint dealer

Q: The picture is totally White / Black / Fuzzy **Q: The detection is bad** **Q: I m loosing the tracking**	

Answer = The picture quality is a very important thing to set before starting using ZebraLab.
You can improve it by two methods :

A.Setting the Camera

1. Launch ZebraLab
2. Select a location
3. Go in tracking mode
4. Set the threshold so you see red spots on the subject
5. Turn the ring of the camera :
   - The Light ring if you have a dark or light picture
   - The focus one if you have a fuzzy picture
6. Get back to item 4 until you have red spots ONLY on the animal

B. Improving the lighting and aparatus

The detection is based on difference between the observed subject and the apparatus.
Therefor :

1. Check you have a white subject on a dark background or a dark subject on a light background
2. Put as much light as possible
3. The light needs to be diffused (no spot light)
4. Avoid reflections (on the maze for example)
5. If you are still experiencing problems you may order an infra red lighting from ViewPoint

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Properties and Acknowledgement

Note on Plublications : Before including informations on ViewPoint's Hardware and Software technology please contact and notify ViewPoint. Certain informations on our technology may be part of patents or ongoing patents an shall note be released.

Trademarks

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Restriction on Hazardous Substances (RoHS) and Waste Electrical and Electronic Equipment (WEEE) Compliance Statement

ViewPoint is committed to the highest quality standards and is dedicated to protecting the environment. We are striving to implement the WEEE/RoHS programs and are working with our suppliers and designing our in-house processes to meet these standards, where applicable.

Restrictions on Hazardous Substances (RoHS) Compliance:
ViewPoint products are classified as laboratory devices and categorized with the WEEE/RoHS directives as "Monitory and Control Equipment" (category 9). At this time, Category 9 products are exempt from the July 1, 2006 effective date. No effective date has been established for when Category 9 products are required to meet the RoHS directive.

Waste Electrical and Electronic Equipment (WEEE) Compliance:
ViewPoint products subject to the WEEE Directive will be marked with the "crossed out wheelie bin" symbol. These products must be properly recycled for recovery and disposal of materials. Contact ViewPoint or ViewPoint's authorized distributor for information regarding where to return these products. Please provide the equipment model identification and serial number for those items being recycled.