

Help (PDF version)

EthoVision® XT

Version 14

Noldus
Information Technology

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Dependent Variables in Detail

Main topics and tasks

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Why use dependent variables?

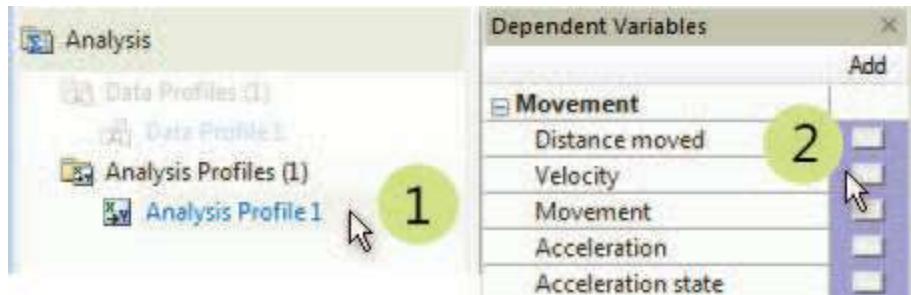
You can use dependent variables:

- In Trial Control. For example to start tracking when a specific behavior or event occurs.
- In the Data profile. For example, to select the intervals when the subject was moving.
- In the Analysis profile. For example, to calculate statistics of distance, velocity or behaviors.

IMPORTANT Some dependent variables are only available if you have one of the Modules installed: Multiple Body Points, Trial and Hardware Control, Social Interaction and Rat/Mouse Behavior Recognition.

TIP If none of the dependent variables cannot help quantify behavior, please contact Noldus, so we can discuss your needs. EthoVision XT contains additional analysis functions which can process the raw data based on JavaScript code.

Movement



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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. It 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.

How to specify Distance moved

1. Click **Add** next to **Distance moved**.
2. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- If your experiment is set to Center-point, nose-point and tail-base detection, click the Body points tab and select the body points for which you want to calculate the distance.
- Because it is based on change in X,Y coordinates, Distance moved needs two valid (non-missing) samples. A missing sample in your track results in missing Distance moved values in that and in the next sample. Make sure that the proportion of missing samples is low (less than 1%).
- Sample rate influences the values of 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 (and total) 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 per-sample (and total) Distance moved. See Track Smoothing for a description of how to filter out small movements.

Applications

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.

Velocity is obtained by dividing Distance moved 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**.
2. Under **Outlier filter**, specify the **Averaging interval**. Leave 1 if you want to keep the raw data; enter a value to smooth the values using a running average method. See Averaging interval
3. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- If your experiment is set to Center-point, nose-point and tail-base detection, click the **Body points** tab and select the body points for which you want to calculate the velocity.
- Sample rate influences the calculation of Distance moved, and therefore Velocity. When tracking at too low a sample rate, parts of the actual path are cut off, resulting in an underestimation of per-sample Velocity. If, on the other hand, the sample rate is too high, EthoVision XT catches all random movements and wobbling of the body point of the walking subject, causing extra apparent displacement, therefore an overestimation of Velocity.

See Track Smoothing for a description of how to filter out small movements.

- Because it is based on change in distance moved, Velocity needs two valid (non-missing) samples. A missing sample in your track results in missing Velocity values in that and in the next sample. 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).
- **TIP** Define Velocity and Movement in the same Analysis profile to find out which averaging interval results in the best match between movement bouts and video.

Averaging interval

Aim

To smooth the values of a dependent variable.

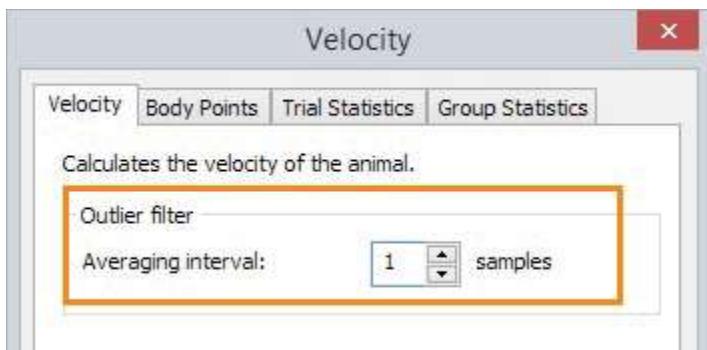
This topic applies to

Dependent variables: Acceleration state, Activity, Activity state, Body angle state, Body elongation, Body elongation state, External data (resampled), External data - state, Mobility, Mobility state, Movement, Velocity.

The dependent variable can be in a condition defined in the Trial Control Settings, in the Analysis profile and in the Data profile. Setting the Averaging interval in one part of EthoVision XT does not influence the value of the same variable in the others.

How to access this option

In the Trial Control Settings, in the Analysis profile or in the Data profile select the dependent variable and locate **Outlier filter**.



How Averaging interval works

- When Averaging interval is 1, the outlier filter is off, thus the values of the variable are not smoothed.
- When Averaging interval is 2 or larger, EthoVision XT replaces the per-sample value of the dependent variable with the average calculated over the number of samples specified by the interval.

The table below shows how EthoVision XT re-calculates a variable V in a few samples when Averaging interval is set to 2. Note how the average (avg) is obtained when values of the dependent variable are missing.

Sample	Data point (●) or missing (○)	Original value	Smoothed value when averaging interval = 2
1	●	V_1	V_1
2	●	V_2	avg (V_1, V_2)
3	●	V_3	avg (V_2, V_3)
4	○	-	avg (V_3 , [no value]) = V_3
5	○	-	avg ([no value], [no value]) = [no value]
6	●	V_6	avg ([no value], V_6) = V_6
7	●	V_7	avg (V_6, V_7)

Notes

- Note the difference between Track Smoothing and the Outlier filter (this topic):

With **Track Smoothing**, you smooth the *raw x,y coordinates*. This has also an effect on the dependent variables calculated based on those coordinates, for example *Distance moved*. See Smooth the Tracks

With **Outlier filter**, you smooth the values of the *dependent variable*, for example velocity or mobility, after they are calculated from the raw data. The Outlier filter is useful when you want to calculate state variables. Apply the Outlier filter, for example, when you want to smooth *Velocity* to calculate the *Movement* states, which are based on velocity; or smooth *Mobility* when you want to calculate *Mobility state*.
- If you combine Track Smoothing with Outlier filter, the dependent variable is calculated with the Outlier filter *after* the raw coordinates are smoothed with Track Smoothing.

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.

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 for more than three samples, the current Movement state ends and the remaining missing samples are ignored.

In the following example, 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'.



How to specify Movement

1. Click the **Add** button next to **Movement**.

2. In 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.

3. Under **Calculate statistics for**, select either one of them, or both.

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.

4. Complete the procedure to add the variable See Calculate statistics: procedure.

Notes

- If your experiment is set to Center-point, nose-point and tail-base detection, click the **Body points** tab and select the body points for which you want to calculate movement.
- 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. See Averaging interval
- Values of velocity between Start velocity and Stop velocity result in no change in the current state of the subject (moving or not moving). 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, *Movement* provides information on the subject's locomotor activity.

Acceleration

Definition

Acceleration at sample n is obtained by dividing the difference in Velocity, by the time difference between that sample and the previous one:

$$A_n = \frac{V_n - V_{n-1}}{t_n - t_{n-1}}$$

where A_n = Acceleration at sample n , and V_n = Velocity at sample n .

How to specify Acceleration

1. Click the **Add** button next to **Acceleration**.
2. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- If your experiment is set to Center-point, nose-point and tail-base detection, click the **Body points** tab and select the body points for which you want to calculate acceleration.
- Because it is based on change in velocity, Acceleration needs three valid consecutive samples. A missing sample in your track results in missing Acceleration values in that and in the next two samples. See also the notes under Velocity.
- Acceleration is sensitive to random changes in velocity between consecutive samples due to noise. Check the track and velocity plot to see if changes in velocity represents true acceleration. If necessary, apply Lowess smoothing to the tracks.

Acceleration state

Definition

A discrete variable, related to one of the body points, with two possible states, *High acceleration* and *Low acceleration*. At any sample time:

- The state is *High acceleration* when the running average acceleration exceeds the High acceleration above threshold.
- The state is *Low acceleration* when the running average acceleration is below the High acceleration above t threshold.

The running average acceleration is calculated according to the formula for Acceleration for each sample, over the number of samples specified by the Averaging interval.

How to specify Acceleration state

1. Click the **Add** button next to **Acceleration state**.
2. In the **Acceleration State** tab, enter the following:

Averaging interval. This is the number of samples over which the running average acceleration is based. The default value is 1, that is, acceleration is not smoothed before calculating the Acceleration state variable.

High acceleration above. The acceleration above which the animal must be considered in the High acceleration state.

3. Under **Calculate statistics for**, select the state you want to analyze, **High acceleration** and/or **Low acceleration**.
4. Under **State duration threshold**, next to **Exclude instances shorter than**, enter the minimum duration of the Acceleration state (see the note below).
5. In the **Body Points** tab (if present), select the body points for which you want to calculate Acceleration state.
6. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

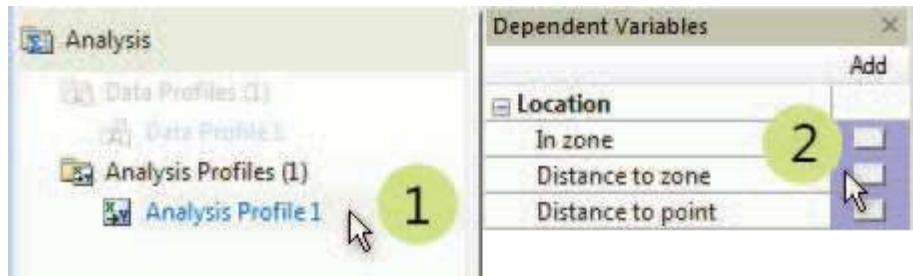
- Enter zero as **High acceleration above** to distinguish between positive and negative acceleration.

- To find the optimal **High acceleration above** threshold, run a few test trials and in the Integrated Visualization plot the values of Acceleration and Acceleration state. Adjust the threshold value in such a way that the state High acceleration is active only when the animal shows bursts of rapid movement in the video.
- We advise you to use an Averaging interval greater than 1 to remove the effect of random changes in velocity between consecutive samples that would result in false transitions between Low and High acceleration. However, the greater the Averaging interval, the longer the delay in the determination of a state transition. See Averaging interval
- The **State duration threshold** is the minimum duration of the set of consecutive samples with Acceleration above (or below) the **High acceleration above** threshold required in order to be scored as the corresponding state. If the set of samples passes the threshold, the samples are scored as the state. If the set of samples does not pass the threshold, the previous state ends, but no new state is defined. Use this option to filter out brief transitions between High and Low acceleration caused by body-point jitter or noise detection.

Applications

Use *Acceleration state* to mark bursts of rapid movement, like swimming bursts in fish.

Location



- In zone 819
- Distance to zone 823
- Distance to point 826

In zone

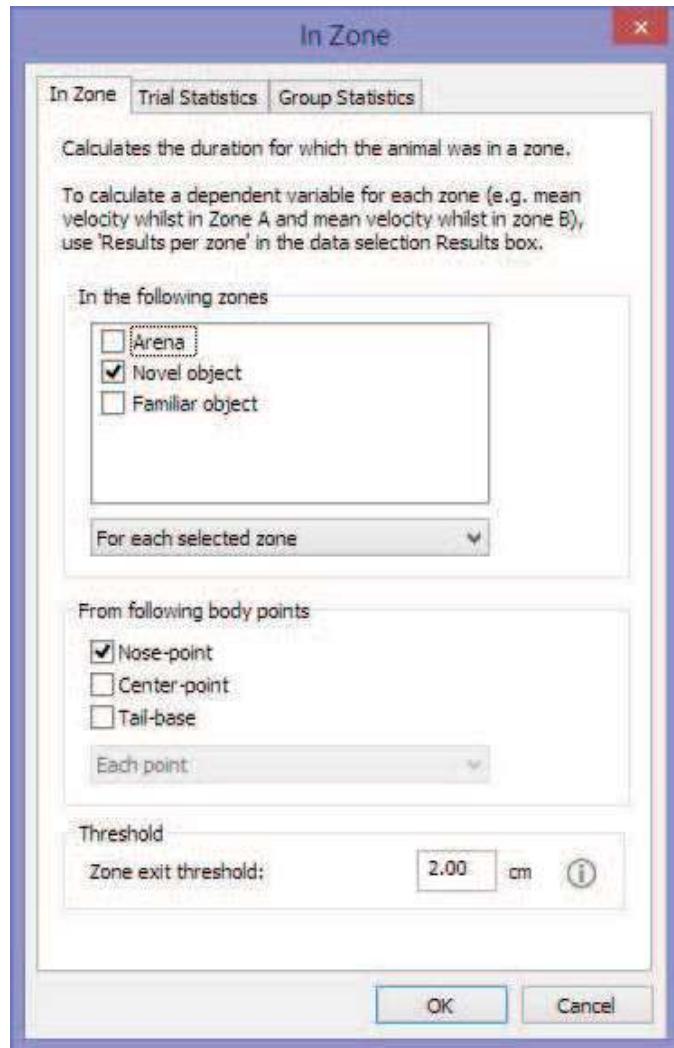
Definition

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.

How to specify In zone

1. Click the **Add** button next to In zone and click the **In zone** tab in the window that appears.



- 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.

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.

- 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.

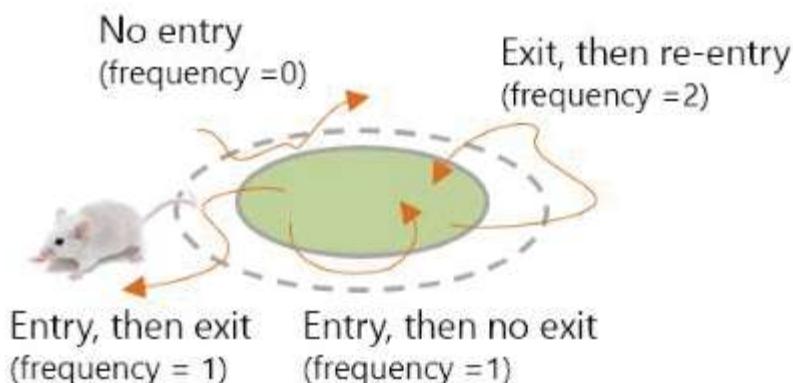
If you have chosen two or more body points, select one of the following from the list:

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.

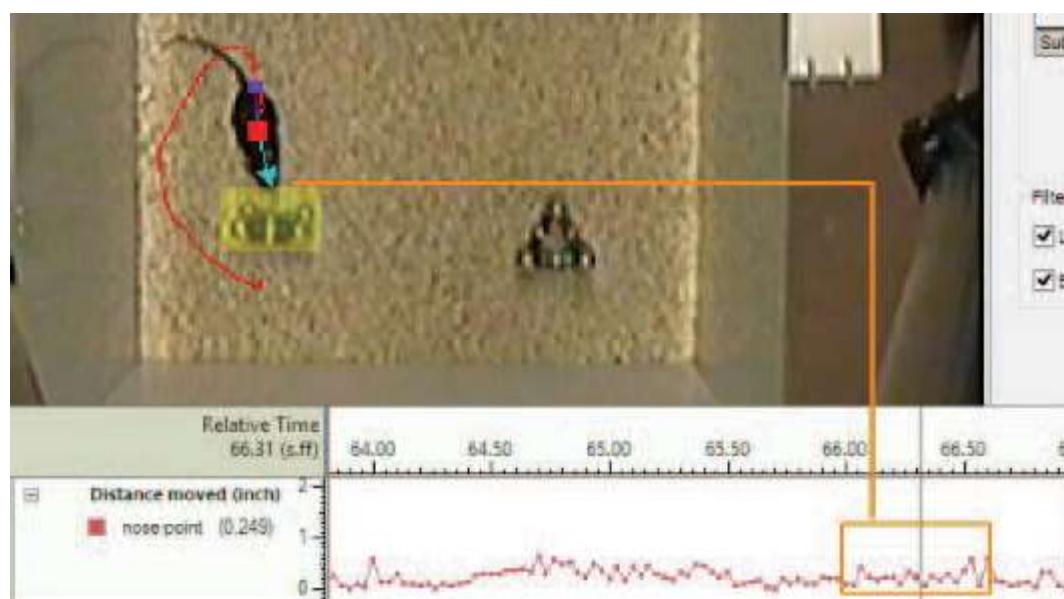
- Under **Threshold**, enter the **Zone exit threshold**. That is, once the animal's body point is detected in the zone, the animal is considered to be in the zone until its distance from the zone border (when outside the zone) exceeds that threshold. Default: 0 cm. Use this option to remove false re-entries resulting from random movements of the body point around the zone border. The following example shows the effect of the threshold for different trajectories. The frequency is shown for *In zone*.



5. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- If your experiment is set to Only center-point detection or Color marker tracking, the *In zone* variable is calculated for the center-point.
- To find a good Zone exit threshold for *In zone*, plot the values of Distance moved for the body point you are interested in. For instance, in a Novel object test, plot the distance moved for the nose-point while the subject explores an object, that is, while its nose-point is within the border of the zone "object". Take note of the variation in the per-sample distance moved and set the Zone exit threshold higher than this range.



- If two zones are adjacent, that is, not overlapping, the subject's body point is supposed to be either in one or the other zone. However, when the zone exit threshold is greater than zero, the subject's body point may not yet be outside zone 1, and (by definition) be already in zone 2. To prevent this, redefine the two zones in such a way there is a gap between the two, with its width at least the same as the threshold.
- When you export *In zone* as raw data, the values are exported for each sample time, with possible values 0 (*Not in zone*), 1 (*In zone*), or “-” (*unknown*). See Export raw data (track and dependent variables)
- When a body point is missing for more than three consecutive samples, the *In zone* state ends and the remaining missing samples are not assigned to any state (neither *In zone* or *Not in zone*).

Application

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 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** 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.

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 Notes 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.

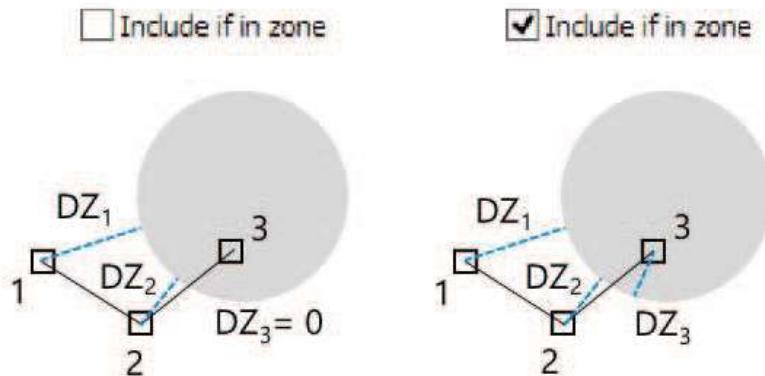
Shortest distance to any point: For each sample, EthoVision XT chooses the body point that is currently closest to the zone, and uses the resulting distances for calculating the statistics.

5. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- 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 is greater than 0.

EXAMPLE Effect of the option **Include if in zone** on distance to zone (DZ, dotted lines) for three consecutive samples 1, 2 and 3. The zone is shown in gray. When the option is not selected, and a sample is within the zone, DZ for that sample is zero. When **Include if in zone** is selected, DZ has a value larger than zero.



- 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.

Application

Two examples of how you can use *Distance to zone* with **Include if in zone** not selected:

- 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). See also the chapter The Morris water maze test in the EthoVision XT Application Manual for the exact procedure. 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 resident's territory could be defined as a zone. You can then measure how close the intruder comes to that area.

One example of how you can use *Distance to zone* with **Include if in zone** selected 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 an Elevated 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** 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.

4. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

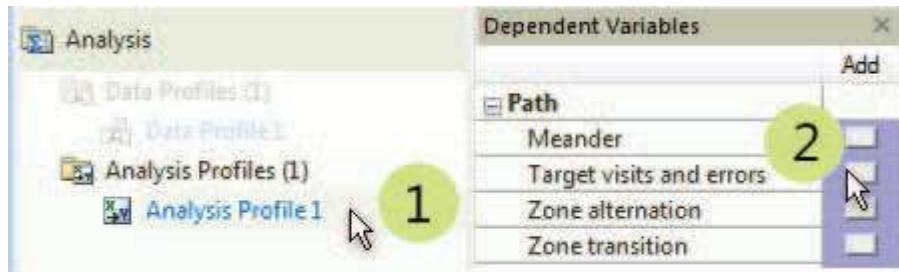
- If your experiment is set to Only center-point detection or Color marker tracking, the body point options are not available. Calculations are based on the center-point.
- The center point of a zone can lie outside the zone itself. This occurs when the zone is ring-shaped or very asymmetrical.

Applications

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.

Path



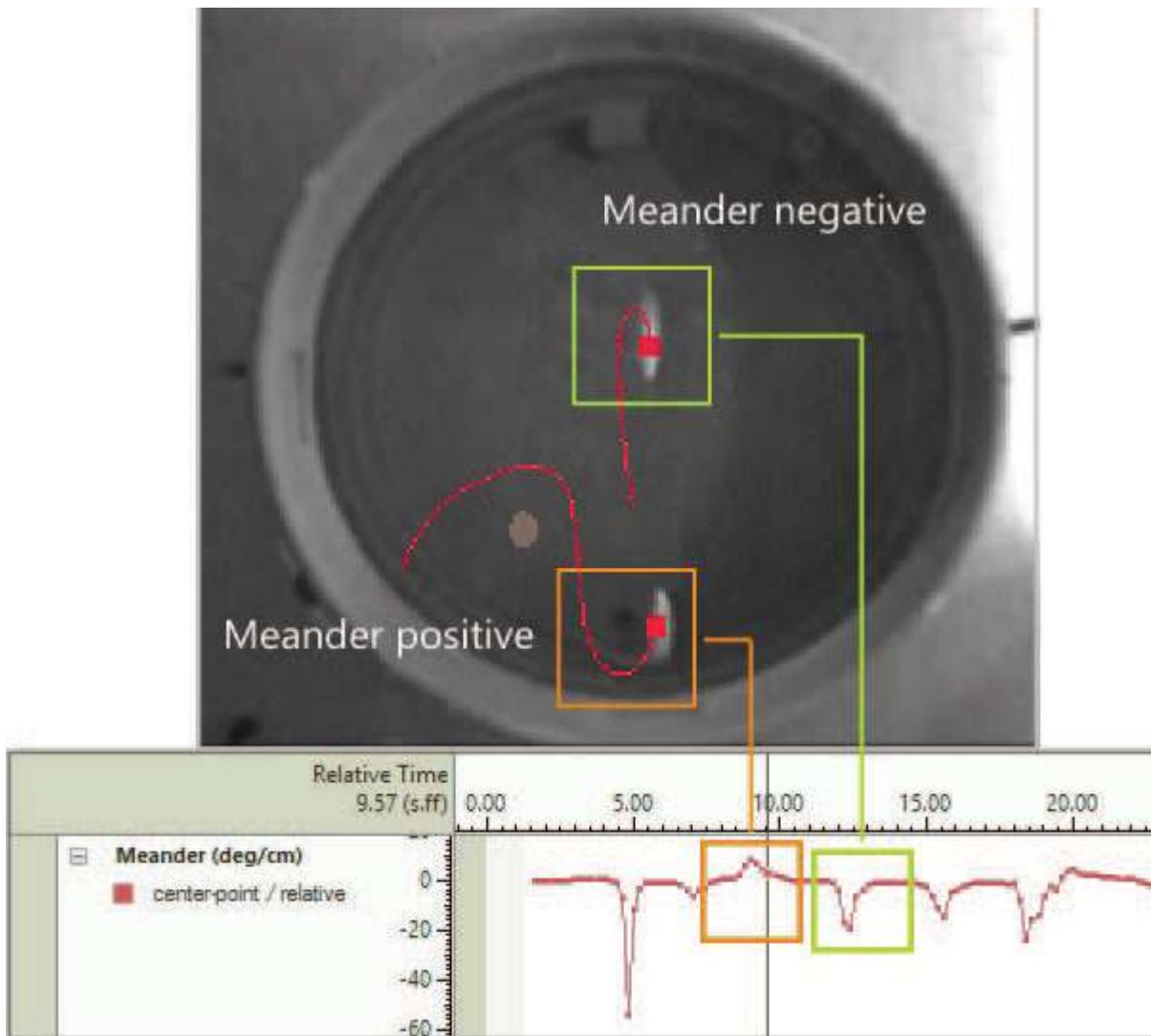
- Meander 829
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- Zone transition 839

Meander

Definition

Meander is the change in direction of movement of a subject relative to the distance moved by that subject. It provides an indication of how convoluted the subject's trajectory is. **Meander** can be relative or absolute:

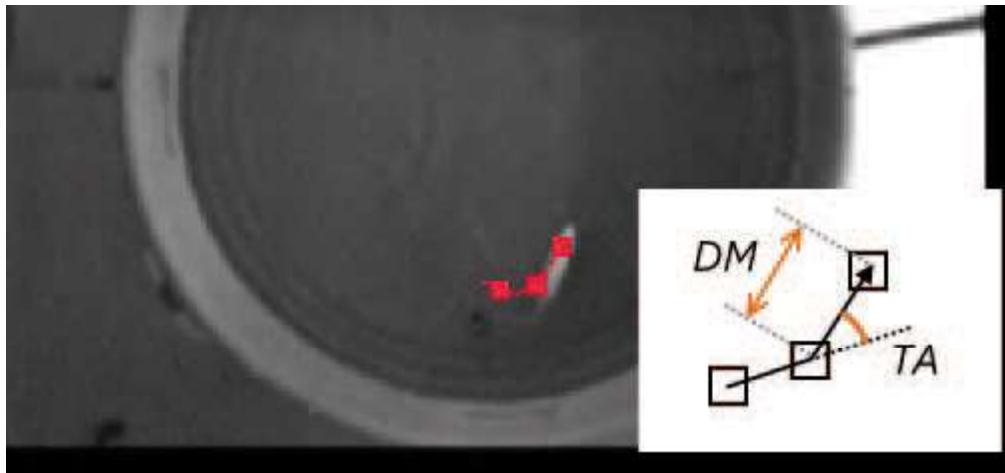
- *Relative Meander*: The change in direction is signed. With a default position of the Calibration axes (x-axis pointing to the right; y-axis pointing upward), a clockwise turn is scored as negative value because turn angle is negative; a counterclockwise turn is scored as positive. With other orientations of the x- and y-axes, the same turn may have different sign, depending on the relative turn angle. See Turn angle for how turns are given positive vs. sign.
- *Absolute Meander*: The change in direction is unsigned.



Calculation

$$RM_n = \frac{RTA_n}{DM_n}$$

where RM is *Relative Meander* for sample n , RTA is *Relative Turn angle* and DM is *Distance moved*. Absolute Meander is the absolute value of the relative Meander.



You can calculate *Meander* in two ways:

- Based on body points: *Meander* is calculated from the turn angle of the specified body point.
- Based on Head direction: *Meander* is calculated from the turn angle based on the Head direction line. 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.

Range

Given the formula above, Relative Meander can range from $-\infty^{\circ}/\text{cm}$ to $+\infty^{\circ}/\text{cm}$, while Absolute Meander from $0^{\circ}/\text{cm}$ to $+\infty^{\circ}/\text{cm}$. When DM is very small, Meander can get high, unrealistic values. One case when DM is very small is when you use a high sample rate and the subject does not move significantly.

TIP Before running analysis, make sure you use Track Smoothing to remove very small values of distance moved from your tracks.

See also Troubleshooting: Statistics > I get unrealistic values of path shape and direction

How to specify Meander

1. Click the **Add** button next to **Meander** and click the **Meander** tab. Select **Absolute** or **Relative**.
2. Select Head direction meander (body point is ignored) if you want to calculate meander based on the head direction line.

3. Click the **Body points** tab and select the body points for which you want to calculate meander. By default, **Center-point** is selected.
4. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- 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.
- **Meander** is very sensitive to small, random movements of the body points. When the animal sits still, **Meander** can get very high, unrealistic values. To remove such small movements from your data, Smooth the Tracks, then run analysis.

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. See Bell (1991). *Searching Behaviour: The Behavioural Ecology of Finding Resources*. Chapman & Hall, London.

Target visits and errors

Definition

An event marking the time when the animal visits a zone defined as target, or non-target (error) zone.

How to specify Target visits and errors

1. Click the **Add** button next to **Target visits and errors**.
2. In the **Target Visits and Errors** tab, under **Settings**, choose the **Target zones** and the **Non-target zones**.

Under **Threshold**, enter the **Zone exit threshold**. That is, once the animal is detected in the zone, the animal is considered to be in the zone until its distance from the zone border exceeds that threshold. Use this option to remove false re-entries resulting from random movements of the body point around the zone border. Click  for an example.

3. Under **Calculate Statistics for**, select the options you require:

Target first visits: Visits to zones defined as targets.

Target revisits: Revisits to the target zones (to analyze working memory).

Non-target first visits: First visits to non-target zones.

Non-target revisits: Revisits to non-target zones (to analyze reference memory).

Total errors: The total number of non-target zone visits and target zone revisits.

4. In the **Body points** tab, select the body point(s) you want to use for calculation.

If you have chosen two or three body points, select one of the following from the list:

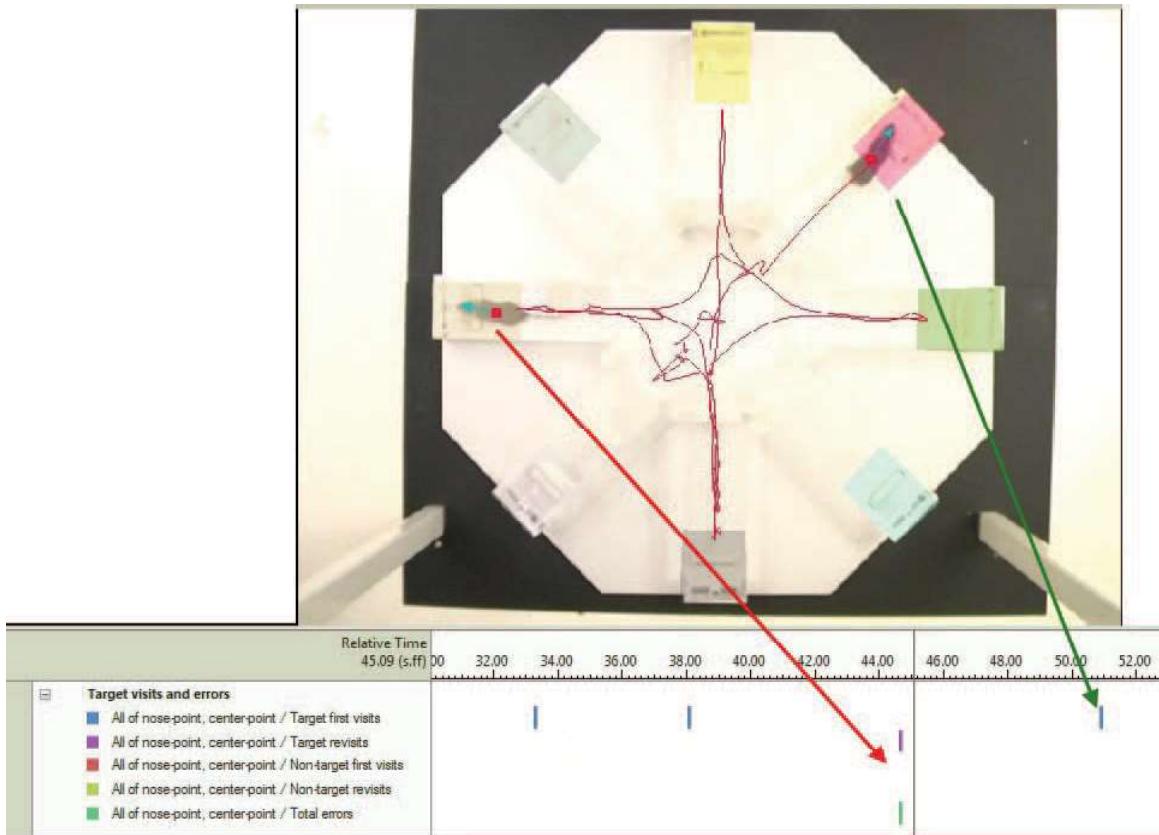
Each point: Visits are scored for each point separately. This results, for example, in one value of Successes for Center-point, and one value of Successes for Nose-point.

Any selected point: Visits are scored no matter which selected point enters a zone.

All selected points: Visits are scored when all the selected points are in a zone simultaneously.

5. Complete the procedure to add the variable. See Calculate statistics: procedure.

Below: Visualization of Target visits and errors scores in a radial-arm maze. Left: the mouse visits an arm for the second time. This is scored as **Target revisit**; **Total errors** is also scored. Right: the mouse visits an arm for the first time. This is scored as a **Target first visit**.



Application

- In a Barnes maze experiment, use *Target visits and errors* to analyze spatial reference memory. Calculate the number of successes and errors and their latencies, and compare those figures between probe trials (with escape hole being closed) and training trials. **TIP:** Select **Nose-point** to estimate nose pokes.
- In a Radial-arm maze experiment, use *Target visits and errors* to analyze working memory. Define the baited ends of the maze as targets. Calculate the number of target first visits and target revisits. To calculate the time

needed to visit all arms, choose the Trial Statistic **Latency to Last** and in the results locate this statistics in the **Target first visits** column.

- See also the EthoVision XT Application Manual for more information on the Radial-arm maze test.

Zone alternation

Definition

An event scored when the animal visits specific zones in:

- Alternations: Multiple entries into different zones, with no reentries, in overlapping sets of zone entries. For example, for a Y maze with three zones A, B and C, the sequence of zone entries ABC is an alternation.
- Revisits: Multiple entries into the same zone. They can be direct (for example, AA) or indirect (for example, ABA).

EXAMPLE

Consider, the zone entry sequence in a Y-maze: ABCBACBCAB. The eight overlapping 3-zone entry sequences are: ABC, BCB, CBA, BAC, ACB, CBC, BCA, CAB.

Of these, six are alternations (underlined): ABC, BCB, CBA, BAC, ACB, CBC, BCA, CAB

Alternation is often calculated together with the maximum possible number of alternations for the given sequence (that is, the total number of zones entries minus 2). In this example, it is $10-2 = 8$.

The sequence above results in zero direct revisits, and two indirect revisits:

ABC, BCB, CBA, BAC, ACB, CBC, BCA, CAB

How to specify Zone Alternation

1. Click the **Add** button next to **Zone alternation**.
2. In the **Zone Alternation** tab, under **Settings**, choose the zones that define the alternation.
3. Under **Threshold**, enter the **Zone exit threshold**. That is, once the animal is detected in a zone, the animal is considered to be in the zone until its distance from the zone border exceeds that threshold. Use this option to remove false re-entries resulting from random movements of the body point around the zone border. Click  for an example.
4. Under **Calculate Statistics for**, select the options you require:

Alternations: To calculate statistics of alternations (ABC, ACB, etc.).

Max alternations: To count the maximum possible alternations given the sequence of zone entries in your data (see Definition above).

Direct revisits: To calculate statistics of the direct revisits (AA, BB, etc.).

Indirect revisits: To calculate statistics of the indirect revisits (ABA, ACA, etc.).

5. Complete the procedure to add the variable. See Calculate statistics: procedure.

Applications

In a T-maze or Y-maze experiment, you can use *Zone alternation* to analyze Spontaneous Alternation.

- In the **Zone alternation** tab, select the arm zones. If the arm zones are separated by a Center zone in the same zone group, do not include the Center zone.
- Also select **Max alternations**. The ratio Alternations/Max alternations expressed in percentage gives the spontaneous alternation index (Conrad *et al.* (1996). *Behav. Neurosci.* **110**: 1321–1334). An alternation index around 50% indicates random arm selection.

Notes

- If your experiment is set to Center-point, nose-point and tail-base detection, click the Body points tab and select the body points you want to use for calculation.

If you have chosen two or three body points, select one of the following from the list:

Each point: Alternations and revisits are scored for each point separately.

Any selected point: Alternations and revisits are scored no matter which selected point enters a zone.

All selected points: Alternations and revisits are scored when all the selected points are in a zone simultaneously.

- In Integrated Visualization, a **Max alternations** event is scored from completion of the first set of entries and for each new zone entry from that point. In the Statistics results, the total number of those events is shown under **Frequency**.
- **Direct revisits** are evaluated in 2-zone entry sequences, also ignoring entries in zones not selected in step 2 above. For example, the sequence ADA results in one direct revisit (AA) when D is not selected.
- **Indirect revisits** are evaluated in 3-zone entry sequences, also ignoring entries in zones not selected in step 2 above. For example, the sequence ABDAB results in the indirect revisits ABA and BAB when D is not selected.

- **Direct revisits** and **indirect revisits** are calculated when you select at least two and three zones, respectively.

Zone transition

Definition

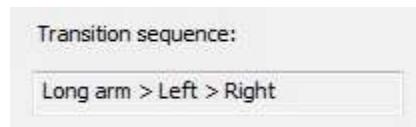
This is the number of times an animal visits two or more zones in a sequence. For example, in a T-maze study, the transition Long Arm > Left Arm > Right Arm.

A **Zone transition** is scored when the animal enters the last zone of the sequence.

How to specify Zone transition

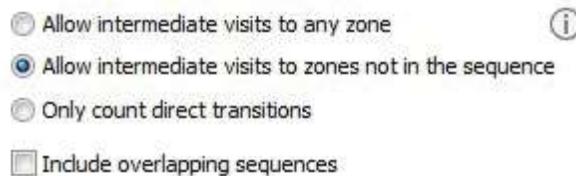
1. Click the **Add** button next to **Zone transition**.
2. In the **Zone transition** tab, to add a sequence click the **Add** button.
3. Select a zone and click or double-click a zone to include it in the sequence you want to define. Repeat this step to add more zones and complete the sequence.

Under **Zone sequence** you can view the current selection.



If the sequence is not correct, click and select the correct zones.

4. When ready click **Add**. Repeat steps 2-3 to add more sequences.
5. Depending on which option under Settings you select, you can get different results. See the notes below.



6. Under **Threshold**, enter the **Zone exit threshold**. That is, once the animal is detected in a zone, the animal is considered to be in the zone until its distance from the zone border exceeds that threshold. Use this option to remove false re-entries resulting from random movements of the body point around the zone border.

Click for an example.

7. Click the **Body points** tab, select the body point(s) you want to use for calculation.
8. Complete the procedure to add the variable. See Calculate statistics: procedure.

Zone transition counting options

For all options below, the zone transitions found in the data are marked in green. Zone transitions ignored are marked in red. The numbers in blue show the statistic Total number.

- **Allow intermediate visits to any zone**

With this option the sequence ABC is found in the zone visit data ABDC, where D represents any zone defined.

In the following example, the zone sequence CAB has been defined. A total of four transitions have been found.



- **Allow intermediate visits to zones not in the sequence**

With this option the sequence ABC is found in the zone visit data ABDC, just like in the example above, because D does not belong to the sequence defined. However, the same sequence ABC is not found in the zone visit data ABAC, because the second A is defined in the sequence.

In the following example, two transitions CAB have been found.



- **Only count direct transitions**

With this option, sequences with intermediate visits to any zone are ignored.



Do not use this option if your focal zones are not adjacent (for example Novel object and familiar object), and multiple zone groups have been defined. In that case, direct transitions from the focal zones are never counted, because when going from one focal zone to another, the animal visits zones of other zone groups.

- **Include overlapping sequences**

You can apply this additional option to any of the options above. Overlapping sequences may occur if the first zone of the sequence defined is visited again before the previous instance is completed. Consider the following zone visit data:

...BABAB...

When looking for the sequence BAB, two overlapping sequences BAB are found:

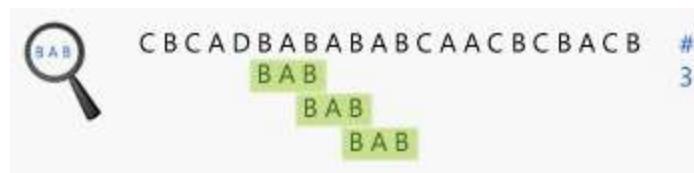
...BABAB...

...BBABA...

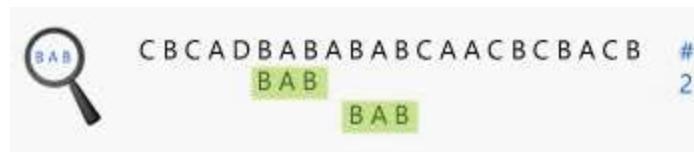
Consider the following zone visit data:

...BABABAB...

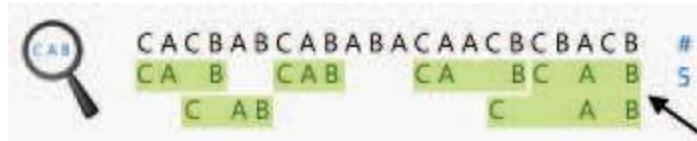
If the option **Include overlapping sequences** is selected, the sequence BAB is found three times.



If the same option is not selected, the sequence BAB is found two times.



However, overlapping sequences that are completed at the same time point (that is, the same zone visit) are counted as one. In the following example, the sequence CAB is defined, and the method **Allow intermediate visits to any zone** is used. The two sequences CAB ending at the same visit to B are scored as one. Note that transitions are scored at the end of the last zone entry.



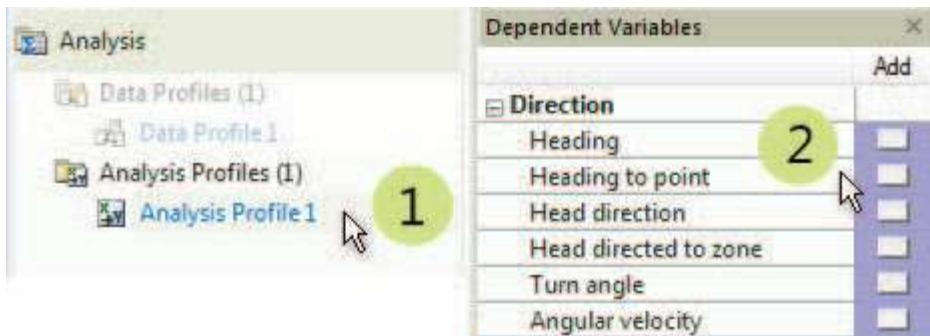
Notes

- The **Body Points** tab is only available if your experiment is set to **Center-point**, nose-point and tail-base detection.
- When you visualize the *Zone transition* variable, only the end of the transitions (which equals the last zone entry) is marked on the time plot. EthoVision does not calculate the duration of the transition.
- To delete a sequence, select that sequence under Settings and click the **Delete** button.

Applications

- In a Novel object test, calculate the number of transitions from the zone Familiar object to the zone Novel object, and from Novel object to Novel object. Or in a PhenoTyper or home cage test, calculate the transitions between the different corner visits. When the focal zones are not adjacent, select Allow intermediate zone visits.
- For Y-maze tests, see Zone alternation.

Direction



- Heading 844
- Heading to point 848
- Head direction 851
- Head directed to zone 855
- Turn angle 857
- Angular velocity 863

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. 'Compass heading' and 'compass angle' are synonyms for *Heading*.

See also

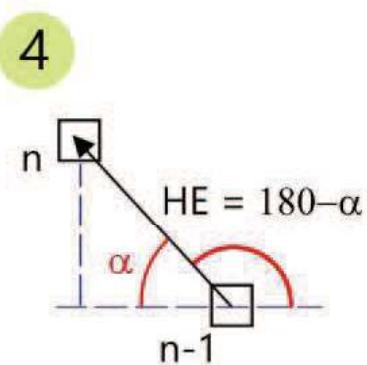
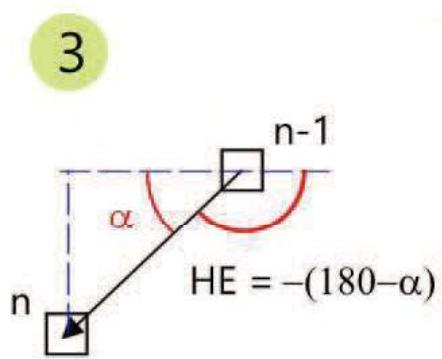
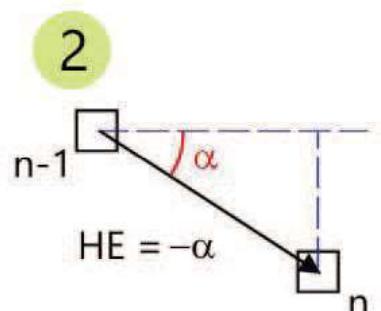
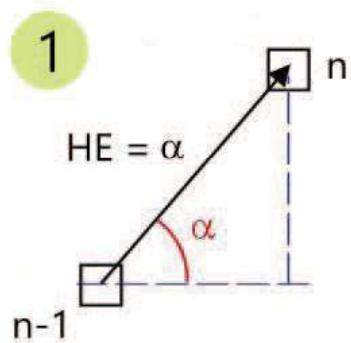
- Heading to point

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 .

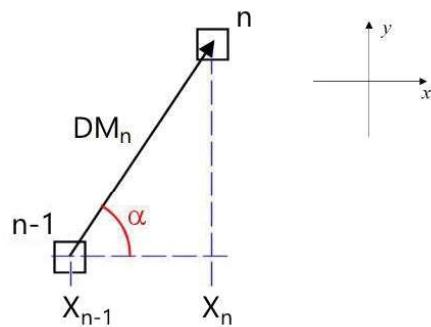
The figure below shows the relationship between the angle α formed by the segment joining samples $n-1$ and n , the horizontal line parallel to the x-axis, and the dependent variable Heading (HE). Four cases are illustrated, corresponding to the possible directions an animal's body point can be moving 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.



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 . 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 the figure above).
 - 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 $\Delta Y = Y_n - Y_{n-1}$.

NOTE Because the way α is defined, it can only range from 0° to 90° .

Range

Heading ranges from -180° to $+180^\circ$.

How to specify Heading

1. Click the **Add** button next to **Heading**.
2. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- If your experiment is set to Center-point, nose-point and tail-base detection, click the **Body points** tab and select the body points for which you want to calculate heading.
- The mean, standard deviation and variance are calculated with circular statistics. See Statistics available.
- Heading is calculated relative to the orientation of the x axis you have chosen in the Arena Settings used for that trial. 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 based on that axis direction.

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.

Heading to point

Definition

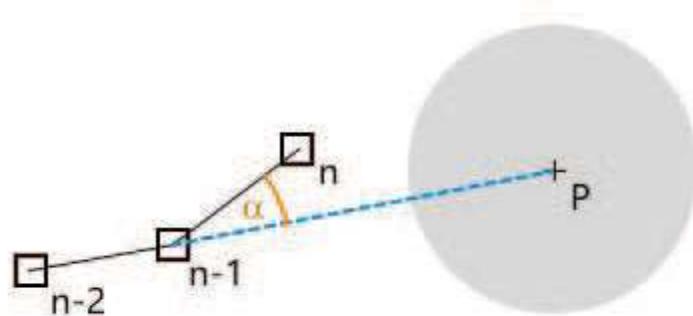
The direction of movement of the nose, center or tail-base point of the current sample relative to a point of interest.

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. 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.

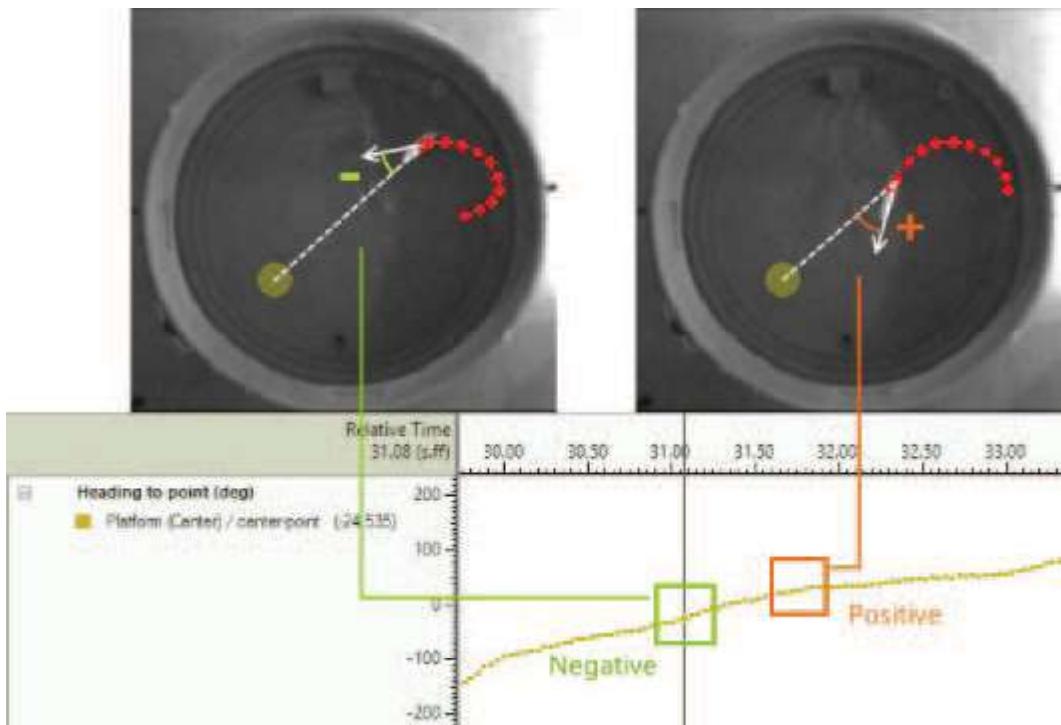
Heading to point for sample n is the angle α formed by the segment joining the samples $n-1$ and n , with the line connecting sample $n-1$ and the point P (a point or the center of a zone).



Range

Heading to point ranges from -180° to $+180^\circ$. The closer to zero *Heading to point* is, the straighter the subject moves toward the point. Negative values occur when the subject moves to the right of the point; positive values in the other case.

Below: Example of Heading to point with positive and negative values in a water maze test. The platform has been specified as a target point. The chart shows *Heading to point* in the Integrated visualization (**Analysis > Results > Integrated Visualization**).



How to specify Heading to point

1. Click the **Add** button next to **Heading to point**.
2. 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.
3. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

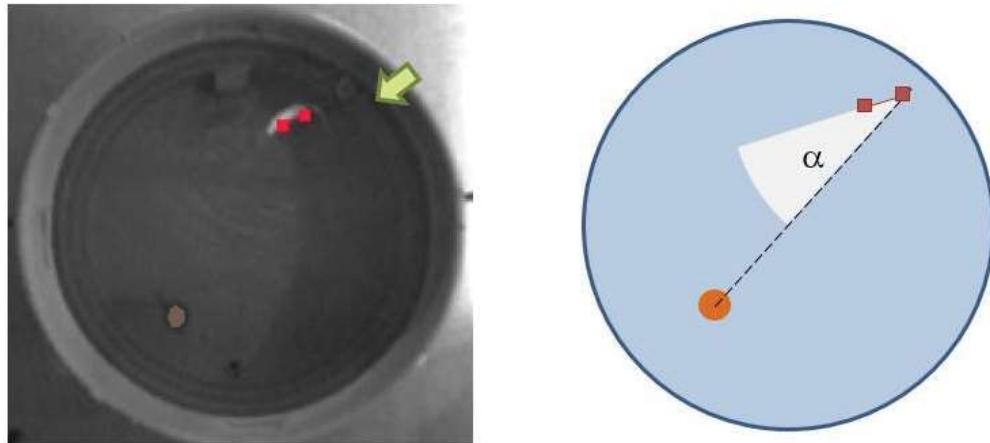
- If your experiment is set to Center-point, nose-point and tail-base detection, click the **Body points** tab and select the body points for which you want to calculate heading to point.
- The mean, standard deviation and variance are calculated with circular statistics. See Statistics available

Applications

Use *Heading to point* to measure the subject's orientation relative to a point of interest.

- *Morris water maze.* Use **Heading to point** to determine the Heading angle error. The Heading angle error is usually determined after the animal has traveled a minimum distance, or after the first few seconds of each track. The Heading angle error at this point is the deviation from a direct line from starting point to center of the platform.

Below: The Heading angle error (α) in a water maze. The first two samples of the track are displayed. The arrow indicates the release point.



How? First, in the Data profile under **Nesting** choose **Time**, and select for example from 0 to 2 seconds. This means that analysis is done on the data points of the first two seconds of the track. Next, in the Analysis profile choose **Heading to point** and select the platform as point of interest. As Trial Statistic, choose **Mean**. For other water maze output variables, see also the EthoVision XT Application Manual.

- *Novel object test.* Use **Heading to point** to determine the subject's movement relative to an object. The assumption is that an animal is interested in a novel object when it is heading towards the center of the object.

Head direction

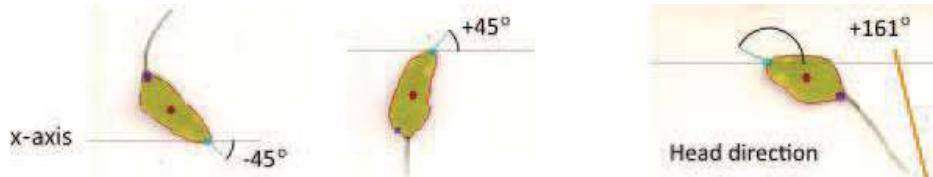
Definition

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. See also Head directed to zone.

Below: An example of *Head direction* when exported in the raw data file (**Analysis > Export > Raw Data**; locate the **Head direction** column).

The angle is negative $(-180^\circ, 0]$ when the Head direction line departing from the nose-point lies left to the line parallel to the x-axis. The angle is positive $[0, +180^\circ]$ in the opposite case (in this example the x-axis is in the default position; it is horizontal and points to the right). The top-left picture shows the Head direction at the sample highlighted below.

IMPORTANT Note that the value of *Head direction* depends on the orientation of the x-axis set in the Arena Settings.



Trial time s	X center cm	Y center cm	X nose cm	Y nose cm	X tail cm	Y tail cm	Area cm ²	Areachan cm ²	Elongatio cm ²	Head direction deg
28.8	5.67234	13.4769	3.54792	14.3752	7.26182	12.3656	8.36306	3.49318	0.503501	158.119
28.88	5.36834	13.5889	3.05876	14.2998	7.02722	12.7162	8.40166	1.62115	0.546821	161.036
28.96	5.18908	13.6375	2.96613	14.2082	7.0215	12.909	8.43382	1.02287	0.524414	161.753
29.04	5.28841	13.6406	3.09116	14.1343	7.07325	13.046	8.1186	0.817006	0.515517	164.261
29.12	5.57874	13.5124	3.54817	14.0439	7.27878	12.8036	7.62968	1.63401	0.458652	165.256
29.2	5.72202	13.376	3.87971	14.1019	7.34167	12.6858	7.28873	1.20299	0.414854	154.753
29.28	5.69759	13.3465	3.65675	13.8059	7.36981	12.762	7.37879	0.41172	0.450797	164.645
29.36	5.65117	13.3683	3.60968	13.9266	7.35515	12.7376	7.48172	0.30879	0.443904	162.502
29.44	5.60349	13.3928	3.53746	13.9413	7.29646	12.7079	7.54605	0.321656	0.463216	164.544
29.52	5.58964	13.3942	3.54929	13.8701	7.24315	12.7085	7.56535	0.096497	0.467954	165.522

Range

Head direction ranges from -180° to $+180^\circ$. Therefore, values like -179 and $+179$ represent very similar orientations (head pointing to the left). Take this into account when interpreting the raw data.

How to specify Head direction

1. Click the **Add** button next to **Head direction**.
2. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- *Head direction* is not available if your experiment is set to Only center-point detection or Color marker tracking.
- *Head direction* is a very different thing than *Turn angle* based on the nose point. The latter measures the direction of movement, not the orientation of the head. See Turn angle
- You can check the head direction line during data acquisition. Click the **Show/Hide** button on the toolbar, 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* values are shown in the Analysis Results and Scoring pane.

During acquisition, the head direction line is calculated from the Subject's contour, therefore its value also depends on the shape of the Subject at that sample.

- The mean, standard deviation and variance are calculated with circular statistics. The mean *Head direction* represents the average orientation of the animal relative to the x-axis. See Statistics available
- *Head direction* is calculated relative to the orientation of the x-axis you have chosen in the Arena Settings used for that trial. 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 based on that axis direction.
- **NOTE** If you swap nose- and tail-base points, or interpolate those two points, the Head direction line is recalculated as the prolongation of the segment joining the nose-point and the center-point (see the figure below). Note that this differs from how the line is calculated during acquisition, using the subject's contour. Once the line is determined, the *Head direction* angle is calculated as usual, relative to the x-axis.



- If you change the position of the nose-point, *Head direction* is unchanged.
- Be careful when interpreting values of Head direction relative to objects, for example two zones, or values extracted from different arenas.

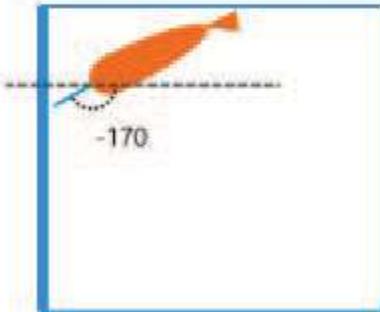
EXAMPLE In a mirror test, we want to measure the head direction of the fish, and compare the data between two arenas. However, the mirror is placed at opposite sides in Arena 1 and 2. If we want to compare angles, with the mirror kept as a reference, we must take into account that the same orientation corresponds to opposite angles:

For a negative angle in Arena 1: Angle in Arena 2 = Angle in Arena 1 + 180° (see the figure below, top).

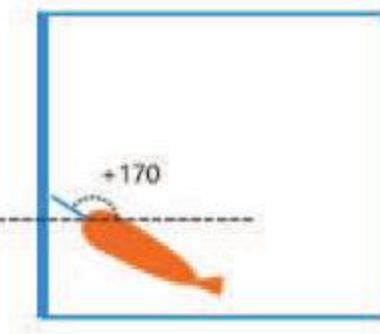
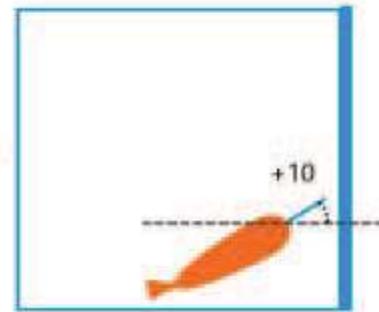
For a positive angle in Arena 1: Angle in Arena 2 = Angle in Arena 1 - 180° (bottom).

To compare results, convert angles from Arena 2 to Arena 1 (or vice versa).

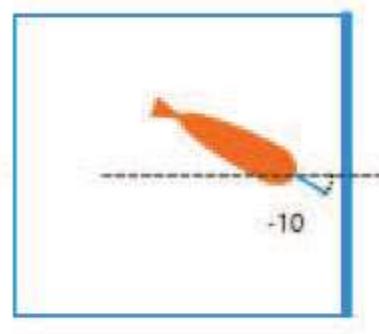
Arena 1 (mirror on the left)



Arena 2 (mirror on the right)



-180 =
→



+180 =
→

Applications

Head direction is useful for studies of spatial orientation and searching behavior. For this you can, for example, use the average and variation in Head direction.

Head directed to zone

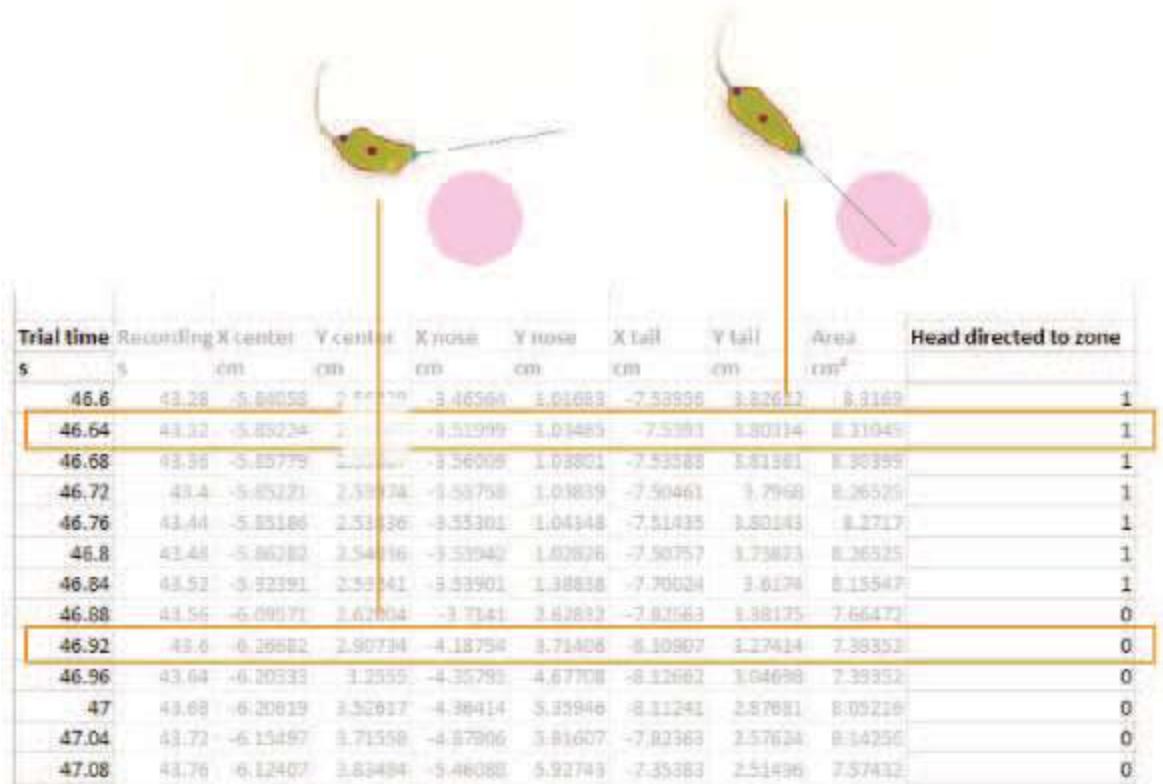
Definition

A discrete (state) variable which marks the time when the subject's head is directed towards a zone or a circular area around a point.

Calculation

1. The Head direction line is calculated based on the Subject's contour.
2. If the Head direction line crosses the zone of interest (or a circular area around a point), the value of Head directed to zone for that sample is set to 1. The samples with 1 are used to calculate the duration of *Head directed to zone*.

The figure below shows Head directed to zone when exported in the raw data file (**Analysis > Export > Raw Data**). Two samples are highlighted, one when the head is directed to the zone, the other when it is not (see the last column; the circle indicates the zone).



How to specify Head directed to zone

1. Click the **Add** button next to **Head directed to zone**.
2. In the **Head directed to zone** tab, under **Zone of interest** select a **Zone** or a **Point**. For the latter, you can 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. If you select **All detected body points**, Head directed to zone is only calculated when all three body points are in the zone selected in the In list.

In: Select one of the zones from this list.

4. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- *Head directed to zone* is not available if your experiment is set to Only center-point detection or Color marker tracking.
- The head direction line is not the same as the segment connecting the nose-point and the center point.
- **NOTE** The Head direction line is calculated using the subject's contour. If you swap nose-point and tail-base, the Head direction line is not calculated using the contour, instead as the prolongation of the segment joining the nose-point and the center-point. *Head directed to zone* is calculated based on the new angle.

Applications

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) *Behavioural Brain Research*, **31**, 47-59). 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.

Turn angle

Definition

The change in direction of the nose, center, tail-base point or Head direction line between two consecutive samples.

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 of the specified body point:

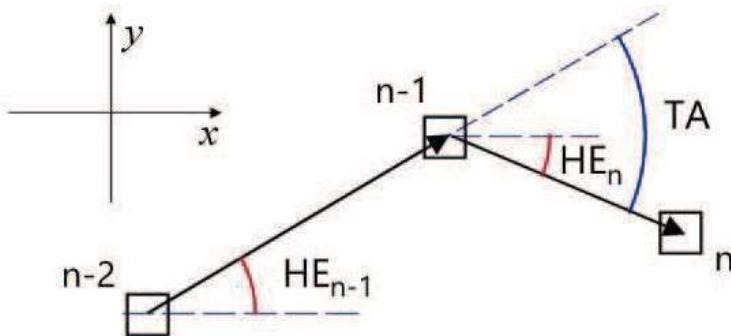
$$\Delta\text{Heading} = \text{Heading}_n - \text{Heading}_{n-1}$$

If $\Delta\text{Heading} < -180^\circ$, then Relative Turn angle = $\Delta\text{Heading} + 360^\circ$.

If $\Delta\text{Heading} \geq +180^\circ$, then Relative Turn angle = $\Delta\text{Heading} - 360^\circ$.

Else Relative Turn angle = $\Delta\text{Heading}$.

Below: 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 the figure below, left). 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 = $\Delta\text{Head direction} + 360^\circ$.

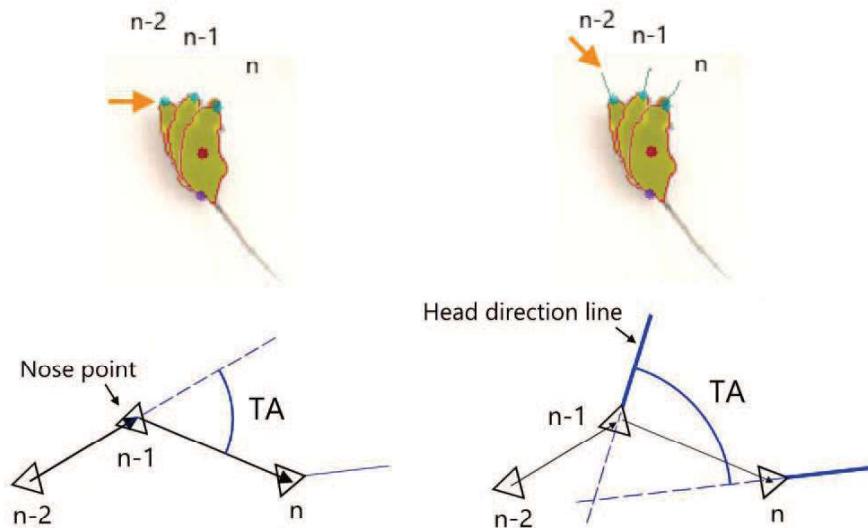
If $\Delta\text{Head direction} \geq +180^\circ$ then relative Turn angle = $\Delta\text{Head direction} - 360^\circ$.

Else relative Turn angle = $\Delta\text{Head direction}$.

This option is available only if your experiment is set to Center-point, nose-point and tail-base detection.

The Turn angle based on the head direction line is not the same as Turn angle based on a body point. The *Turn angle* based on a body point at sample n depends on the last three samples ($n-2$, $n-1$ and n ; see the figure below, left), while *Turn angle* based on the head direction line depends only on the samples $n-1$ and n (right).

Below: An example of the difference between Turn angle (TA) calculated from the nose-point (left) and Turn angle calculated from the Head direction (right). Triangles represent nose-point samples. Triangles represent the nose-point. Thick segments departing from the triangles are the Head direction lines.



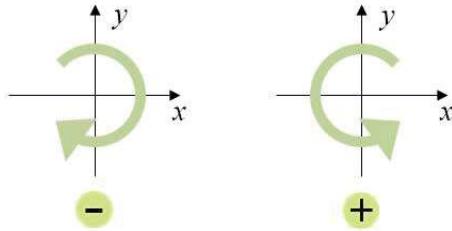
NOTE The Head direction line is calculated using the subject's contour. If you swap nose- and tail-base points, or interpolate those two points, the Head direction line is not recalculated using the contour, instead as the prolongation of the segment joining the nose-point and the center-point. *Turn angle* is then calculated based on those head direction lines.

Absolute vs. Relative Turn angles

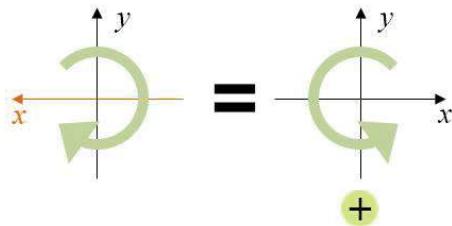
- Absolute Turn angle: The difference in direction is unsigned. Absolute Turn angle ranges from 0° to $+180^\circ$.
- Relative Turn angle: The difference in direction is signed. With a default axis orientation, a clockwise turn is signed negative, and a counterclockwise is signed positive. Therefore, the Relative turn angle ranges from -180° to $+180^\circ$. Relative angles help you distinguish between clockwise turns and counterclockwise turns.

Absolute and Relative turn angles result in different averages. Consider for example two angles, -10° and $+40^\circ$. The average relative turn angle is $(-10^\circ + 40^\circ)/2 = 15^\circ$. The average absolute turn angle is $(10 + 40)/2 = 25^\circ$.

TIP Use the picture below to know the sign of a turn. With the default axis orientation, a clockwise turn means a negative turn angle.



However, if the axes orientation in your Arena Setting is different from default, rotate the image to know the sign of the turn.



How to specify Turn angle

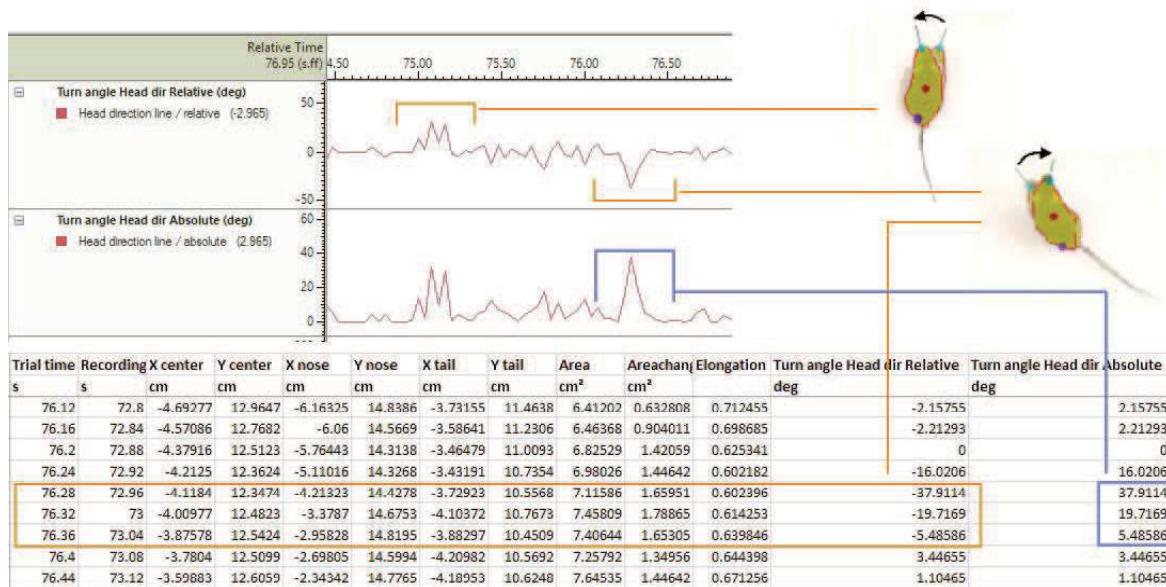
1. Click the **Add** button next to **Turn angle** and click the **Turn angle** tab. Select **Absolute** or **Relative**.
2. Select **Head direction turn angle (body point is ignored)** if you want to calculate turn angle based on the Head direction line.
3. Click the **Body points** tab. Select the 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.

This tab is not available if you choose the option in step 2.

4. Complete the procedure to add the variable. See Calculate statistics: procedure.

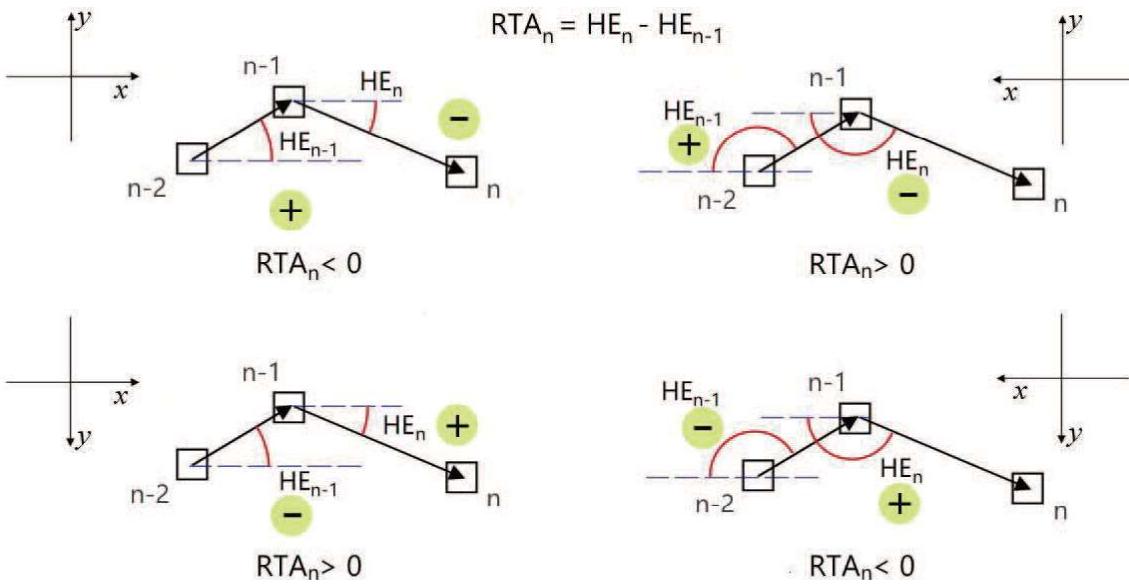
Below: Relative and absolute turn angle based on the change in direction of the Head direction line. Top: dependent variables visualized in Integrated visualization (**Analysis > Results > Integrated Visualization**). First chart: Relative turn angle, showing positive and negative values for left and right turns of the head, respectively. Second chart: Absolute turn angle. Bottom: dependent variables when exported in the raw data file (**Analysis > Export > Raw Data**).



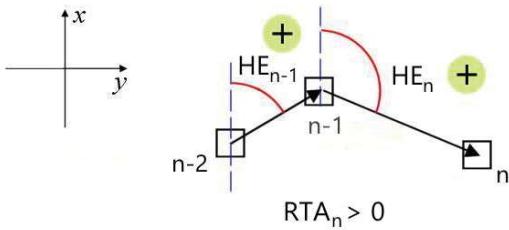
Relative Turn angle and axis orientation

Relative Turn angle depends on the orientation of the x and y axes in the Arena Settings used for that trial. 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.

Below: The sign of the *Relative Turn angle* (RTA) depends on the orientation of the x and y axes. The example illustrates the effect of four different axis orientations on a clockwise turn. The sign inside the circles indicates the sign of Heading (HE) values. When the difference $HE_n - HE_{n-1}$ is larger than $+180^\circ$ or smaller than -180° (examples on the right), the rules described in Heading apply.



If the axes are swapped, the reference line is now vertical. the Relative Turn angle is calculated relative to the vertical axis. Compare the following figure with the example at the top-left corner of the figure above.



Notes

- The mean, standard deviation and variance are calculated with linear statistics.
- 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.
- **Turn angle** is very sensitive to small, random movements of the body points. When the animal sits still, **Turn angle** can get very high, unrealistic values. To remove such small movements from your data, Smooth the Tracks, then run analysis.
- *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.

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). For better quantification of biases in the left/right direction, first filter the data with the Minimal Distance Moved method, to ignore points where the animal does not move significantly.

Cumulative Turn angles are used to calculate rotations. See Rotation

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 ($^{\circ}/s$) and can be either positive or negative (see below).

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 , and $t_n - t_{n-1}$ is the time difference between the current and the previous sample.

Data used for calculation

Angular velocity can be calculated 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 of the specified body point (see below).
- Based on Head direction: For the Head direction line, the angular velocity is calculated from the turn angle based on Head direction. 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.

IMPORTANT Note that calculating *Angular velocity* from Head direction is not the same as calculating it from the nose-point. This is because Turn angle (and therefore Angular velocity) at sample n based on a body point depends on the last three samples ($n-2$, $n-1$ and n), while Turn angle based on Head direction depends only on the samples $n-1$ and n .

Range

The minimum and maximum angular velocity depend 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 $180/0.04 = 4500^{\circ}/s$.

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.

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	Absolute Ang. vel.	Relative Ang. vel.
0.04	10	-10
0.08	45	45
0.12	35	-35
Mean	30	0

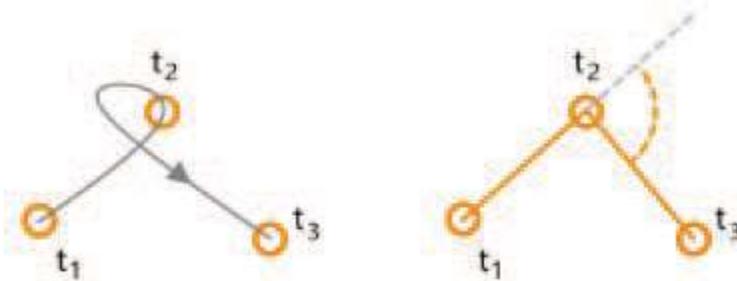
The mean *absolute Angular velocity* reflects the amount of turning, whereas the mean *relative Angular velocity* gives a better indication of the overall direction of turning.

How to specify Angular velocity

1. Click the **Add** button next to **Angular velocity** and click the **Angular velocity** tab. Select **Absolute** or **Relative**.
2. Select **Head direction angular velocity (body point is ignored)** if you want to calculate angular velocity based on head direction.
3. Click the **Body points** tab. Select the body points for which you want to calculate the velocity. By default, **Center-point** is selected.
4. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- 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.
- If the body point turns more than 180° between one sample and the next, the direction of turning is calculated incorrectly. In the example below, the subject makes a fast counterclockwise turn of 270° between time 2 and 3 (left). The program interprets this as a 90° clockwise turn (right).



As a result, *Angular velocity* gets a value smaller than expected, and in case of relative Angular velocity a false sign. This kind of error occurs when the sample rate is too low and the subject makes very fast turns. 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.

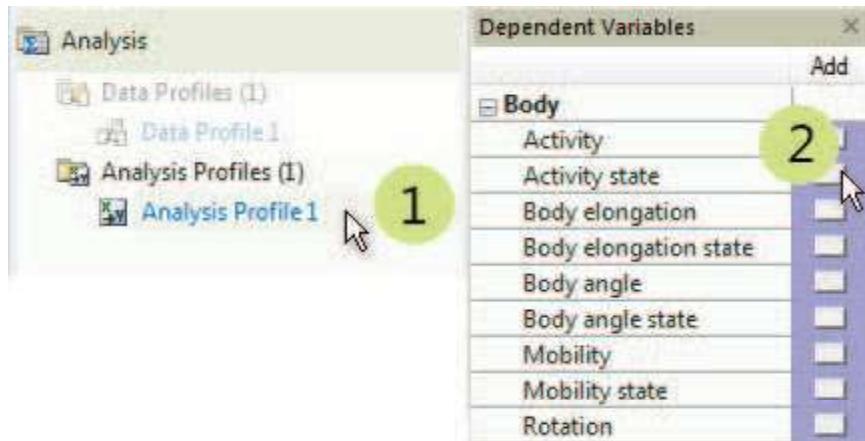
- The Head direction line is calculated using the subject's contour. However, if you swap nose- and tail-base points, or interpolate those two points, the Head direction line is not recalculated using the contour, instead as the prolongation of the segment joining the nose-point and the center-point. *Angular velocity* is then calculated based on those head direction lines.

Applications

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 (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.).

Body



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Activity

Definition

The dependent variable **Activity** is only available when you selected **Activity analysis** in the Experiment Settings and you track only one subject per arena. Activity gives the percentage changed pixels in the entire arena between current sample and previous sample.

Calculation

See step 1 and 2 in Activity state for information on how this percentage of changed pixels is calculated. With the dependent variable Activity state, you can calculate how much time and how often your subject was inactive, moderately active and active and highly active. With the dependent variable Activity, you can calculate the average activity of your subject.

How to specify Activity

1. Click the **Add** button next to **Activity**.
2. Under **Outlier filter**, select the **Averaging interval**. This is the number of samples over which the running average mobility is based. The default value is 1, that is, the current value of Activity is only based on the current image. See Averaging interval
See Activity state for more information.
3. Complete the procedure to add the variable. See Calculate statistics: procedure.

Activity state

The dependent variable *Activity state* is only available when you selected Activity analysis in the Experiment Settings and you track only one subject per arena. With Activity state you can calculate how long and how frequent your subject has been in different activity states. These states depend on the total pixel change within the arena between a sample and the previous sample. The number of states and their thresholds are user-defined.

When the subject goes missing for more than three samples, the current *Activity state* is ended and the remaining missing samples are ignored.

Calculation

Step 1 - Calculation of proportion of pixel change

All pixel coordinates in the arena, so not only of the detected subject, are determined immediately after they have been detected. The gray scale values of all pixels are compared with the previous sample to determine the number of changed pixels in the arena between the two. The formula for Activity is simply the number of changed pixels for the current sample divided by the total number of pixels in the arena:

$$\text{Activity} = \frac{CP_n}{P_n} \times 100$$

Activity is calculated by taking every pixel and comparing it between the current image and the previous one. If all the pixels are the same, there is zero activity. If all the pixels are different, there is 100% activity. If the animal is moving and increases its velocity (whilst keeping the same shape) there will be an increase in activity, because the pixels belonging to the animal are increasingly different as it moves faster.

Step 2 - Running average

To smooth the values of Activity, an Averaging interval is used. This gives you the option to specify the number of samples for calculating a running average of Activity. The Activity percentage is summed over the number of samples that you specify, and divided by the number of samples. This way, sudden changes in surface area caused by such factors as the animal entering a shadowed area, or a reflection, are smoothed out.

NOTE When you export the dependent variable, each row in the export file contains the Activity value, but not the averaging interval.

Step 3 - Calculation of the Activity state dependent variable

The *Activity state* variable is established for each sample, according to the value of running average Activity relative to the thresholds. You can define between two and four states, varying from *Inactive* (below the lowest threshold) to *Highly active* (above the highest threshold).

How to specify Activity state

1. Click the **Add** button next to **Activity State**.
2. Enter the following:

Averaging Interval: The number of samples over which the running average mobility is based. The default value is **1**, that is, the current value of Elongation is only based on the current image.

3. Under **Number of states**, select the number of activity levels you are interested in. The following options are available, dependent on the number of states:

2 states: Highly active above and Inactive below only.

3 states: Highly active above, Moderately active between and Inactive below.

4 states: Highly active above, Active between, Moderately active between and Inactive below.

4. Under **Thresholds**, specify thresholds for the states defined in step 3.

You can enter a number with up to two decimals.

Under **State duration threshold**, in the field next to **Exclude instances shorter than**, enter how long a state must last before it is scored as one of the four Activity states. If the duration passes this threshold, the samples in this time length are scored as one of the four states according to the criteria above. If the duration does not pass the threshold, the previous state ends, but no new state is defined. When you visualize *Activity state* in the Integrated Visualization, this is displayed as a gap between two adjacent color bars, which represent the scored activity states.

Under **Calculate statistics for**, select at least one of the states defined in step 3.

5. Complete the procedure to add the variable. See Calculate statistics: procedure.

To find the optimal activity thresholds, run a few test trials and check in the Analysis Results and Scoring pane the values of Activity when the animal shows such

behavior. These values are calculated real-time during acquisition. See an example in Fear conditioning: view Activity state.

Notes

- **IMPORTANT** The Activity detection thresholds set in Acquisition are not used in analysis. When you specify *Activity* in your Analysis profile, enter the new values in the appropriate fields.
- 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 Activity for the first sample of the track is always calculated over one sample.
- You set the thresholds during acquisition, but you can override them when calculating statistics to produce new values for Activity state. To see what the original values of Activity state thresholds were (unless you have changed them while acquiring data), open the Acquisition module and click the button under **Activity** in the Analysis results and Scoring pane.

Frequently asked questions about Activity

1. *What is the difference between Activity and Mobility?*

Activity is the percentage change in all pixels in the arena between the current sample and the previous sample. This is independent of the detected subject.

Mobility is the percentage pixel change between the current sample and the previous sample in the detected subject only.

2. *When should I use Activity and when should I use Mobility?*

Use Activity when the dependent variable Mobility does not give satisfying results, or if detection of your subject is difficult. This can be the case when bars of the shock grid floor in the background complicate detection of the animal's surface area, or when the animal is very large compared to the arena size.

3. *Does Activity detection depend on the size of the subject relative to the arena?*

Yes. Activity is calculated as the number of changed pixels divided by the total number of pixels in the arena. The smaller the subject relative to the arena, the smaller the activity will be. When your animal is very large in

comparison to the arena size, for example when you have a rat in a small cage, activity detection is generally more suitable than mobility detection.

4. *Does Activity detection depend on the video resolution?*

Yes. The higher the video resolution, the greater the number of pixels that change when the subject moves. Therefore, when you have a high video resolution, it is less likely that a small movement of the subject results in an abrupt change in activity.

However, this effect is present at very low resolution (for example, when the subject is less than 100 pixels large). We advise you not to compare values of Activity between videos of different resolutions.

5. *Does Activity detection depend on sample rate?*

Yes. Since Activity is detected from the change in pixels between two samples, and the pixel change depends on how frequently the area is acquired (that is, the sample rate), Activity depends on the sample rate. All being equal, the higher the sample rate, the smaller the pixel change. See also the effect of the sample rate on Mobility in Frequently asked questions about Mobility.

Applications

Activity can be used to determine freezing behavior in rodents. Also, it may be used to assess inactivity of rodents in a Porsolt Swim Test. Furthermore, the startle response of zebrafish larvae can automatically be detected with (in)activity.

Body elongation

Definition

The *Body elongation* measure of the subject's detected shape, expressed in percentage (0 to 100%). See the formula in Body elongation state.

Calculation

1. The *Body elongation* measure E is calculated as follows:

$$E = \frac{\sqrt{\left(\sum_x (x - x_c)^2 - \sum_y (y - y_c)^2\right)^2 - 4 \left[\sum_{x,y} (x - x_c) \cdot (y - y_c)\right]^2}}{\sum_x (x - x_c)^2 - \sum_y (y - y_c)^2}$$

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.

E is expressed as a percentage.

2. The Body elongation value is smoothed at each sample using the optional Averaging interval.

Range

Body elongation ranges from 0% (when the subject's shape is perfectly circular) to 100% (when the subject's shape is a line).

How to specify Body elongation

1. Click the **Add** button next to **Body elongation** and click the **Body Elongation** tab.
2. Enter the **Averaging interval**. This is the number of samples over which the running average elongation is based. The default value is 1, that is, the variable is not smoothed using the values of neighboring samples. See Averaging interval
3. Complete the procedure to add the variable. See Calculate statistics: procedure.

Body elongation state

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*.

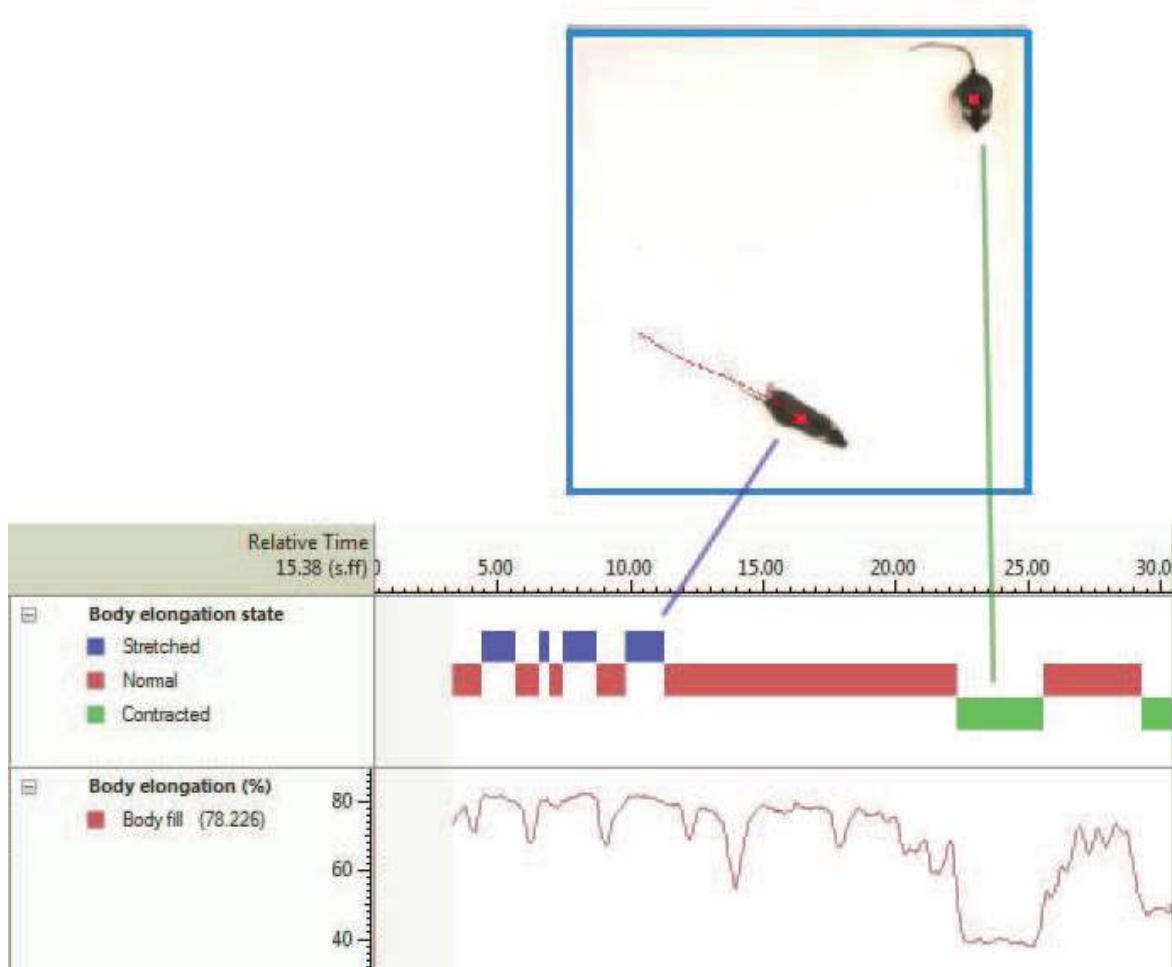
The running average *Body elongation* is calculated for each sample, over the number of samples specified by the Averaging interval.

When a body point is missing for more than three samples, the current Body elongation state ends and the remaining missing samples are ignored.

Calculation

1. The *Body elongation* is calculated for each sample. See Body elongation
2. If you set an averaging interval (see below), the running average is calculated for each sample.
3. The *Body elongation state* variable is determined for each sample, according to the value of running average Body elongation relative to the thresholds you set (see below).

Below: Visualization of *Body elongation state* (top) and *Body elongation* (bottom). The two variables have been set with the same Averaging interval (10 samples). *Stretched* is scored when *Body elongation* is above 80%. *Contracted* is scored when the resampled variable is below 50%.



How to specify Body elongation state

1. Click the **Add** button next to **Body elongation state** and click the **Body Elongation State** 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 *Body Elongation state*.

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*.

To find the optimal Stretched above and Contracted below, run a few test trials and check in the Analysis Results and Scoring pane the values of Body

elongation state when the animal shows such behavior. These values are calculated real-time during acquisition.

Values of elongation percentage between Stretched above and Contracted below result in the subject being scored as *Normal*.

3. Under **Calculate statistics** for, select which state you want to calculate statistic for: **Stretched**, **Normal** o **Contracted**. Select at least one state.
4. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- The elongation measure is independent of video size and the subject's position and orientation.
- The Body elongation thresholds set in Acquisition are not used in analysis. When you specify the Body elongation dependent variable in your Analysis profile, enter the new values in the appropriate fields.
- 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. See Averaging 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.
- In the Detection Settings, use the Dilation and Erosion filter (first erode, then dilate) to remove the animal's tail from the detected image of the subjects. This way you get a more accurate measurement of Body elongation.

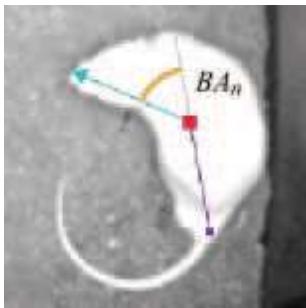
Application

The *Body elongation state* variable can help you assess the frequency and cumulative duration of stretch attend postures in a more objective way.

Body angle

Definition

The angle formed by the prolongation of the segment joining the tail-base and the center-point, and the segment joining the center-point and the nose-point, calculated at the current sample. It is only available with the Multiple Body Points module.



Range

Absolute Body angle ranges from 0° to 180° . *Relative Body angle* ranges from -180° to $+180^\circ$.

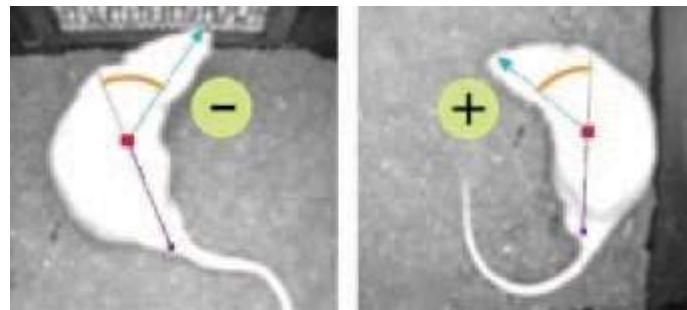
0° occurs when the nose point, center point and tail base point are aligned. Values around $\pm 180^\circ$ are only hypothetical, because they would imply that the animal is completely curled up in such a way that the nose points to the tail base. Values higher than 100° often occur with wrong detection of the body points.

How to specify Body angle

1. Click the **Add** button next to **Body angle**.
2. In the **Body Angle** tab, specify whether you want to calculate:

Absolute angle: The unsigned angle.

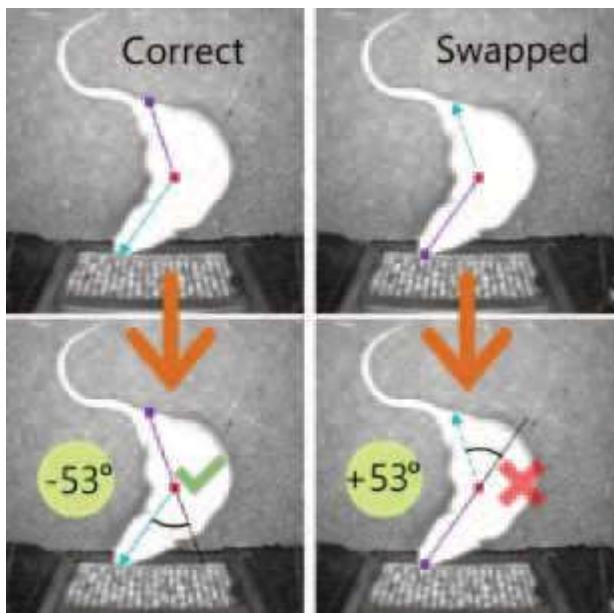
Relative angle: The signed angle. It is negative when the body is bent to the right (clockwise); positive when the body is bent to the left (counterclockwise).



3. Complete the procedure to add the variable. See Calculate statistics: procedure.

Note

When the nose-point and the tail-base point are swapped, and these are not properly corrected in the Track Editor, the value of *relative* body angle is opposite to what it should be. For example, if the actual body angle is $+10^\circ$, EthoVision XT gives -10° . However, *absolute* angle remains correct. Therefore, if your track contains nose-tail base swaps, either correct them in the Track Editor, or use Absolute angle.



Body angle state

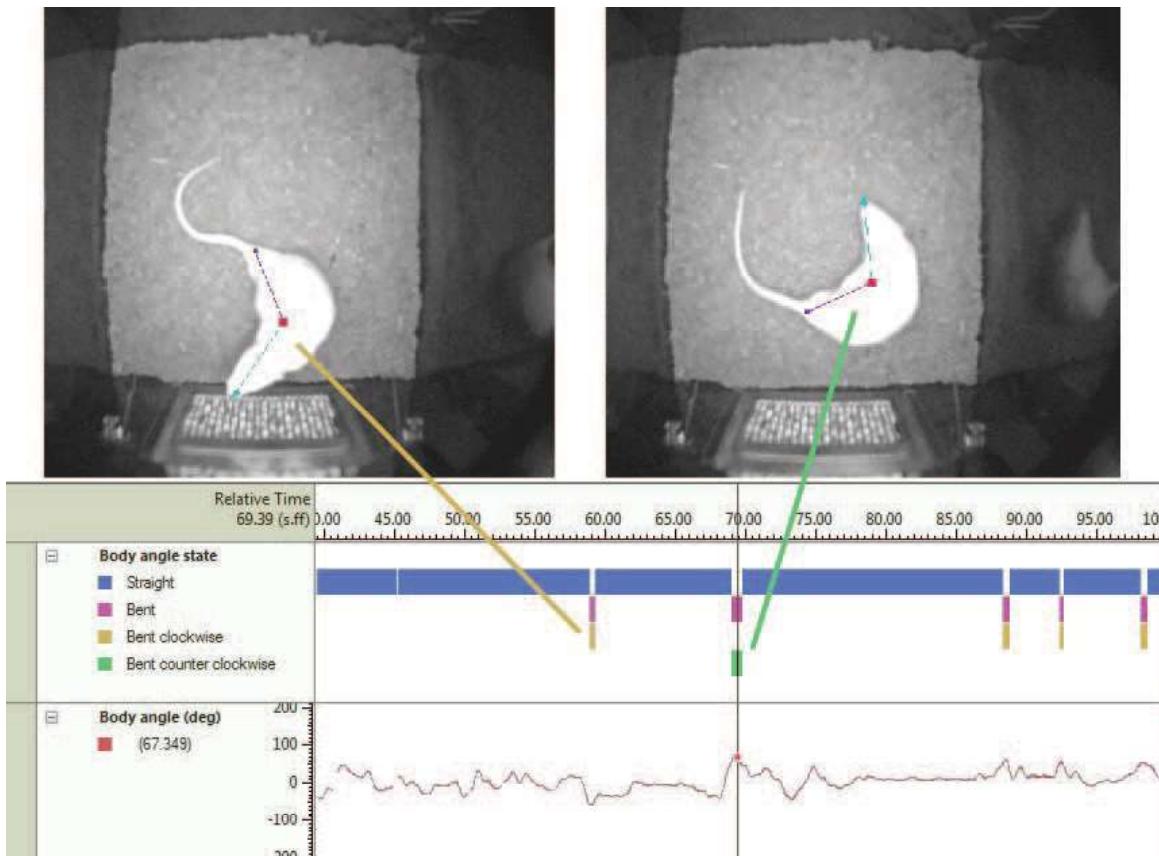
Definition

A discrete (state) variable with two possible states: *Straight* and *Bent*, depending on where the running average body angle (see Body angle for its definition) of the subject calculated for the current sample lays relative to a user-defined threshold:

- If the running average body angle is greater than the threshold value, the state is Bent. This state can be further split in *Bent counterclockwise* and *Bent clockwise* depending on the sign of the angle.
- If the running average body angle is smaller than the threshold value, the state is Straight.

The running average body angle is calculated for each sample, over the number of samples specified by the Averaging interval.

Below: Visualization of *Body angle state* (top chart) and *Body angle* (bottom). Body angle state was based on the Body angle values smoothed with averaging interval of 5.



How to specify Body angle state

1. Click the **Add** button next to **Body angle state** and click the **Body Angle State** tab.
2. Enter the following:

Averaging interval: The number of samples over which the running average body angle is based.

Body bent above: The angle above which the subject is considered to be bent. This value is unsigned and applied to both negative and positive Body angles.

3. Under **Calculate statistics for**, select which state you want to calculate statistic for: **Straight**, **Bent**, **Bent counterclockwise** (that is, when the running average body angle is positive; see Body angle for its definition), and **Bent clockwise** (when the running average body angle is negative).
4. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- When a body point is missing for more than three samples, the current Body angle state ends and the remaining missing samples are ignored.
- **Averaging interval.** The default value is 1. In that case the body angle state is based on the value of *Body angle* at the current sample. If you select a number k higher than 1, EthoVision takes the body angle of the last k samples (including the current sample) and replaces the current value with the average. See Averaging interval

Mobility

Definition

The percentage changed pixels of the detected subject between current sample and previous sample.

Calculation

Step 1 - Calculation of the changed area

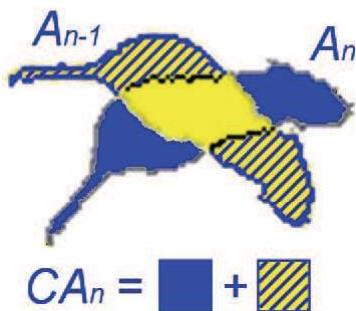
All pixel coordinates of the subjects are determined immediately after they have been detected. Those coordinates are compared with the previous sample to determine the number of changed pixels between the two. The changed pixels are

- 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 $n-1$.



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 increasingly different as it moves faster.

Step 2 - Calculation of Mobility

The formula for Mobility is the changed area for the current sample divided by the sum of the current area and the previous area:

$$\text{Mobility} = \frac{CA_n}{A_{n-1} + A_n} \times 100$$

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 calculated as above is averaged over the number of samples that you specify. 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. See also Averaging interval

When you export data, you do not export any information about which averaging interval was used.

Range

Mobility ranges from 0% (the sample is identical to the one before it) to 100% (no pixel overlap).

How to specify Mobility

Click the **Add** button next to **Mobility**. Select the **Averaging interval**. See step 3-Running average) for more information on the Averaging interval of the Mobility parameter. Click the **Trial Statistics** tab and select the statistics you want to calculate.

Mobility state

Definition

A discrete (state) variable with three possible states: Highly mobile, Mobile and Immobile, depending on where the changed pixels of the detected subject between current sample and previous sample (referred to as changed area) lay relative to two user-defined thresholds.

When the subject goes missing for more than three samples, the current Mobility state is ended and the remaining missing samples are ignored.

- For the difference between Mobility and Movement, see Frequently asked questions about Mobility.
- For the difference between Mobility and Activity, see Frequently asked questions about Activity

Calculation

The *Mobility state* variable is calculated for each sample according to the value of the running average Mobility relative to the thresholds:

- Below the Immobile threshold, the state is *Inmobile*.
- 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 state

1. Click the **Add** button next to **Mobility state** and click the **Mobility State** tab.
2. Enter the following:

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 *Inmobile*. You can enter a number with up to two decimals.

3. 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*.

4. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- To find the optimal Highly mobile and Immobile threshold, run a few test trials and check in the Analysis Results and Scoring pane the values of Mobility when the animal shows such behavior. These values are calculated real-time during acquisition. See an example in Porsolt swim test: view the Mobility state variable.
- Since Mobility is calculated on the detected subject, the gray-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. See Averaging interval
- You set the thresholds during acquisition, 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

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 center-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 center-point (or the nose/tail point). That means that the center-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?*

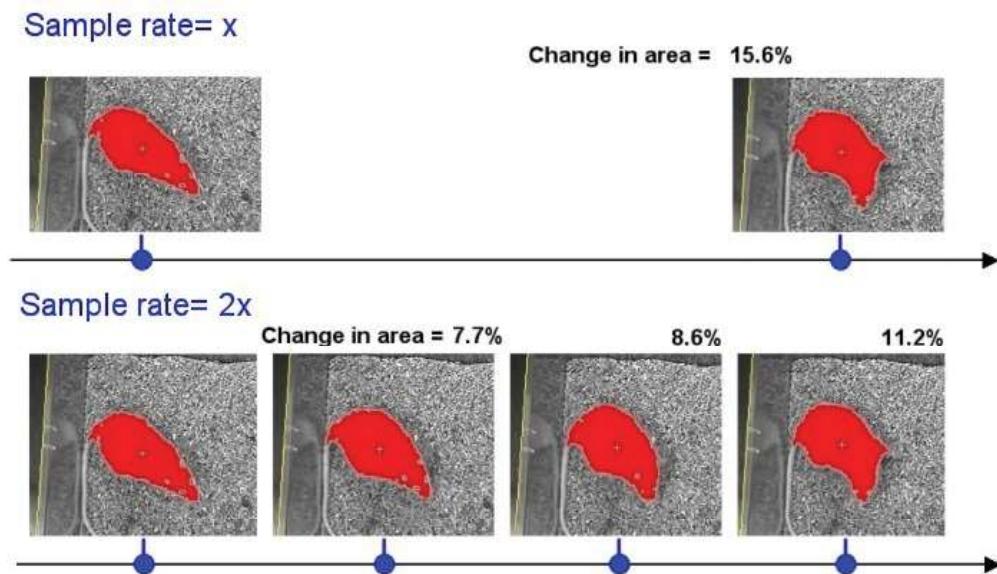
Yes. Since Mobility is detected from the change in area of the subject between two samples, and the change in area depends on how frequently the subject area is acquired (that is, the sample rate), Mobility depends on the sample rate. All being equal, the higher the sample rate, the smaller the change in subject area.

In the example of the figure below, 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.

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.



Applications

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.

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

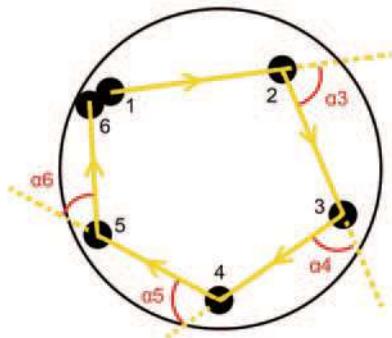
Definition

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.

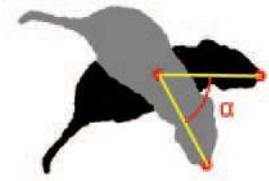
In the figure below, a simplified example of 6 samples from an animal walking around in a circular arena. Rotation is based on the accumulation of the turn angles (α) between heading of the body point (center, nose or tail-base) of the current and previous sample. Also see Notes in Heading.



- *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.

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.

Body axis rotation is based on the accumulation of the turn angles between the axis from center-point to nose-point of the current and previous sample. It is also possible to use the axis from tail-base to center-point (not shown in the picture below).



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 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**.
2. Select whether EthoVision XT should count rotations in a **clockwise** or **countrerclockwise** 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 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:

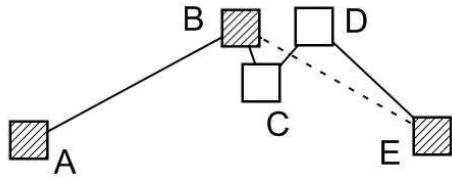
- The Turn angle in the counterclockwise direction does not exceed the Threshold angle: EthoVision XT continues to calculate the cumulative Turn angle until it exceeds the corresponding Rotation count.
- The Turn angle in the counterclockwise direction does exceed the Threshold angle: the cumulative 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 Moved** 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. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

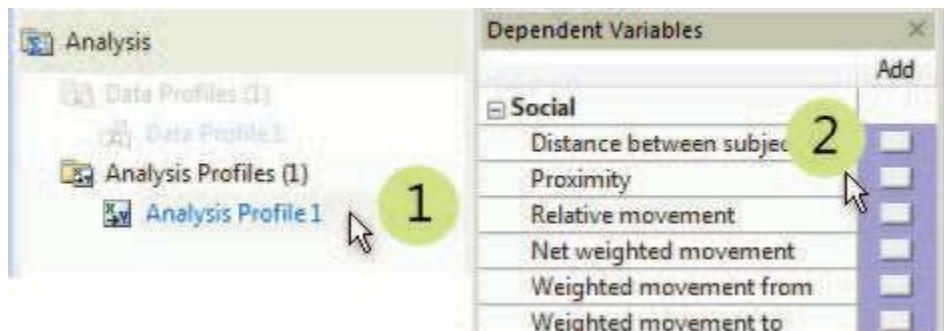
- If you want to count both clockwise and counterclockwise rotations, or if you want to use both methods, add *Rotation* several times. Rename the variables to be able to distinguish between them (Right-click the variable in the Selected Dependent Variables list and choose **Rename**).
- Applying the Minimum Distance Moved filter: Samples are filtered with the Direct method for Minimal Distance Moved. This filter changes the values of turn angle at a specific sample. In the example below, samples C and D are set equal to B 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.



Applications

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.

Social



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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**.
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 **Receivers** tab. Here, you specify the other subjects (*Receivers*).
Under **Select**, choose the subjects you want to calculate the distance from.
Under **Body points**, select the body points of the subjects selected above.
4. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

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'.

Applications

This parameter forms the basis for the Proximity dependent variable used in studies of social or aggressive behavior. For zebrafish shoaling behavior analyzed with Distance between subjects in EthoVision XT, see Green *et al.* (2012) *J. Neurosci. Methods*, **210**, 266– 271.

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 for more than three samples, the current *Proximity* state ends and the remaining missing samples are ignored.

Calculation

For each sample, the program calculates first the Distance between subjects, 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**.
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.
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 **Receivers** tab. Here, you specify the other subjects (Receivers).

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.

6. 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.

- **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.

7. Complete the procedure to add the variable. See Calculate statistics: procedure.

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. 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.

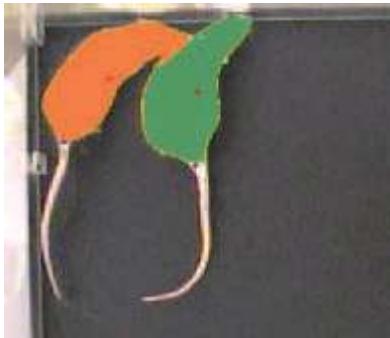
Application

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. Neurosc. Methods* **59**: 157-167).

Body contact

Definition

A discrete variable with two possible states, *In contact* and *Not in contact*, based on whether the subject's body contour is in contact with the body contour of any of the other subjects.



IMPORTANT Body contact is only available when the tracks have been recorded with EthoVision XT 12 or higher. Data from older experiments will not produce results.

How to specify Body contact

1. Click the **Add** button next to **Body contact**.
2. In the **Body contact** tab, select the state you want to analyze.
For example, select **Body Contact** if you want to know how often or how long the subjects were in contact during the test.
3. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- An animal's contour is determined by computer vision, which is unlike human vision. This is exemplified when dark animals are tracked: often their shadow on the background will be considered part of the animal. Thus, the detected contour is regularly a bit bigger than the human eye would observe.
- *Body contact* is not subject-specific. That is, you cannot isolate instances of contact for a specific combination of subjects (for example, Subject 1 in contact with Subject 3). Instead, use *Proximity* and specify the two subjects.

- *Body contact* is based on the subject's contour, while *Proximity* is based on the distance between subject's body points. For example, two subjects next to each other may be in proximity, but not in body contact.
- Body contact is affected by the track smoothing methods The Minimal Distance Moved smoothing method and The Maximum Distance Moved smoothing method. When you apply one of those methods, Body contact is recalculated and may be slightly different with respect to when the method is not applied. The difference is usually limited to one or a few samples.

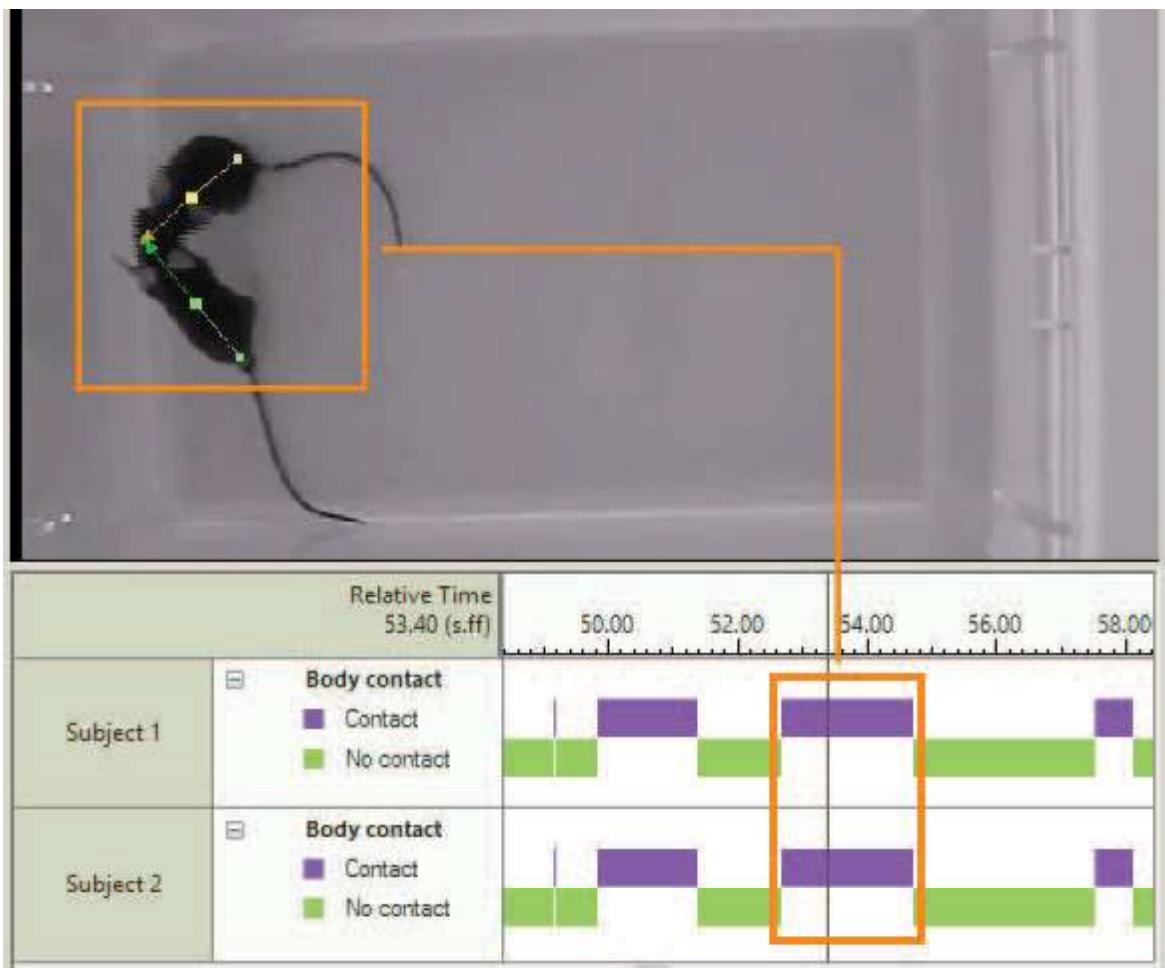
Applications

Body contact can be used in any social interaction tests:

- To quantify the level of interaction between subjects. Add **Body contact** in the Analysis profile (see above).
- To exclude instances of body contact when these result in unreliable tracking. In this case, instead of adding *Body contact* in the Analysis profile, in the Data profile choose Nesting over Body contact and select **Not in contact**.

Below: Integrated Visualization of *Body contact* in a 2-subjects test.

TIP In Integrated Visualization, the body contour is not visible. To view the body contour, open **Acquisition**, click the **Show Hide** button, choose **Detection Features** and select **Body contour**.



Relative movement

Definition

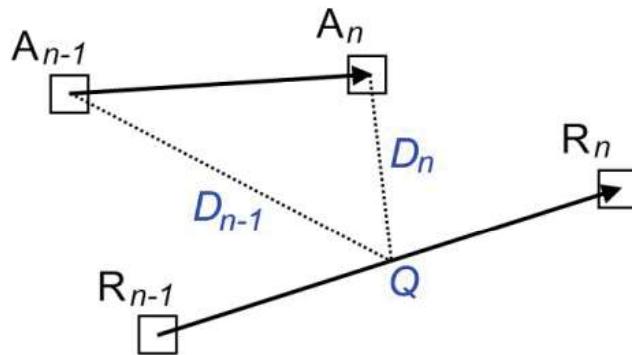
A discrete variable with four possible states, *Moving to*, *Moving from*, *No relative movement*, and *No interaction*.

- 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 .

In this example, 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*

If $D_n >$ Maximum interaction distance (user defined): *No interaction*.

From the figure above, one can see that the outcome depends on which animal is considered as Actor and which as Receiver. In that case, A is Moving 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 R would be considered Moving from A.

How to specify Relative movement

1. Click the **Add** button next to **Relative movement**.
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. 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 **Receivers** tab. Here, you specify the other subjects (Receivers).

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.

- **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).

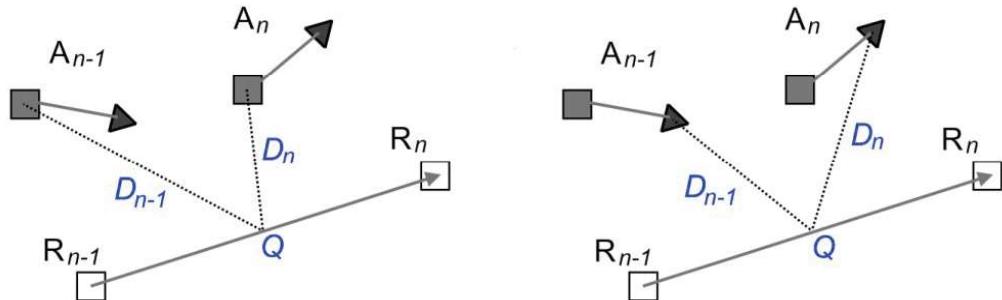
- **Each point:** A state is defined for each body point of the Receiver. Results are shown for each Receiver body point separately.

5. Complete the procedure to add the variable. See Calculate statistics: procedure.

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 the figure below. 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 (see below and also the last note).

Below: 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** 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.



- **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 *Moving to* Subject 2 and *Moving from* Subject 3. In such cases when you select **Any selected Subject**, multiple states are assigned to that sample. You can check multiple states being assigned to one sample time when exporting the dependent variable. 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.
- 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

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.

From the figure in Relative movement it can be seen that the outcome depends on which subject is considered as Actor and which as Receiver. In that case, 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**.
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.

4. Click the **Receivers** 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. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- *Net weighted movement* 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).

Calculation

Formula:

- If $D_n - D_{n-1} > 0$, then

$$WMF_n = (D_n - D_{n-1}) * \text{abs}(DS_n - DS_{n-1}) / (\max(DS_n, DS_{n-1}))$$

- If $D_n - D_{n-1} \leq 0$, or $DS_n = 0$, then

$$WMF_n = \text{missing value}$$

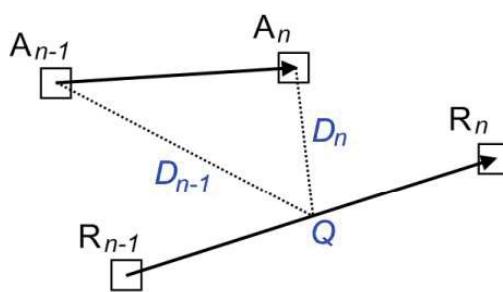
Where:

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.

DS_{n-1}, DS_n is the distance between the subjects' body points for two samples.

Weighted movement from is not calculated when $DS_n=0$, and $DS_n >$ Maximum interaction distance.

Below: Illustration of the dependent variable *Weighted movement from*. 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.
- From the figure above one can see 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 that case, 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**.
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 **Receivers** 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. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- *Weighted movement from* 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).

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. DS_{n-1}, DS_n is the distance between the subjects' body points for two samples (see Distance between subjects).

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 have a lower weight, so they can be distinguished from movements at close distance, which have a different biological meaning.
- From the figure in *Weighted movement from* one can see 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 that case, 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**.
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 **Receivers** 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. Complete the procedure to add the variable. See Calculate statistics: procedure.

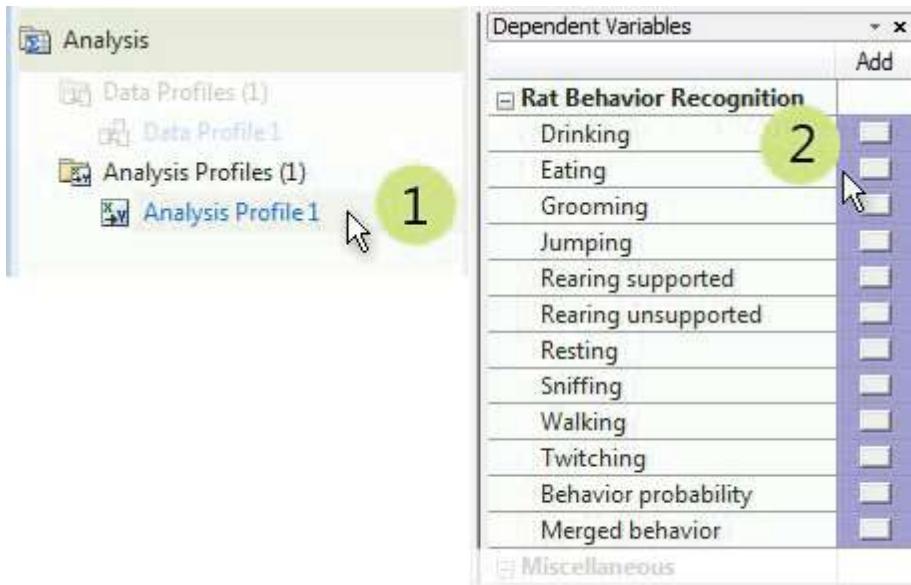
Notes

- *Weighted movement to* 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).

Behavior recognition



The Rat/Mouse Behavior Recognition group of dependent variables is only available when you have the Rat or Mouse Behavior Recognition add-on module of EthoVision XT, respectively.

Behaviors detected with Behavior recognition 911

Behavior probability 920

Merged behavior 921

Behaviors detected with Behavior recognition

Definitions

See also the reference in Notes.

Digging

Rooting with the muzzle or digging with the front paws in the bedding material (for Mouse Behavior Recognition only).

Drinking

The subject licks at the spout of the water bottle.

Eating

The subject eats at the feeder or from the floor, or is eating while holding food in front paws.

Grooming

The subject grooms snout, head, fur or genitals. Includes scratching and licking of paws during a grooming session.

Hopping

The subjects moves forward with both hind limbs at the same time. Head goes first, followed by the rear. Unlike Jumping, Hopping is scored as a point event with no duration, and does not interrupt behavior states (for Mouse Behavior Recognition only).

Jumping

The subject moves quickly forward with both hind limbs at the same time. Jumping interrupts other states like Walking (for Rat Behavior Recognition only).

Rearing unsupported

The subject stands in an upright posture, with front paws not in contact with any object. Includes the rise and descend.

Rearing supported

The subject stands in an upright posture, leaning with front paws against the cage-wall. Includes the rise and descend.

Resting

The subject rests with hardly any moving, either sits or is lying down. Includes sleeping. Apparently no interest in the environment.

Sniffing

The subject makes slight movements of the head, possibly with slight, discontinuous body displacement. Includes sniffing the air, the wall, the floor and other objects.

Twitching

The subject makes sudden and short movements of the body or head. Includes body shake and head shake. Twitching is scored as a point event with no duration, and does not interrupt behavior states (for Rat Behavior Recognition only).

Twitching is defined as a point event, that is, an event marking a point in the time line, but with no duration.

Walking

The subject moves to another place, and hind legs move as well.

Note the difference between *Walking* and *Movement*: Both dependent variables Walking and Movement are based on displacement of the subject's body point in the 2D space. However, Walking is based on many more video frames than Movement, and therefore Walking is a more accurate measure of walking behavior.

How to specify a behavior for Rat or Mouse behavior recognition

1. Click the **Add** button next to the behavior you want to analyze.
2. Under **Behavior decision method**, select:

Default: Statistics will be calculated by using all samples scored as that behavior (no filtering based on the behavior's probability).

Probability greater than: Statistics will be calculated by using only the samples for which the probability of that behavior is higher than a specific value. Select this value from the list (0-99%).

3. Under **Behavior duration threshold**, next to **Exclude instances shorter than**, enter how long the current state must last before it changes from the previous state (in seconds). If the bout length passes this threshold, the previous samples in the bout are included in the new state. If the bout length does not pass the threshold, the previous state ends, but no new state is defined.
4. Under **Calculate statistics for**, select which state you would like to calculate statistics for.
5. Complete the procedure to add the variable. See Calculate statistics: procedure.

Accuracy

According to our tests, the average recall rate, that is, the proportion of ground truth manually-scored behaviors that are correctly recognized, is around 70%. For more information, see the paper mentioned below.

- *Drinking* can be confused with *Sniffing* especially when the Drinking Spout Points are not defined.
- Make sure to draw the Feeder Zones in the Arena Settings to maximize the detection accuracy of *Eating*.
- To maximize accuracy of detection of *Rearing supported* and reduce false positives, carefully draw the Wall zone in the Arena Settings.

Notes

- For *Twitching* and *Hopping*, which is an event with no duration, the Behavior duration threshold and the **Calculate statistics for** options are not available.
- For more information on Behavior recognition in EthoVision XT, see the following paper:

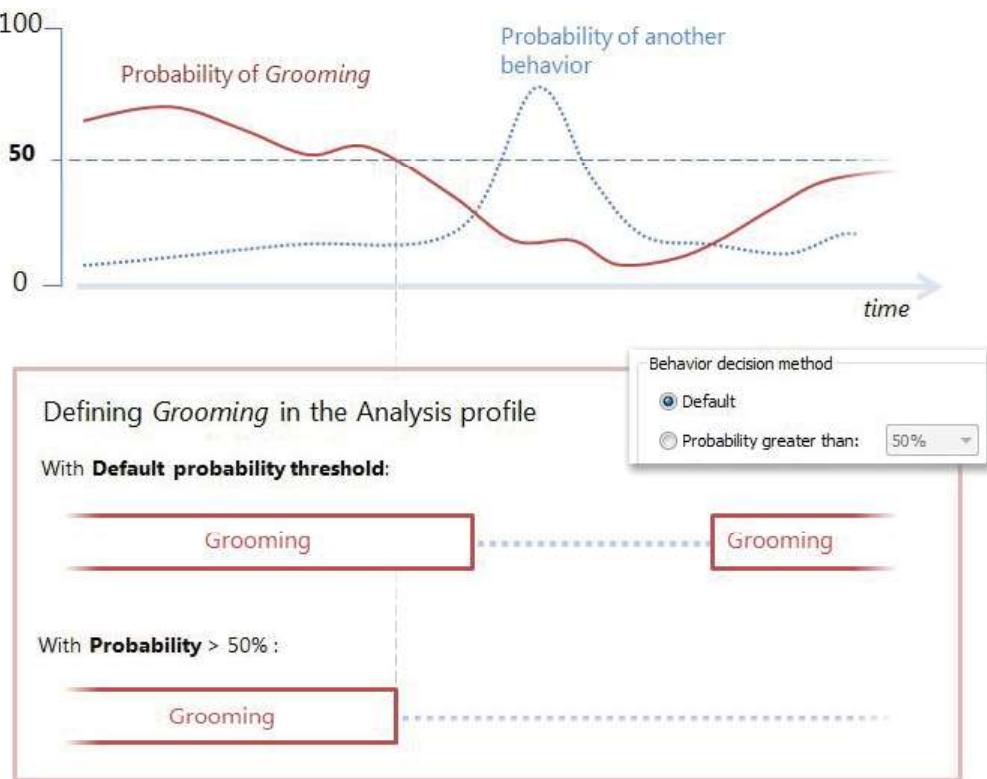
van Dam, E., J.E. van der Harst, C.J.F. ter Braak, R.A.J. Tegelenbosch, B.M. Spruijt, L.P.J.J. Noldus (2013). An automated system for the recognition of various specific rat behaviours. *Journal of Neuroscience Methods* **218** (2), 214–224.

<http://dx.doi.org/10.1016/j.jneumeth.2013.05.012>

- The Behavior decision method acts as a filter to ignore samples for which the behavior is associated with a low probability, making it easier to extract more reliable data. By default, EthoVision XT uses the original scores (thus no filtering based on probabilities). See the figure below for an example.

Top: The probability of *Grooming*, and another behavior plotted against time (for simplicity, other behaviors are ignored here). Bottom: When defining *Grooming* in the Analysis profile, consider the following options:

- With **Default** selected, Grooming occurs every time its probability exceeds the probability of any other behavior (default behavior recognition).
- With **Probability greater than 50%** selected, Grooming is limited to the samples for which its probability is higher than 50%. The higher the value, the more conservative the definition.

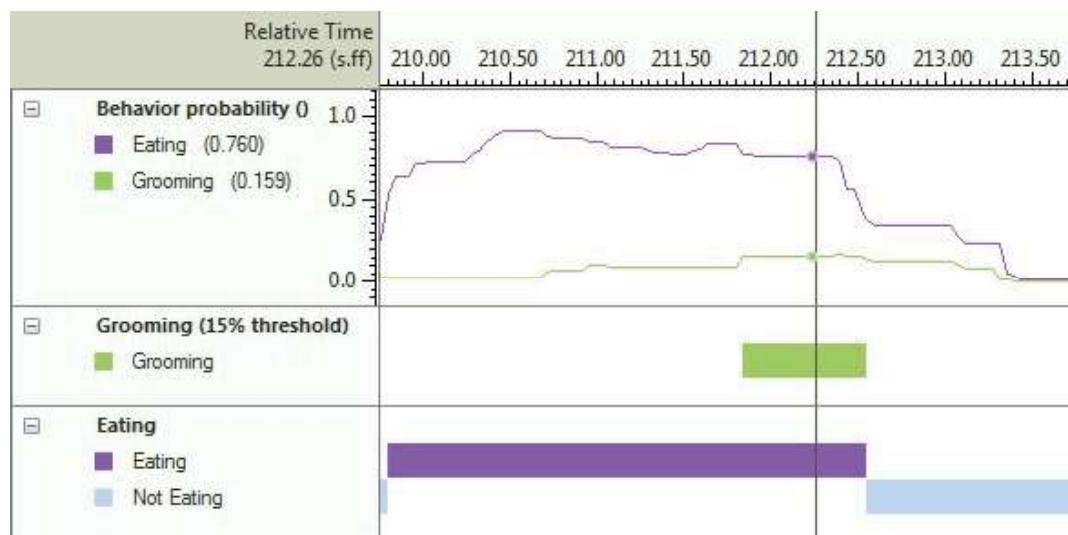


- The **Behavior duration threshold** acts as a filter to ignore short transitions between states. For example, if the behavior *Grooming* is scored for 0.8 s and you select a Behavior duration threshold of 1.0 s, the *Grooming* state does not exceed that threshold, and is therefore excluded from analysis.
- For all behaviors but *Twitching* and *Hopping*, the percentage statistics are calculated including *Unknown*. For example, when *Grooming* lasts 30 seconds, *Not Grooming* 1 min 30 s, and *Unknown* 30 s, then the Cumulative Duration within Track (%) for *Grooming* is $30/150 = 20\%$. If you want to exclude *Unknown* from the percentage, in the Data profile make two

nesting intervals, one with [behavior name] and the other with Not [behavior name], then combine the two Nest boxes in parallel. Next, in the Analysis profile specify the behavior and its statistic, Cumulative Duration within Nesting (%). In the example above, the result would be $30/120 = 25\%$.

- The duration of the behaviors may not add up to 100% of the track duration, also when including Unknown. This could result, for example, when you filter a behavior using a probability threshold. When visualizing behaviors, samples filtered out appear as gaps between behaviors.
- When you specify a Behavior probability threshold for one behavior, the other behaviors (which are mutually-exclusive by definition) are not recalculated according to that setting. Depending on the value of the probability threshold, the behavior may become overlapping with other behaviors. For example, if you select a probability threshold of 15% for *Grooming*, some parts of the track may be scored as *Grooming* where also *Eating* was scored based on the default settings (see below). To prevent this from happening, either use Default or choose the same probability threshold for all behaviors.

Below: Time-event plots of behaviors *Grooming* and *Eating*. Top: Behavior probability. Middle: *Grooming* is defined with a Behavior probability greater than 15%. Bottom: *Eating* is defined with Default probability settings, and is scored for most of the time due to the high probability (see the probability plot). As a result, *Grooming* is partially overlapping with *Eating*, although the two behaviors are supposed to exclude each other. With default settings, *Grooming* would not be scored.



See also

- How behaviors are scored in Behavior recognition

How behaviors are scored in Behavior recognition

Basic rules

For each sample in your track, EthoVision XT calculates a probability value for each behavior (see Behaviors detected with Behavior recognition). The sum of probability values is 100%.

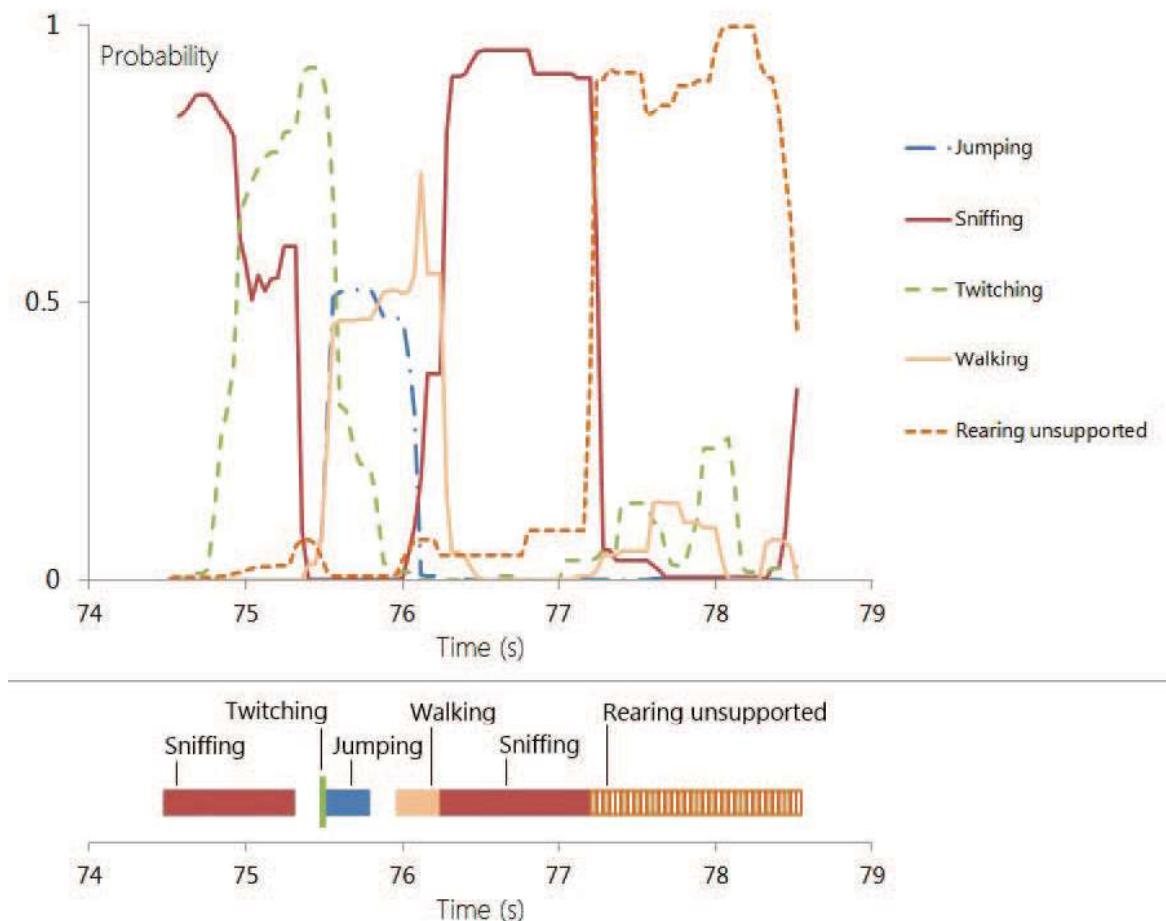
All behaviors but Twitching and Hopping are mutually-exclusive states, that is, only one can be assigned to a sample. A behavior is assigned to a sample based on a number of decision rules that take into account the probability of that behavior relative to that of others, and the behaviors assigned to adjacent samples. See also the figure below.

- *Twitching* and *Hopping* have their own probability, but are not compared with that of the others. As a result, *Twitching* and *Hopping* are scored independent of other behaviors. Each may be assigned to a sample also when one of the other behaviors is scored for that sample. For example, *Hopping* when *Walking* is active.
- When two or more behaviors (excluded *Twitching* and *Hopping*) have similar probability values, and the same behaviors are not scored in adjacent samples, or when all behaviors have probability values below 25%, then none of the behaviors are scored for that sample. This results in gaps between scored behaviors when you visualize them in the Integrated Visualization.

Example

Below you see an example of how behaviors are scored based on their per-sample probability values. Top: X-Y chart of probability (here ranging from 0 to 1) of five behaviors (others are not shown for simplicity). Bottom: The resulting scored behaviors. In general, the behavior is assigned to a sample that has the highest probability (see for example *Sniffing* at the beginning of the time line). However, the state is smoothed based on the values in a number of samples before and after the current sample. For example, at 75 s *Sniffing* has a lower probability than *Twitching*, but the state *Sniffing* is still active. Note that *Twitching* is scored as a point event, with no duration, and is represented with a vertical segment.

The gap between scored states is caused by samples where two or more behaviors have similar probability values (for example at about 75.9 s, with *Jumping* and *Walking* having probabilities around 0.5) or the probability is below 0.25 for all behaviors. The example is taken from Rat Behavior Recognition data.



Behavior, "Not" Behavior and "Unknown"

Each behavior type, except *Twitching* and *Hopping*, has three complementary, mutually-exclusive states. For example, *Grooming*, *Not Grooming* and *Unknown*.

At any sample, the "Not" behavior is automatically scored if the corresponding behavior is not scored, unless Unknown is scored for that sample (see below).

Behavior "Unknown"

A behavior type is scored as *Unknown* in the following cases:

- At the beginning of the track if the Trial Control Settings rule does not include an additional Time condition that waits three seconds before the start of tracking, and in the last portion of the track (see Behavior recognition: Data, performance and accuracy).
- When the nose and tail base points are not detected.

- In all cases when the subject is not found or samples are missed for more than 0.4 s.

Statistics of *Unknown* can be calculated for all behaviors except *Twitching*. The statistics are the same for all behaviors. For example, the duration of *Unknown* calculated for *Grooming* is equal to that of *Unknown* for *Rearing supported*.

Behavior probability

Each behavior is associated with a probability, calculated per sample. Whether a behavior state is scored for a specific sample depends on a set of decision rules (for details see How behaviors are scored in Behavior recognition).

All behaviors but *Twitching* and *Hopping* are mutually-exclusive, therefore the sum of their probability values is 1. *Twitching* and *Hopping* have a probability too, but whether they are scored at a specific time does not depend on the probability of other behaviors.

How to specify Behavior probability

1. Click the **Add** button next to Behavior probability.
2. In the **Behavior Probability** tab, select the state(s) you would like to calculate the probability for.
3. Complete the procedure to add the variable. See Calculate statistics: procedure.

Application

You may want to filter the occurrences of a behavior based on their probability values. If the probability of that behavior is lower than a threshold at a specific sample, that sample is left out and the behavior state is re-calculated (see an example in Behaviors detected with Behavior recognition).

1. In the Analysis profile:
 - Add the behavior you are interested in.
 - Add the Behavior probability variable and, in the window that appears, select the same behavior.
2. Plot the integrated data to view the behavior detected and its probability together with the video. Check how those states correspond with the subject's behavior in the video.
3. To filter data based on probability, in the Analysis profile click the behavior variable, and select a probability value for Behavior probability threshold.
4. Plot the data again to see the result of filtering.

Merged behavior

You can define a behavior state that includes two or more of the behaviors detected automatically. For example, to calculate the overall frequency of Rearing = *Rearing supported* + *Rearing unsupported*.

How to specify Merged behavior

1. Click the **Add** button next to **Merged Behavior**.
2. Under Select behaviors to merge, select which behaviors you would like to merge for analysis.

For example, select Rearing supported and Rearing unsupported to analyze the total occurrences of Rearing.

3. Under **Behavior probability threshold**, select:

Default: Statistics will be calculated by using all samples scored as that behavior (no filtering based on the state's probability).

Probability greater than: Statistics will be calculated by using only the samples for which the probability of one of the merged behaviors is higher than a specific value. Select this value from the list. See a note below.

4. Under **Behavior duration threshold**, next to **Exclude behavior instances shorter than**, enter the minimal duration of the merged behavior to be considered for analysis.

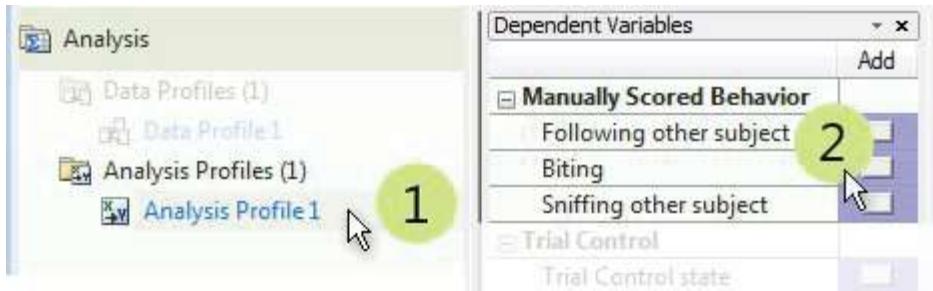
NOTE The threshold is applied to the merged behavior, not the instances of single behaviors. For example, two instances of behaviors Rearing unsupported and Rearing supported follow each other in the time line. The first lasts one second, the other two seconds. When you merge the two behaviors and select a duration threshold of 3 seconds, the two behaviors are considered for analysis.

5. Complete the procedure to add the variable. See Calculate statistics: procedure.

Note

The Frequency of *Merged behavior* may not always be equal to the sum of the frequencies of the single behaviors. This can happen, for example, when an instance of *Rearing supported* is immediately followed by an instance of *Rearing unsupported*. Then the frequency of *Merged behavior* is 1, not 2.

Manually scored behavior



Definition

Behaviors defined in the Manual Scoring Settings are analyzed as dependent variables with discrete states, like *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.
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 name]**. Selecting **Not [behavior name]** means that the program calculates the statistics for the time that the behavior was not active (see below).

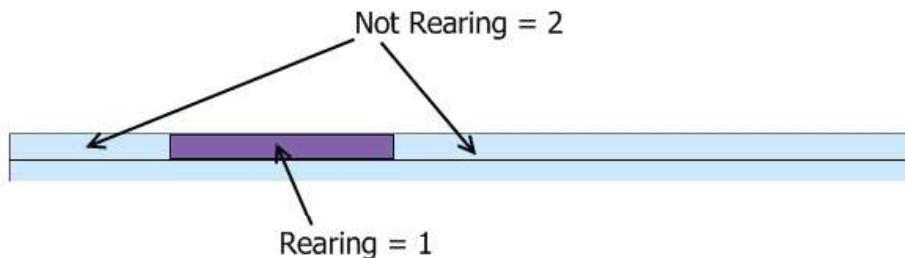
3. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- **Not [behavior name]:** 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*.

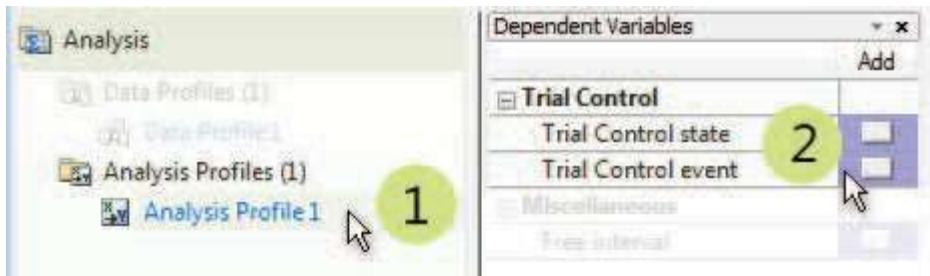


The cumulative duration of *Not [behavior name]* is the sum of all the periods of time that the behavior was not active.

See also

- Export manually-scored behaviors

Trial Control



- Trial Control event 925
- Trial Control state 927

See also

For **Hardware** variables related to devices like the Pellet dispenser, the Lickometer, PhenoWheel or the PhenoTyper Top Unit, see the **Analysis of Trial Control data** in the Reference Manual - Trial and Hardware Control in EthoVision XT. To open this manual, choose **Apps > Noldus > EthoVision XT Other Documentation**.

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).

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**.
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. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- Statistics of Cumulative 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 Trial Control events based on Conditions, the sample time at which a Trial Control event is scored also depends on the statistic used in the condition:

If you use Current to define the condition (for example: "when Current In zone= true"), the Trial Control event is scored at the expected sample time. For example, when the animal enters the zone (and thus Current= true for that sample). See also A in the figure in Trial Control state, where the start of the Trial Control state is like a Trial Control event.

If you use any other statistic to define the condition (for example, "when Frequency of In zone >= 1), consider the following scenarios:

- When the condition becomes true after the condition box is activated, the Trial Control event is scored at the expected sample time. See How the trial control instructions are executed for an explanation of the terms *true* and *active*.
- When the condition is already true when the condition box is activated (for example, a condition "Frequency of In zone =1" is activated when the animal is already in the zone), such statistic is only evaluated at the next sample (or in the second next sample, in the case of Heading). In that case, the Trial Control event is scored one sample (or two) later than expected from the condition (see B in the figure in Trial Control state). However, for the consecutive frequencies of In zone =2, 3, etc, the condition is already active by definition; therefore the Trial Control event is scored at the expected time, when the condition becomes true.

See also

- The Reference Manual - Trial and Hardware Control in EthoVision XT. To open this manual, in the Apps screen under **Noldus** choose **EthoVision XT 14 Other Documentation**.

Application

You can use *Trial control event* 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.

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.
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.
4. Select **Ignore last interval if incomplete** to ignore the interval when the Trial Control event that defines the end of the state is not found. If you do not select this option, and the end criterion is *not* met, EthoVision XT defines an interval up to the end of the trial.

EXAMPLE A Trial Control state is defined "from Trial start to when the condition "Subject in zone A" becomes true. If the subject never enters zone, then the end criterion is never met. If you select the Ignore option, the Trial Control state is not defined. If you do not select the option, the Trial Control state is defined from the start to the end of the trial.

5. 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.
6. Complete the procedure to add the variable. See Calculate statistics: procedure.

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 cumulative 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.
- 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.

Trial Control states based on Conditions

The sample time at which a Trial Control state starts (or ends) also depends on the statistic used in the condition in the Trial Control rule.

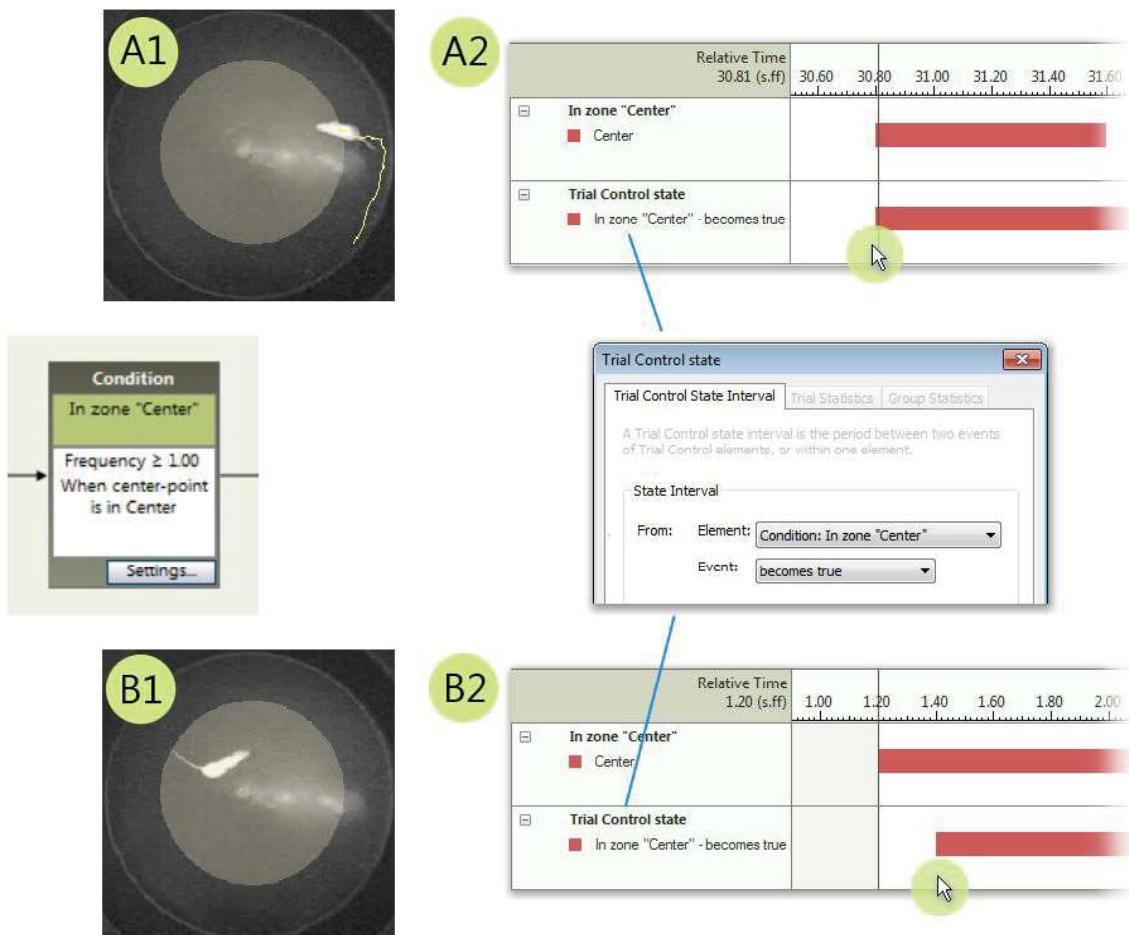
- If you use **Current** to define the condition (for example, "when Current In zone= true"), the Trial Control state starts (or stops) at the expected sample time. For example, when the animal actually enters the zone (and thus Current= true for that sample).
- If you use any other statistic to define the condition (for example, "when **Frequency** of *In zone* >= 1) consider the two scenarios:
 - When the condition becomes true after the condition box is activated (see How the trial control instructions are executed for an explanation of the terms *true* and *active*), the Trial Control State starts (or stops) at the expected sample time (see A in the figure below).
 - When the condition is already true when the condition box is activated (for example, a condition "Frequency of In zone =1" is activated when the animal is already in the zone), such statistic is only evaluated at the next sample (or in the second next sample, in the case of Heading). In that case, the Trial Control state starts (or stops) one sample (or two) later than expected from the condition (see B in the figure below). However, for the consecutive frequencies of In zone =2, 3, etc, the condition is already active by definition; therefore the Trial Control State starts at the expected time, when the condition becomes true.

EXAMPLE A Trial Control condition has been defined in the Trial Control Settings, which checks that the statistic **Frequency** of the dependent variable *In zone* is $>= 1$ for the Center of the open field. A Trial Control state has been defined in the Analysis profile, which starts when the condition “Frequency of *In zone* $>= 1$ ” is true.

A1: The animal enters the Center zone (and therefore the condition becomes true) after the condition is activated. The Frequency statistic is evaluated at the same sample time. A2: Plot of the variables *In zone* for the Center and the Trial Control state. The Trial Control state starts at the expected sample time, that is, when the animal actually enters the zone.

B1: The animal is already in the zone when the condition is activated. Therefore, the condition becomes true at the same time that it is activated, but the Frequency statistic is evaluated at the next sample (here, sample rate = 5/s, thus after 0.2 s).

B2: Same plot as A2; Here, the Trial Control state starts 0.2 s after the *In zone* state.



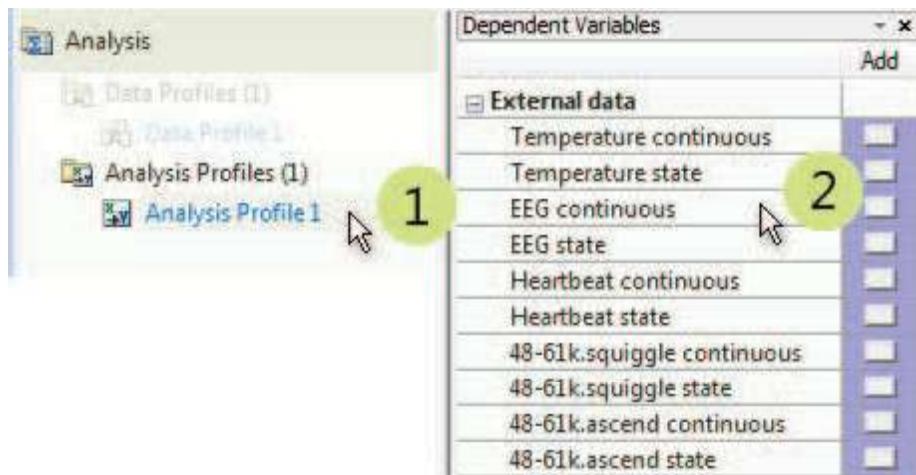
See also

- The Reference Manual - Trial and Hardware Control in EthoVision XT. To open this manual, in the Apps screen under **Noldus** choose **EthoVision XT** **14 Other Documentation**.

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.

External Data



- External data (resampled) 932
- External data - state 936

External data (resampled)

Definition

EthoVision XT only analyzes external data after *resampling*. That is, it does not analyze the original data, but a Dependent variable representing the imported signal, resampled to the same sample rate as the EthoVision XT sample rate. This is accomplished by combining upsampling and downsampling (see below).

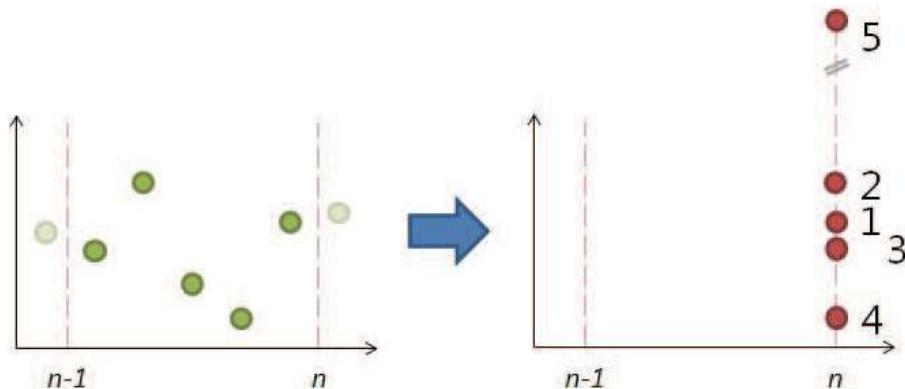
Downsampling and Upsampling

Downsampling

If the sample rate of the external data signal is higher than the EthoVision XT sample rate, the signal is downsampled. For each sample n , a new value is calculated from the values of the original signal in the interval $(n-1; n]$ (or in multiple preceding sample intervals, depending on the Averaging interval chosen). The interval is half-closed, that is, values at the time of n , not $n-1$, are also considered. The new value gets the time stamp of the corresponding sample n .

Five methods are available to downsample the signal.

Below: Effect of downsampling of an external data signal. Left: original signal imported in EthoVision XT. Vertical hatched lines represent the EthoVision XT sample intervals. Right: Signal upsampled in EthoVision XT using the following five methods (for simplicity, only the values for sample n are shown). **1** Last value, **2** Maximum, **3** Mean, **4** Minimum, **5** Total value.

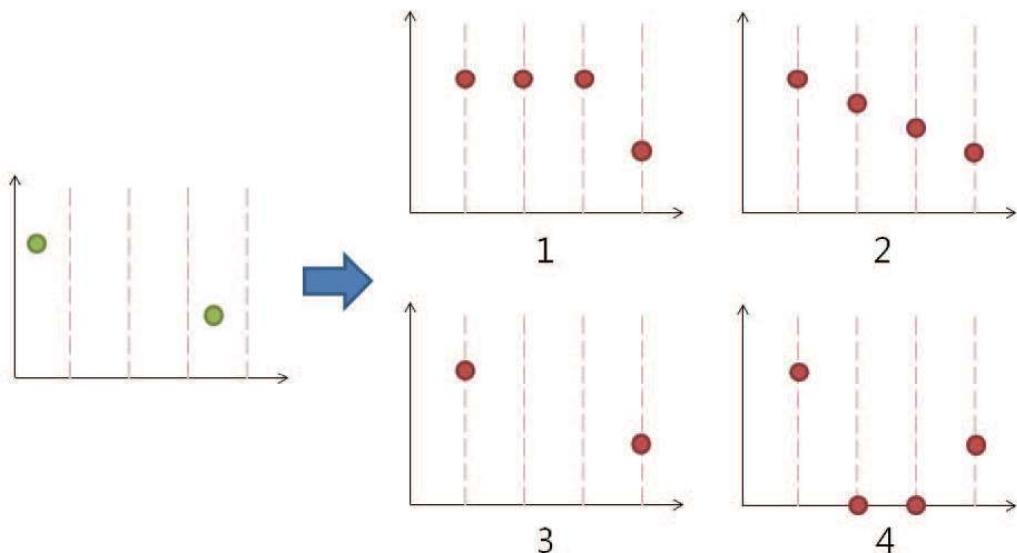


Upsampling

If the sample rate of the external data signal is lower than the EthoVision XT sample rate, the signal is upsampled.

For each sample n , a new value is calculated from the values present in the sample interval $(n-1; n]$ (or in multiple preceding sample intervals, depending on the Averaging interval chosen). If no value is found, A new value is interpolated using one of the methods available, or replaced by "missing sample" or a zero value.

Below: Effect of upsampling of an external data signal. Left: original signal imported in EthoVision XT. Vertical hatched lines represent the EthoVision XT sample intervals. Right: Signal upsampled in EthoVision XT in the following four methods: **1** Last value, **2** Linear interpolation, **3** Missing value, **4** Zero value.



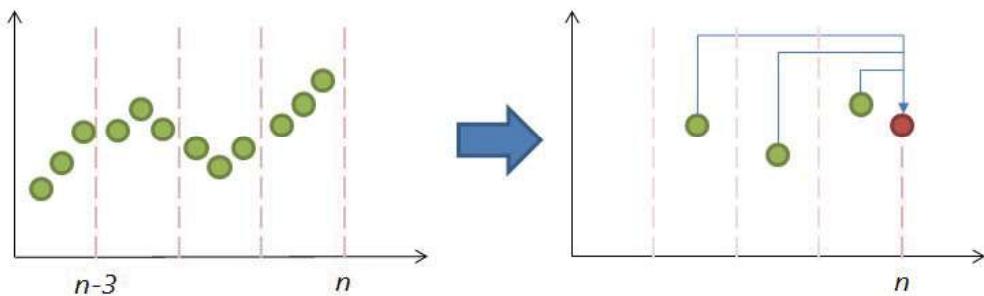
Averaging interval

With Averaging interval you can smooth the values of the converted signal. This has an effect on both resampled and state variables.

- With Averaging interval = 1, the new value for sample n only depends on the original values of the signal in the interval $(n-1, n]$.
EXAMPLE Downsampling a EEG signal with the Mean value method and the Averaging interval of 1. See **3** in the figure under Downsampling.
- With Averaging interval = x (where $x=2, 3, \dots$), the new value for sample n depends on the original values of the signal in the interval $(n-x, n]$.

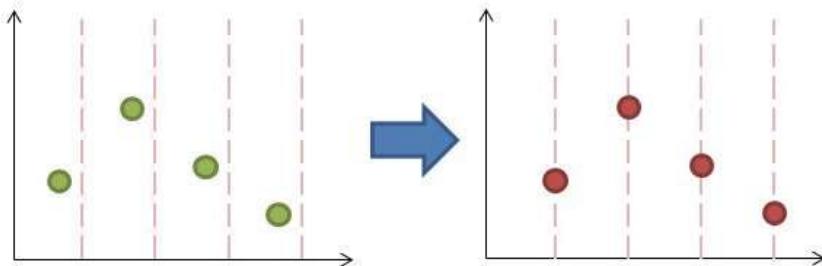
EXAMPLE Downsampling a EEG signal with the Mean value method and the Averaging interval of 3.

Left: Original EEG signal. Right: Signal downsampled with Mean value and Averaging interval =3 (for clarity only sample n is shown). Green dots= averages in the 3 sample intervals. Red dot= the average of the three.



Special case: Resampling

If the sample rate of the external data signal is the same as the EthoVision XT sample rate, but with different time stamps, the signal is re-sampled to align its values to the EthoVision XT samples. Values are shifted to the right.



How to specify an external data variable (resampled)

1. Click the **Add** button next to the [data set name].
2. Under **Select**, choose the **Downsampling method** (default: **Mean value**) and the **Upsampling method** (default **Last value**).
3. Under **Outlier filter**, select the **Averaging interval** (default = 1).
4. Complete the procedure to add the variable. See Calculate statistics: procedure.
5. Plot Integrated Data or calculate the statistics.

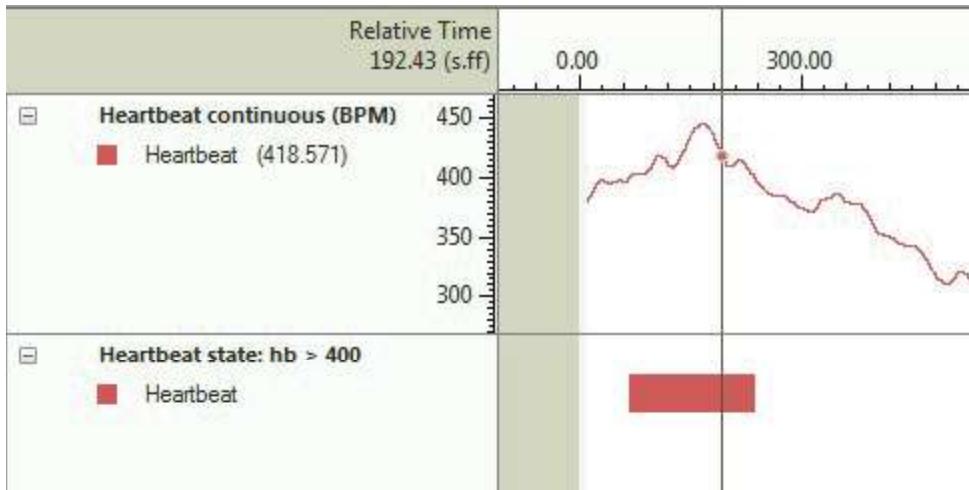
Notes

- If you upsample a signal with a sample rate much lower than the EthoVision sample rate, and you choose **Missing value** as the **Upsampling method**, it may be difficult to see the data points. In Integrated Visualization, choose **Show/Hide > Show Graph Data Points** and zoom in the plot.
- See also Averaging interval

External data - state

Definition

Dependent variable with two possible values (0 and 1) calculated from an imported data signal, resampled to the same sample rate as the EthoVision XT sample rate. The value of the state variable is calculated per sample from the values of the corresponding resampled variable, relative to one or two thresholds.



Top: Heartbeat. Bottom: Heartbeat state, defined in the Analysis profile as "Heartbeat > 400 bpm, with Downsampling= Mean value, Upsampling= Last value, Averaging interval =1". For clarity it is renamed to Heartbeat state: hb >400.

How to specify an external data variable "state"

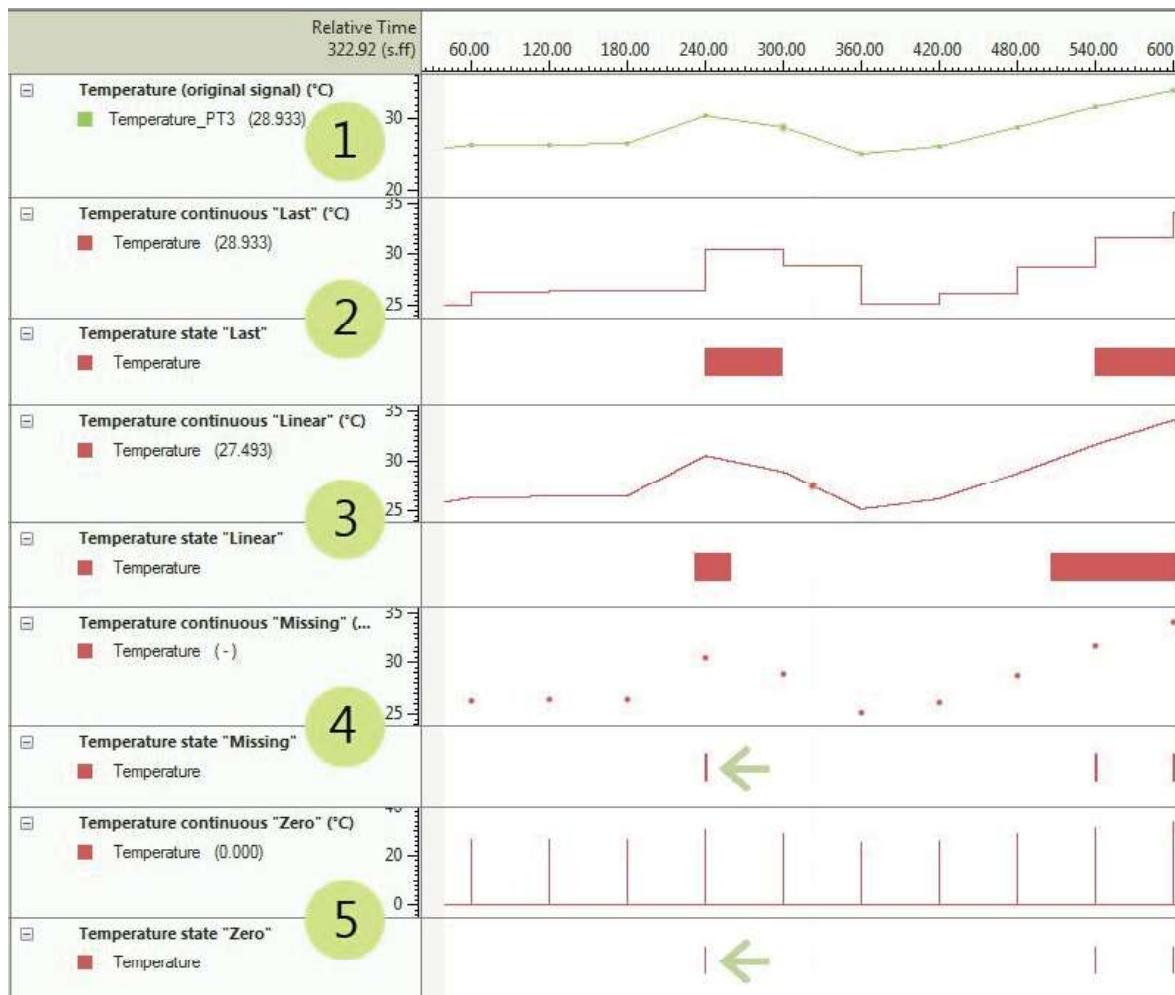
1. Click the **Add** button next to the [data set name] state.
2. Under **Select**, choose the Downsampling method (default: Mean value) and the Upsampling method (default Last value).
3. Under **Outlier filter**, select the **Averaging interval** (default = 1). See Averaging interval in External data (resampled).
4. Under **State threshold**, specify that the state is active when the **Signal is**:
 - above (\geq) or below (\leq) a threshold value x .
 - within a range ($\geq x_1$ and $\leq x_2$) or outside a range ($\leq x_1$ and $\geq x_2$).

Enter the thresholds you require.

5. Complete the procedure to add the variable.
6. Plot Integrated Data, or calculate the statistics.

The resulting state (frequency and duration) is much dependent on what you choose as downsampling/upsampling methods.

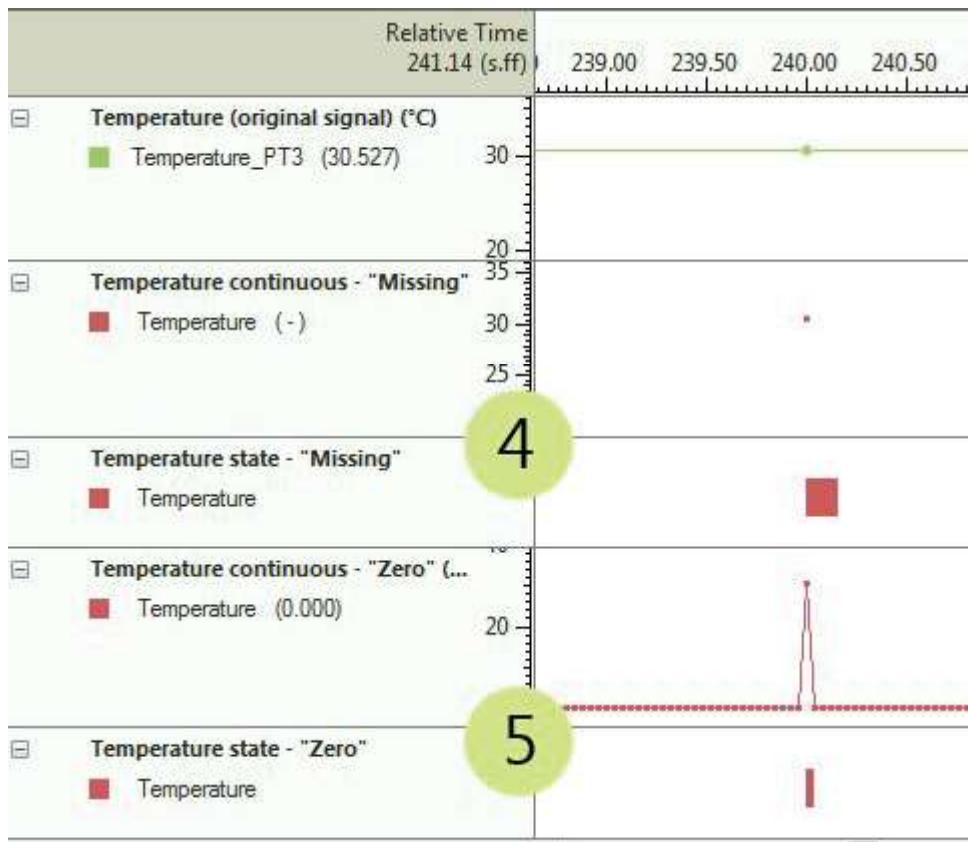
Below: Effect of different upsampling methods on a state variable Temperature state. **1** Original temperature signal sampled every minute. **2-5** Variables resampled at 25 Hz. The state variable is scored when the resampled value is $\geq 30^{\circ}\text{C}$, using the methods Last value (**2**), Linear interpolation (**3**), Missing value (**4**), and Zero value (**5**). Note that Temperature state in cases 4 and 5 gives very short states (indicated by the arrows), because most of the data points in the corresponding Temperature resampled variable are missing or zeroes, respectively. See the next picture for (**4**) and (**5**).



NOTE When calculating states, if the sample with a valid variable value is followed by missing samples, EthoVision XT keeps the calculated value of the state for the next three missing samples.

Below: Zoomed-in view of the previous picture for Temperature state upsampled with Missing value (**4**), and Temperature state upsampled with Zero value (**5**),

around 240 s. A state is scored when the resampled value is $\geq 30^{\circ}\text{C}$. For simplicity, Averaging interval = 1. When calculating states, if the value of the resampled variable is followed by missing values, like in Temperature - "Missing", the calculated value of the state is kept for next 3 samples. This is the reason why the Temperature state - "Missing" around time 240 s lasts 4 samples (see 4). In the case of upsampling with Zero value, the state is assigned only to the current sample.



How to retrieve states from an imported signal

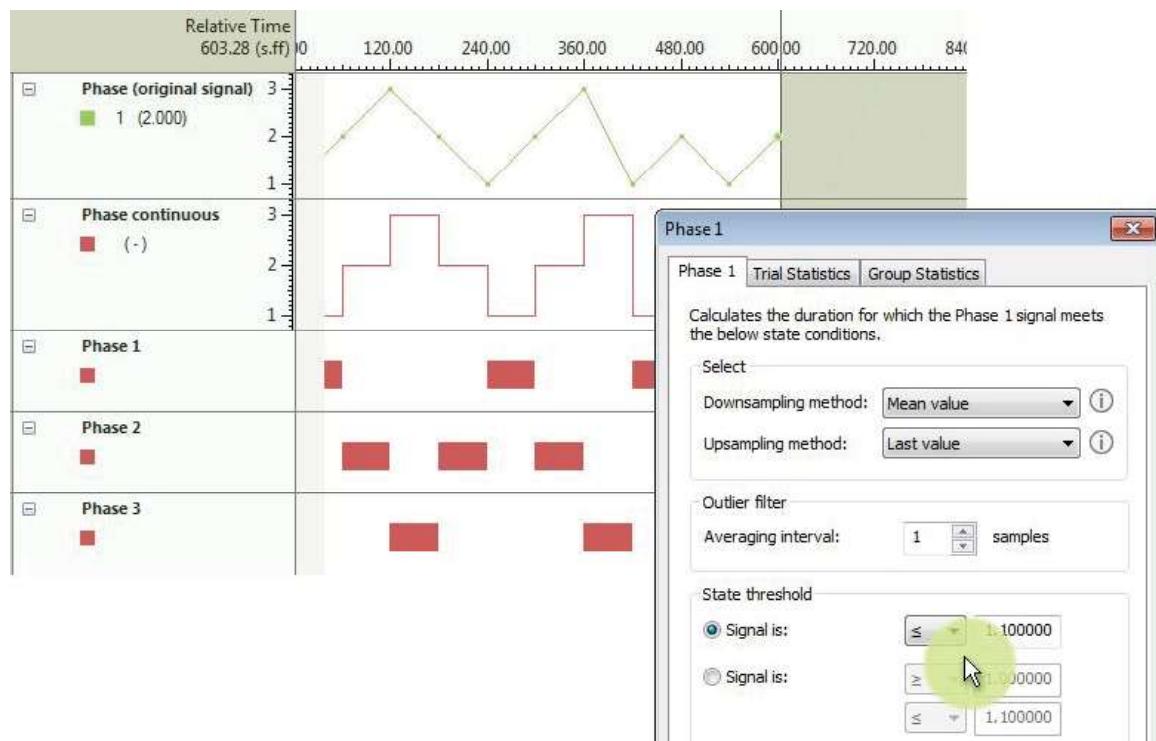
In the following example, the file containing the data set Phase with possible values 1, 2 and 3 is imported:

```
Start date; 12/18/2012
Start time; 11:31:55.5
Phase_PTS
time; Phase
s; Value
0;1
60;2
120;3
180;2
240;1
300;2
360;3
420;1
480;2
```

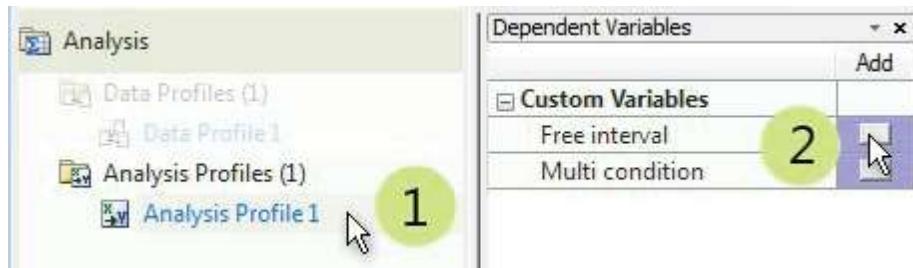
If you want to retrieve the original states with their duration:

1. In the Analysis profile, define three Phase state variables, and rename them to, for example, Phase 1, Phase 2, and Phase 3.
2. For each state variable, set the appropriate thresholds. For example, for Phase 1: **Signal is** ≤ 1.5 .
3. Plot the variables to compare them with the original signal.

From top to bottom: original signal, resampled signal, states calculated using thresholds. Right: threshold for Phase 1.



Custom Variables



- Free interval
- Multi condition

Free interval

Definition

A segment of track from sample A to sample B, where A and B are determined by a time, the value of another dependent variable, a Trial Control event, or the value of a signal from a hardware device.

Use cases

See Examples of Free intervals in the Analysis profile

Calculation

A Free interval is defined by a *Start criterion* and a *Stop criterion*. Depending on whether and how often the criteria are met within a track, a Free interval may result in zero, one or more *instances* of the interval within the same track.

NOTE Like all intervals defined with Nesting, a Free interval is left-closed [A, B). That is, the start sample A, not the stop sample B, is included in the analysis.

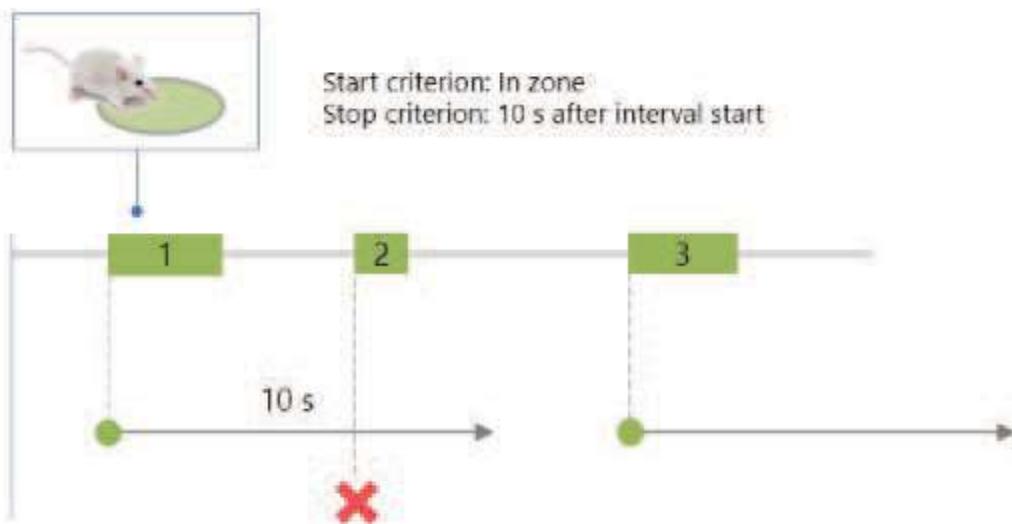
Multiple instances of the same interval definition

When you define a Free interval, this may result in zero, one or more (up to 2000) instances of an interval depending on where the Start and Stop criteria are met.

An instance of a free interval starts at the sample time when the Start criterion is met, and stops at the sample time when the Stop criterion is met.

A new instance is defined if the Start criterion and Stop criterion are met again, after the time in the track that the last Stop criterion was met.

NOTE Multiple instances of the same free interval definition cannot overlap. An interval is NOT defined when its start criterion falls within a previous instance of the same interval definition. For example, you define a Free interval that starts when the subject enters zone A, to 10 seconds after that time. If a new instance of *In zone A* occurs before the end of the interval (see the green bar marked with 2 in the figure below), that instance of In zone is ignored.



Similarly, if you define a Free interval that goes from track start to when the external data *Heart rate* > 400, then one interval is defined, from Track start to the time when Heart rate reaches 400 bpm for the first time. This because the "Track start" criterion is met once in each track, no matter how many times Heart rate reaches 400 bpm.

Multiple free intervals in the Analysis profile

You can define multiple intervals in the Analysis profile, by repeating the procedure below. The interval definitions are independent of each other, therefore the resulting intervals may be overlapping.

To specify a Free interval

1. In the Analysis Profile, under **Custom Variables**, click the button next to **Free Interval**.
2. Under **Start criterion** select the starting point of the interval:

Time. To select a time. For example, **Track start** (that is, the first sample in a track) or an **Elapsed time** after the track start (format: H:mm:ss.fff). For example 0:01:00.00 for one minute after the start. **NOTE** Track start corresponds to the first sample in the track (Recording time = 0.0), not the Trial time. For multiple-subject setups, Track start is the first sample of the animal detected first.

Dependent Variable. To select the time that a dependent variable has a specific value. For example, *Distance moved* >= 1 m, or frequency of *In zone*

≥ 10 . This list also includes behaviors scored manually and external data. See Free interval based on a dependent variable

Trial Control. To select the time that an event in your trial control procedure occurs. For example, when a condition becomes true, or a command to a hardware device is given. See Free interval based on a Trial Control event

Hardware. To select the time that a Hardware-related variable has a specific value. For example, when the number of food pellets dropped by a Pellet Dispenser is greater than 10. This option is only available when hardware devices are defined in your experiment. See Free interval based on hardware

3. Under **Stop criterion** select the end point of the interval. See above for most of the options.

If you choose **Time**, and under **Interval stop** you choose **Elapsed time**, you have two options: **after track start** to stop the interval at a specific time after the track start (that is, the first sample in the track); **after start event** to stop the interval a specific time after the event selected as start criterion. Note that this “start event” is not the same as the start time of the resulting interval, if you set some time before the event (for example, 10 s before the frequency of *In zone* becomes 1). The “start event” is when the Frequency of *In zone* becomes 1. However, the interval starts 10 s earlier.

4. Select **Ignore last interval if incomplete** to ignore the interval when the stop criterion is not met. If you do not select this option, and the stop criterion is not met, EthoVision XT defines an interval up to the end of the track.
5. A free interval may occur once or more in a track (see above). To analyze each instance separately, select **Calculate statistics per interval** and enter the number of intervals you want to view in the results (1 to 100). To be sure you to view all intervals, enter a large number that exceeds a maximum possible number of instances per track.

To analyze all instances as one, de-select **Calculate statistics per interval**.

6. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- Note the difference between Free Interval in the Analysis Profile (this topic) and Nesting over a Free interval in the Data Profile.

You define a Free Interval in an Analysis Profile when you want to analyze the interval itself, not other dependent variables within that interval. For example to calculate the duration of an interval.

You define a Free Interval in a Data Profile when you want to analyze any dependent variable *within the resulting track segment*. For example, to calculate the average velocity of the subject in the interval.

- If a start criterion is not met in a track, EthoVision XT does not define the Free intervals in that track.
- If a stop criterion is not met in a track, EthoVision XT does the following:
If you selected **Ignore last intervals if incomplete**, it does not define the interval.
If you did not select that option, it defines an interval up to the end of the track.
- *Free intervals in multiple-subjects setups.* A free interval is calculated for each subject separately, based on the data (dependent variables, behavior etc.) for that subject. See Free interval based on multiple subjects
- *Free intervals and missing samples.* A free interval is not interrupted if one, two or three consecutive missing samples occur. With four or more consecutive missing samples, the current interval is interrupted and a new one starts when the Start criterion is met.
- *Contiguous intervals.* When two instances of a Free interval are contiguous, for example when the Interval Start criterion is supposed to be met immediately after the Interval Stop criterion for the previous instance is met (see Example 3 in Examples of Free intervals in the Analysis profile, where a track is split in segments of equal path length), one sample is always excluded between the end of an instance and the start of the next instance. For example, the first instance goes from time 0 (included) to sample 100 (excluded), then from sample 101 (included) to sample 250 (excluded). Samples 100 and 250 are not included in the analysis. The reason is that the statistic used to find the start of the interval is updated one sample after the end of the previous interval.

More details

- Free interval based on a dependent variable
- Free interval based on a Trial Control event
- Free interval based on hardware
- Free interval based on multiple subjects
- Examples of Free intervals in the Analysis profile

Free interval based on a dependent variable

Background

This topic contains details about defining a Free interval. For the main procedure, see Free interval.

NOTE Dependent variable here also includes external data and manually-scored behaviors, not behaviors of Rat and Mouse Behavior Recognition.

To specify the dependent variable

Stop criterion:

Time
 Dependent variable
 Trial Control

Interval stop

Stop at: H:mm:ss.fff after event:

Select variable: Distance moved

Statistic: Total

of: Distance moved of the center-point

is: \geq cm

Settings...

In the Free Interval window, under **Start criterion** or **Stop criterion**, choose **Dependent variable**.

1. In the **Start at / Stop at** field, enter the time of the start (or stop) of the interval relative to the event described by the variable.

EXAMPLE 1 To start an interval 1 s before the animal enters a zone, next to **Start at** enter 0:00:01:000 **before event**.

EXAMPLE 2 To stop an interval 10 s after the total distance moved reached 10 m, next to **Stop at** enter 0:00:10:000 **after event**.

2. From the **Select variable** list, choose the dependent variable.

For example **In zone** if you want the interval to start or stop when the animal enters a zone for the nth time, or has spent a specific time in that zone.

3. From the **Statistic** list, choose the statistic of the dependent variable. For example:

- **Frequency** if you want the interval to start/stop when the animal has entered a zone for the 10th time,

- **Cumulative Duration** if you want the interval to start/stop when the animal has spent a specific total time in that zone.

See Statistic: When to use Frequency, Current, or others

4. Click the **Settings** button to specify the dependent variable more in detail.

For example, which zone the animal should enter to define the stop of the interval, or which body points should be in the zone. See Dependent Variables in Detail

5. Next to **is:** specify which value the statistic should have to define the start/stop of the interval, using the operators available (\leq , \geq or **false/true**).

Return to To specify a Free interval

Notes

- The following dependent variables are not available as start/stop criteria: *Activity state*, *Acceleration state*, and the behaviors of Rat or Mouse Behavior Recognition.
- A Free interval can only start at the time that, or *before* (not after) a variable reaches the specified value. Similarly, a Free interval can only end at the time that, or *after* (not before) a variable reaches the specified value.
- If you have imported external data in an experiment, you can define an interval based on the values of the resampled external data, not the original (imported) external data. Choose the external data from the **Select variable** list.

See External data (resampled) and Examples of Free intervals in the Analysis profile

- If you want to define a free interval based on two values of external data, for example Heart rate between 300 and 400 bpm) then do not use Free

intervals. Instead, use the dependent variable under External data in the Analysis profile.

Statistic: When to use Frequency, Current, or others

- **Frequency.** To define an interval that starts/stops when the animal has entered a zone or has shown certain behavior (*Moving*, *Highly mobile*, *Grooming*, etc.) a number of times.
- **Current.** To define an interval that starts/stops when a variable reaches a specific value. For discrete variables like *In zone*, the Current statistic can be false or true.

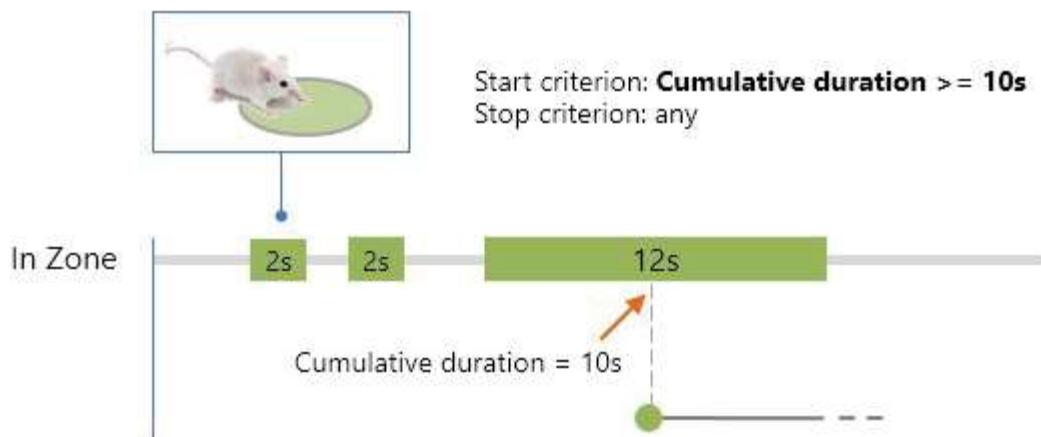
EXAMPLES

For *In zone*, Current= true means that the interval starts/stop as soon as the animal enters the selected zone. Current= false means that the interval starts/stops as soon as the animal is outside the selected zone.

For *Velocity*, Current ≥ 10 means that the interval starts/stops as soon as the animal's velocity is higher then or equal to 10.

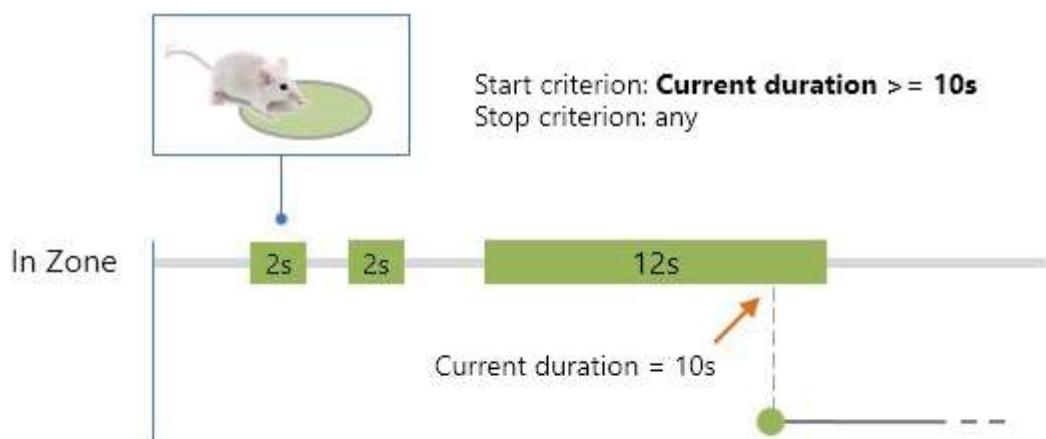
- **Total.** This is used especially with *Distance moved*. This makes the interval starts/end when the animal has covered a specific distance since the start of the track or the start of the interval.
- **Cumulative Duration.** To define an interval that starts/stops when the animal has been in a certain state (that is, within a specific zone, or in the state *Highly mobile*, etc.) for a specific total time since the start of the track or the start of the interval. It does not matter if the subject's state was sometimes interrupted.

EXAMPLE The interval starts when Cumulative duration of *In zone* ≥ 10 s. The green dot indicates where the interval starts.



- **Current Duration.** To define an interval that starts/stop when the animal has been in a certain state (within a specific zone, or in the state *Moving*, etc.) for a specific time without interruption.

EXAMPLE The interval starts when Current duration of *In zone* = 10 s. The green dot indicates where the interval starts.



- **Latency to first.** To define an interval that starts/stops when the time to the first instance of an event (for example, the animal being in a certain state or within a zone) has reached a specific value.

How dependent variables are calculated to find the stop of an interval

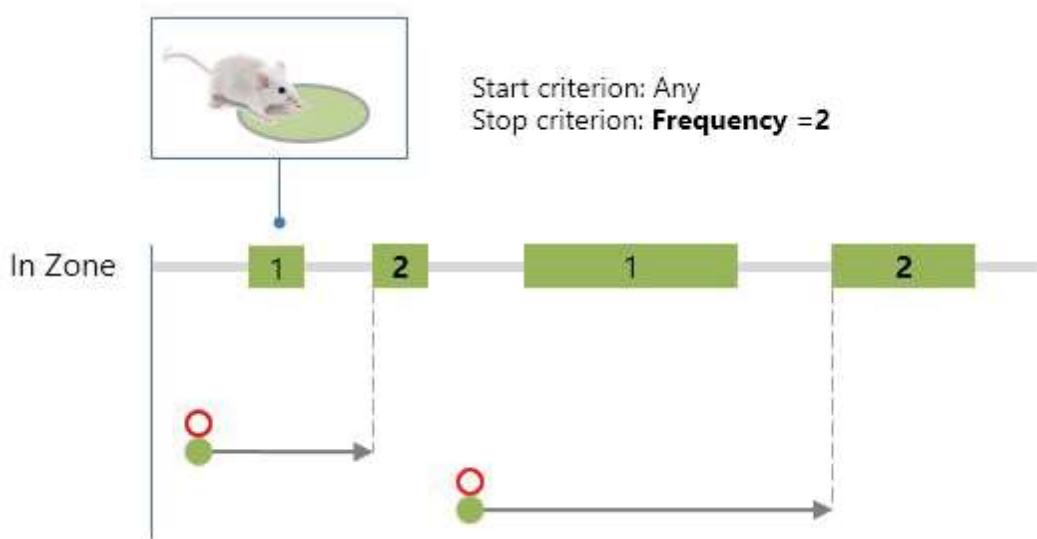
Where in the track an interval ends depends on the value of the statistic of the dependent variable used. The following information applies to the statistics Total, Frequency, Current Duration, Cumulative Duration, and Latency to first.

Once the start point of an interval is found, EthoVision XT resets the statistic at that point, and starting from the next sample time, updates its value until it matches the stop criterion.

- If the stop criterion is met, the interval is fully defined.
- If the value of the statistic does not match the stop criterion before the end of the track, the result depends on whether you select the option **Ignore last interval if incomplete**. See Free interval

EXAMPLE 1 The statistic Frequency for the dependent variable In Zone. A free interval is defined that ends when the Frequency of *In zone* is equal to 2. In the figure below, the green bars represent the time when the subject is in the specified zone (numbers indicate frequency). The green dots indicate when the Start criterion is met (for sake of simplicity, it can be any criterion). The arrows represent the free

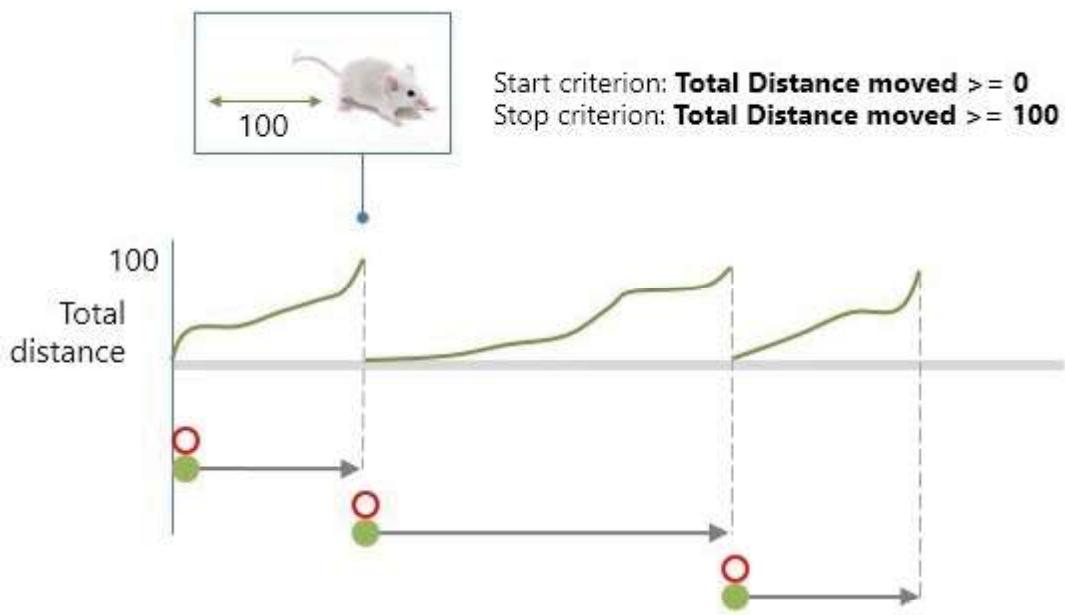
intervals defined. The red circles show when the statistic Frequency for *In zone* is reset to zero.



EXAMPLE 2 The statistic Total for the dependent variable Distance moved. A recurring free interval is defined to split the track in segments of 100-inch distance moved.

- Interval start: Dependent variable Distance moved, Statistic Total ≥ 0 .
- Interval stop: Dependent variable Distance moved, Statistic Total ≥ 100 .

When the second, third, etc. interval starts, the total Distance moved is reset to zero (see the red circles) and updated, starting from the next sample time, until the Stop criterion is met.



Other statistics

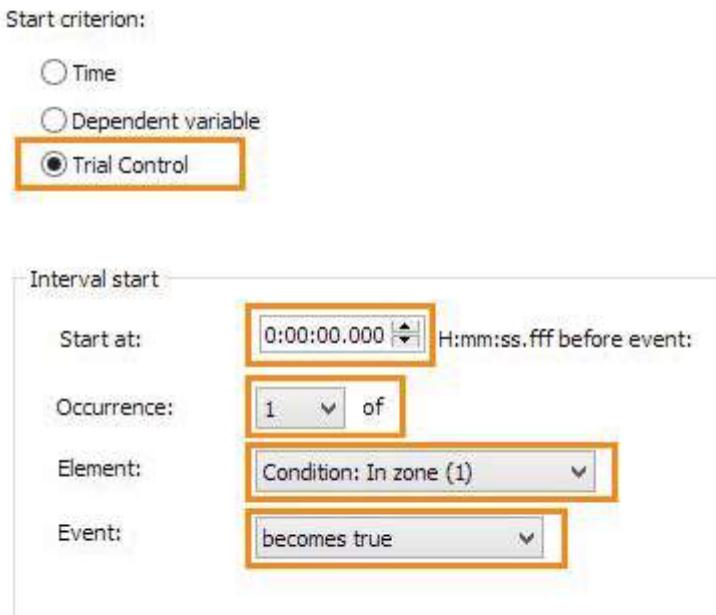
For other statistics like Current (for example In zone or Not in zone) EthoVision XT evaluates the value at the current sample, and checks that the Stop criterion is met.

Free interval based on a Trial Control event

Background

This topic contains details about defining a Free interval. For the main procedure, see Free interval.

To specify the Trial Control event



In the Free Interval window, under **Start criterion** or **Stop criterion**, choose **Trial Control** and select the following:

1. In the **Start at / Stop at** field, enter the time of the start (or stop) of the interval relative to the Trial Control event specified in the next steps.
EXAMPLE 1 To start an interval 1 s before a Trial Control condition becomes true, next to **Start at** enter 0:00:01:000 **before event**.
EXAMPLE 2 To stop an interval 10 s after the Trial Control action "Drop pellet", next to **Stop at** enter 0:00:10:000 **after event**.
2. From the **Occurrence** list, select which occurrence should mark the start of the interval (1st to 9th).

Select **1st** if you want to define an interval from the first time that a trial control event occurs. Select **2nd**, etc. when the trial control event occurs repeatedly, for example in sub-rules.

3. From the **Element** list, select the trial control category (condition, action, sub-rule, etc.).
4. From the **Event** list, select the event. The options differ depending on the Element chosen.

For actions, sub-rules: **becomes active/inactive**.

For conditions: **becomes active/inactive**, and **becomes true/false**.

For sub-rule references: **becomes active/inactive**, and **makes sub-rule active/inactive**.

Return to [To specify a Free interval](#)

Notes

- A Free interval can only start at the time that, or *before* (not after) a Trial Control event occurs. Similarly, a Free interval can only end at the time that, or *after* (not before) a Trial Control event occurs.

See also

- For information on the meaning of active/inactive, false/true, see [How the trial control instructions are executed](#).
- For information on sub-rules and analysis of trial control data, see the [Trial and Hardware Control - EthoVision XT Manual](#).

Difference between Free Intervals based on Trial Control and Nesting over a Trial Control state

With Free intervals you can select the same track segments that you can define with Nesting over a Trial Control state in the Data profile. The main differences are that:

- With a Free interval, you can also specify the number of occurrences of an event in the Interval start criterion. For example, define an interval that starts when an action is carried out the 3rd time. With Trial Control state that is not possible. There, you can only specify the number of occurrences in the Interval stop criterion.
- With a Free interval, you can combine Trial Control events with other criteria (time, dependent variable values, hardware log values). For example, define

an interval that goes from the time a light switches on, to 1 minute after that time.

Free interval based on hardware

Background

This topic contains details about defining a Free interval. For the main procedure, see Free interval.

To specify the hardware state or value

Start criterion:

- Time
- Dependent variable
- Trial Control
- Hardware

Interval start

Start at:	0:00:00.000 H:mm:ss.fff before event:
Select device type:	Top Unit (Standard)
Select name:	Top Unit Cage 1
Select signal/command:	Is white spot on
Signal value:	true

In the Free Interval window, under **Interval start** or **Interval stop**, choose **Hardware** and select the following:

1. In the **Start at / Stop at** field, enter the time of the start (or stop) of the interval relative to the Hardware event specified in the next steps.

EXAMPLE 1 To start an interval 1 s before the PhenoTyper's white light switches on, next to **Start at** enter 0:00:01:000 **before event**.

EXAMPLE 2 To stop an interval 10 s after a pellet was dropped, next to **Stop at** enter 0:00:10:000 **after event**.

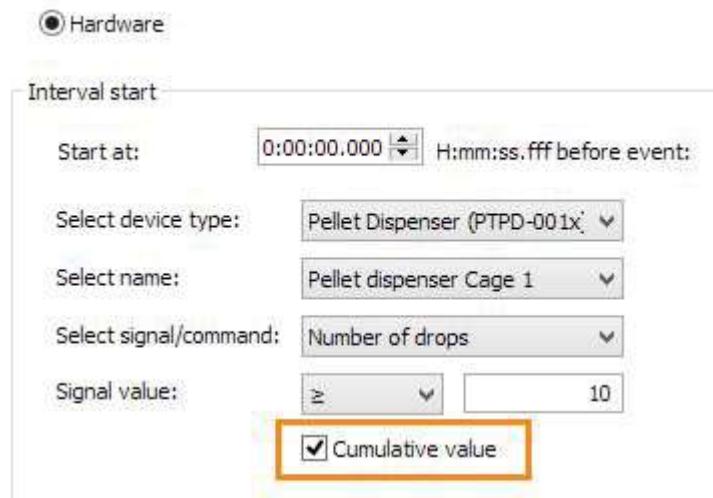
2. From the **Select device type** list, choose the type of device you want to use.
3. From the **Select name** list, select the name of the device of the chosen type. The names in this list have been assigned to that device type in the Arena - Hardware mapping window in the Arena Settings.

4. From the **Select signal/command** list, select the variable or the command you are interested in.
5. From the **Signal value** list, choose which value the signal should have to define the start/stop of the interval, using the operators available (\leq , \geq or **false/true**).

Return to To specify a Free interval

Notes

- The **Cumulative value** option is available for numerical variables like Number of pellet drops, Number of licks, Duration of licks, or counts (for example, Input 1 L->H count).



When **Cumulative value** is selected, hardware events are summed up and EthoVision "remembers" that at any time. Keep this option selected if, for example, you want to start an interval when the **Signal value** is ≥ 10 .

When **Cumulative value** is not selected, each hardware event is a state that lasts one sample time (for example, 0.04 s if the sample rate is 25). At any time, EthoVision does not "know" how many events have occurred up to that time. De-selecting **Cumulative value** provides results when the **Signal value** selected is 1. De-select **Cumulative value** when you want to specify multiple intervals, for example for each time a pellet is dropped.

- The options under **Interval start** and **Interval stop** are based on the **Hardware state** dependent variable. For more information, see also **Hardware state** under **Analysis of Trial Control data** in the Trial and Hardware Control - EthoVision XT Manual.

See also

- The Reference Manual - Trial and Hardware Control in EthoVision XT. To open this manual, on the Windows **Apps** screen choose **Noldus > EthoVision XT 14 Other documentation**.

Examples of Free intervals in the Analysis profile

For all the examples below, define the interval first, then choose **Analyze > Results** to calculate the statistics or visualize the results. See Free interval

TIP If you want to define a free interval, to further analyze the events within that interval, choose Nest over a Free interval in the Data profile.

Examples

- Example 1: From the track start to when the subject is in a zone for a specific time
- Example 2: From the start of a conditioning routine to when 10 rewards are given to the subject
- Example 3: Split a track in segments of a specific path length
- Example 4: Detect bouts of a behavior
- Example 5: From the track start to when external (physiological) data reach a specific value

Example 1: From the track start to when the subject is in a zone for a specific time

Aim

This Free interval can be used, for example:

- Exploratory behavior. Calculate the time that the subject takes to explore the novel object for at least 30 s (Cumulative duration).
- Learning. Calculate the time me that the animal takes to stay in a zone for 30 s uninterrupted (Current duration).

Solution

Define a Free interval with:

- Start criterion: Track start
- Stop criterion: Dependent variable *In zone*. The Statistic depends on the type of question you want to answer.

In the Novel object test, you want to stop the interval when the subject has explored the object for a *total* of 30 s. Choose then *Cumulative duration*.

In the learning example, you want to stop the interval when the subject enters a zone and stays for 30 s without exiting. Choose then *Current duration*.

Example 2: From the start of a conditioning routine to when 10 rewards are given to the subject

Aim

In a conditioning experiment, the subject has to stay in a specific zone to obtain a food pellet. The action-reward sequence is repeated a number of times in a subrule. The researcher wants to know how long it took the subject to get 10 rewards.

Solution

Create a Free interval with:

- Start criterion: Trial Control. Element: Subrule. Event: becomes active.
- Stop criterion: Hardware. Device type: Pellet dispenser. Select signal: Number of drops ≥ 10 . The option Cumulative is selected.

Example 3: Split a track in segments of a specific path length

Aim

Suppose you want to split your tracks in segments of a specific distance moved by the subject, for example 1 m, and you want to calculate the duration of each segment.

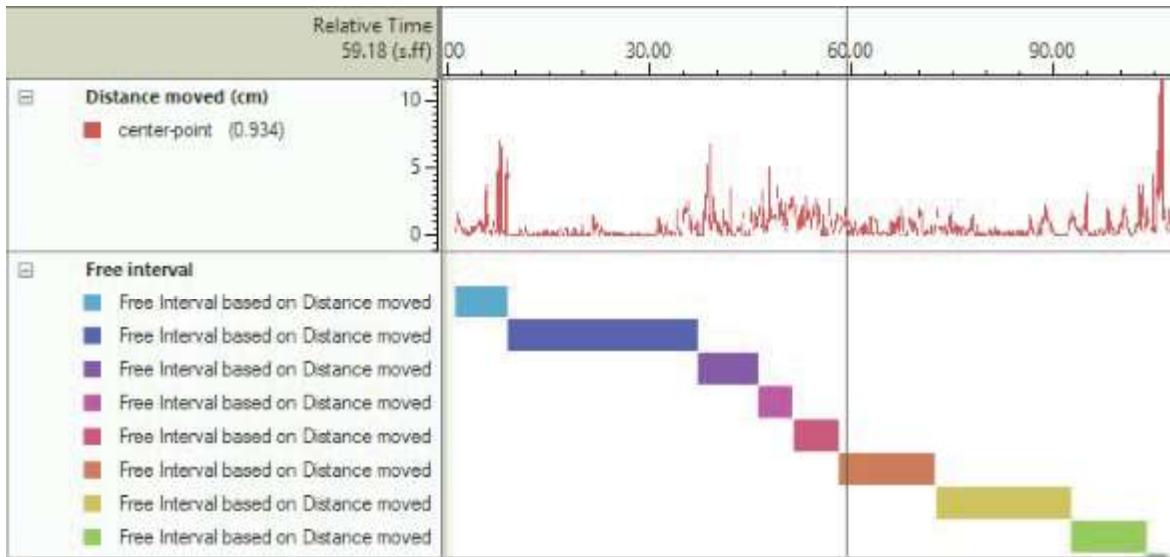
Solution

Create a Free interval with:

- Start criterion: Dependent variable *Distance Moved*. Statistic: Current ≥ 0 cm.
- Stop criterion: Dependent variable *Distance Moved*. Statistic: Total ≥ 100 cm.

To calculate the duration of individual instances, select **Calculate statistics per interval** and enter a number that exceeds the maximum number of interval per track.

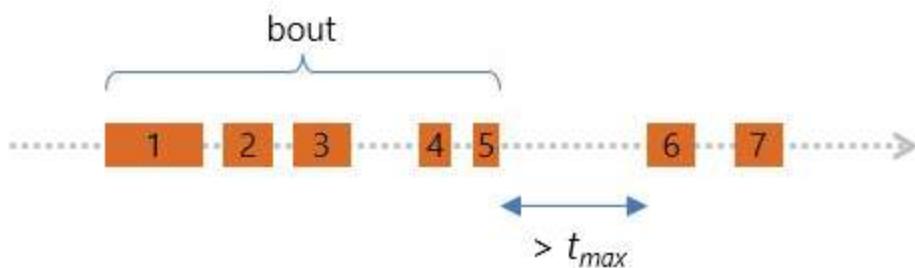
Below: Plot 1: Per-sample distance moved. Plots 2: Free interval that runs from Current Distance moved ≥ 0 to Total Distance moved ≥ 100 cm. Note that the faster the animal (i.e. the longer the per-sample distance moved), the shorter the resulting interval.



Example 4: Detect bouts of a behavior

Aim

Calculate the number and duration of bouts of a behavior, for example *Movement* or *Activity*. The single instances of Movement being part of a bout may be separated by up to time t_{max} . In the example below, instances 1 to 5 are grouped in a bout.



Solution

Create a free interval with:

- Start criterion: Dependent variable *Movement* with state *Moving*. Statistic: Current.

- Stop criterion: Dependent variable *Movement* with state *Not moving*. Statistic: Current Duration ≥ 5 s (t_{max}). This ensures that the interval ends when the animal has not moved for five seconds without interruption.

In the Trial Statistics tab of the same window, select **Mean** if you want to know the average duration of a bout.



NOTES

- Because each free interval also includes t_{max} (see the length of the orange bars in the figure above, which represent the free intervals), you have to subtract that time to obtain the true average duration.
- If the event which specifies the end of a bout (in this example, *Not moving* for at least 5 seconds) is never found, the result for that trial is that there is no bout at all. The result is one long bout if you de-select the option **Ignore last interval if incomplete**.
- For the same reason as described above, if the Stop criterion is not met, the last bout of a trial is not detected. For example, when the time between the start of *Not moving* and the end of the trial is less than t_{max} . You can have the last bout detected by selecting the option **Ignore last interval if incomplete**, but the duration of this bout is truncated.

Example 5: From the track start to when external (physiological) data reach a specific value

Aim

To calculate the time that it takes for a physiological variable (e.g. heart rate) to reach a specific value.

Prerequisite

The physiological variable has been imported in the trial. See External Data

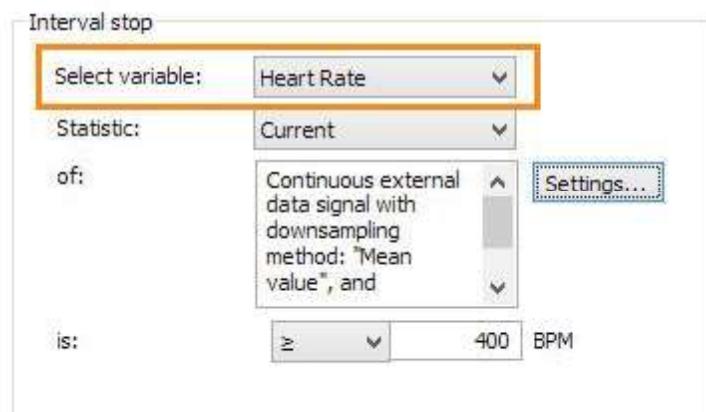
Solution

Create a Free interval with:

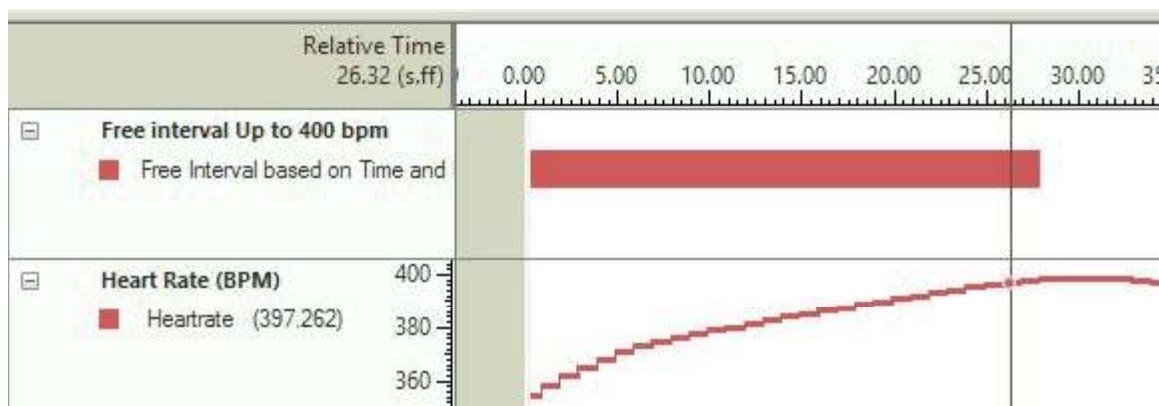
- Start criterion: Track start
- Stop criterion: Dependent variable *Heart rate*. The Statistic chosen is *Current*.
If necessary, click **Settings** and choose how to resample the variable from the original signal. See External data (resampled)

Stop criterion:

- Time
 Dependent variable
 Trial Control



The result is shown in the first plot below, together with the plot of Heart rate. Choose **Analysis > Results > Statistics and Charts** to obtain the length of the free interval.



TIP If you want to analyze the time between two values of external data, for example from Heart rate = 300 to Heart rate = 400, then in the Analysis profile choose External data (state) instead, not Free interval.

See also

- Analysis advisor

Free interval based on multiple subjects

Procedure

When working with multiple subjects per arena, you can define free intervals in two ways:

- Under Nesting, choose Free interval to define a Free interval for each subject separately. The interval is based on the behavior of that subject, not the other(s).

As a result, for each subject a specific time period is selected.

- Under Nesting over Subjects, choose Free interval to define an interval based on a combination of conditions based on two or more subjects. The resulting interval is applied to all subjects.

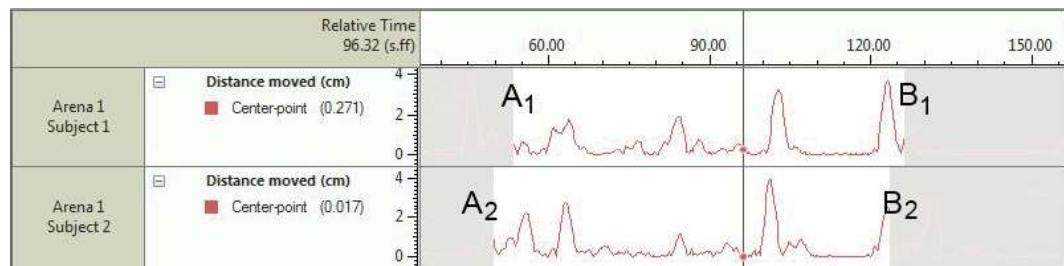
As a result, the same time period is selected for all subjects.

Example

Your arena contains two subjects. A free interval is defined that goes from Total Distance moved ≥ 100 to Total Distance moved ≥ 200 .

- If you choose **Free interval** from **Nesting**, the start/stop criteria are applied per subject, resulting in most cases in different time periods being analyzed for each subject.

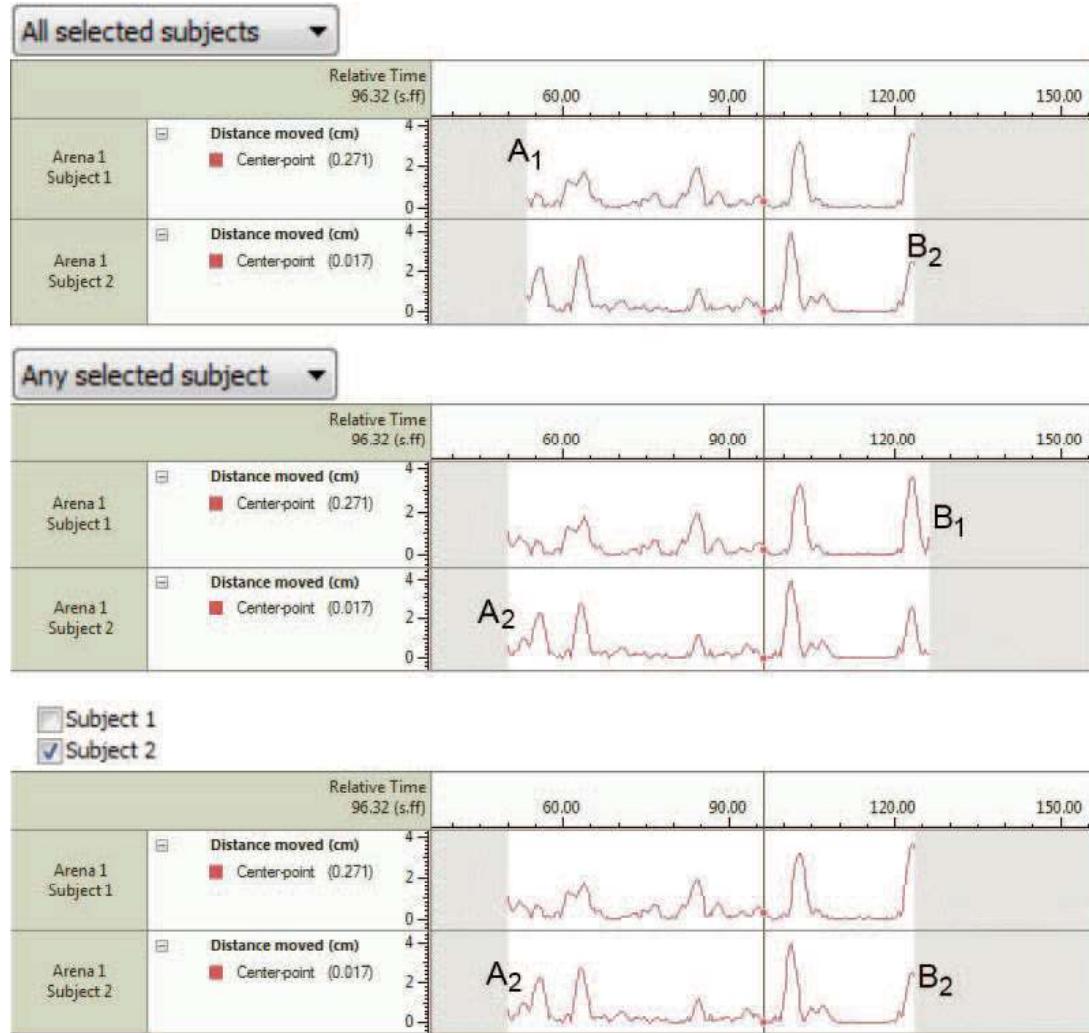
Below: Time plot of a dependent variable (in this example, *Distance moved*) after defining the Free interval from Nesting. Different time periods are analyzed for Subject 1 (from A₁ to B₁) and Subject 2 (from A₂ to B₂).



- If you choose **Free Interval** from **Nesting over Subjects**, the start/stop criteria are applied per subject, but the interval are defined after combining the criteria based on what is selected in the **Actors** tab.

Below: Time plots of a dependent variable (in this example distance moved) after defining a Free interval from **Nesting over Subjects** (compare with the previous figure). Depending on what is selected in the **Actors** tab:

- With **All selected subjects** (top). The time period shared between subjects (from A1 to B2).
- With **Any selected subject** (middle). The time period defined by at least one subject (from A2 to B1).
- With one subject selected (bottom). The time period defined by the criterion met by that subject (in this example, subject 2; therefore, from A2 to B2).



Multi condition

Definition

A segment of track that corresponds to the combination of the value of one, two or more dependent variables.

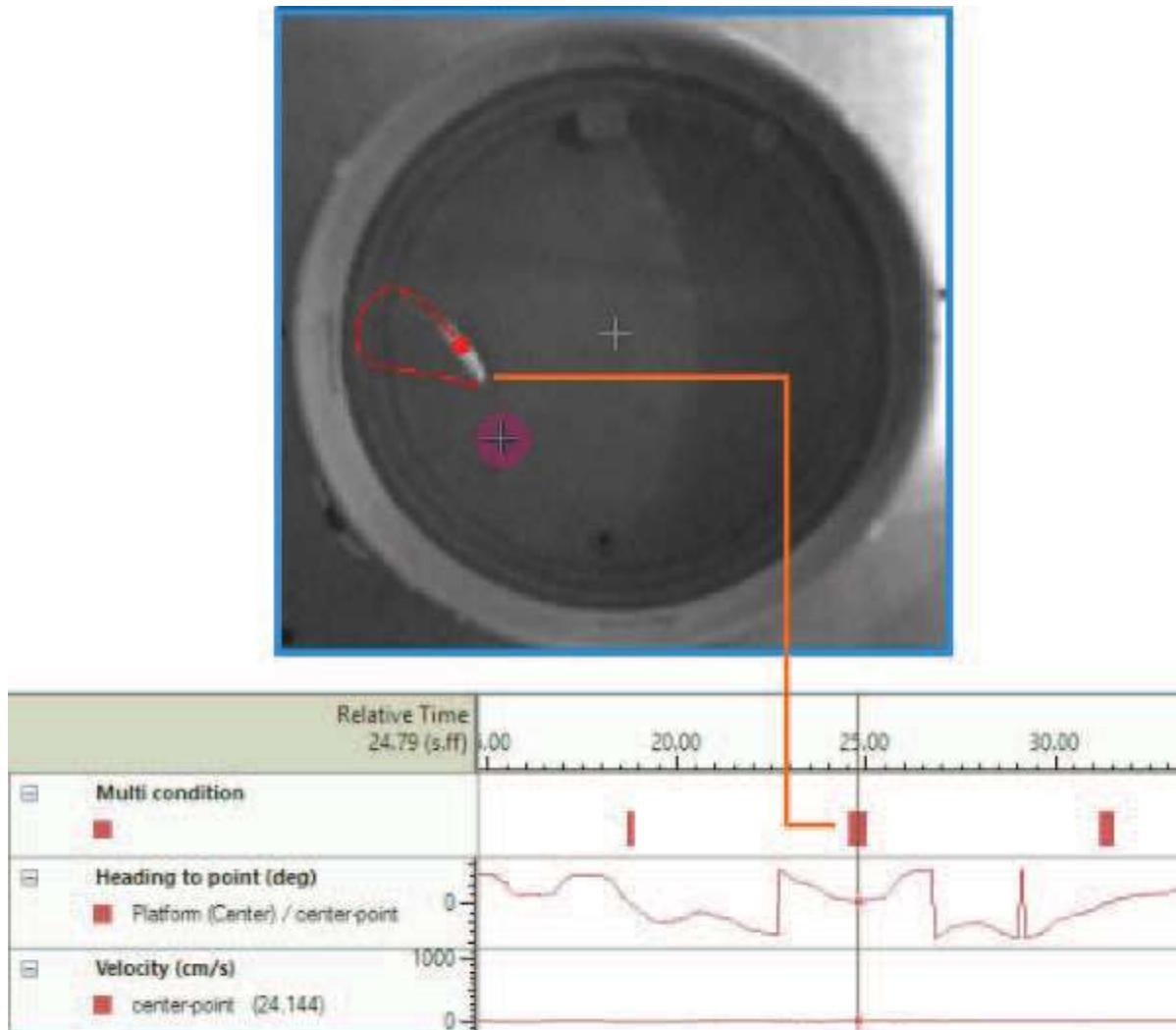
Use cases

- *In Trial Control: Stop the trial when exploration time reaches a specified value.*
In a Novel object test, stop the trial when the exploration of an object reaches 30 seconds. Exploration is defined as (a) Nose point in the zone "object sniffing zone", and (b) Head direction pointing to the "object sniffing zone". When the total time that (a) *and* (b) are simultaneously true reaches 30 s, stop the trial.
TIP Define this condition in the Trial Control Settings.
- *In Analysis: Calculate the time that the animal swims pointing to the platform.*
In a probe trial of the Morris water maze test, the subject swims for a number of seconds around the platform. You want to know how long the animal points to the platform while it is swimming. This can be translated to (a) Heading to platform less than a user-define angle (for example 10 degrees) *and* (b) Velocity higher than a user-define value (for example 10 cm/s). **TIP** Define this in the Analysis profile.

Calculation

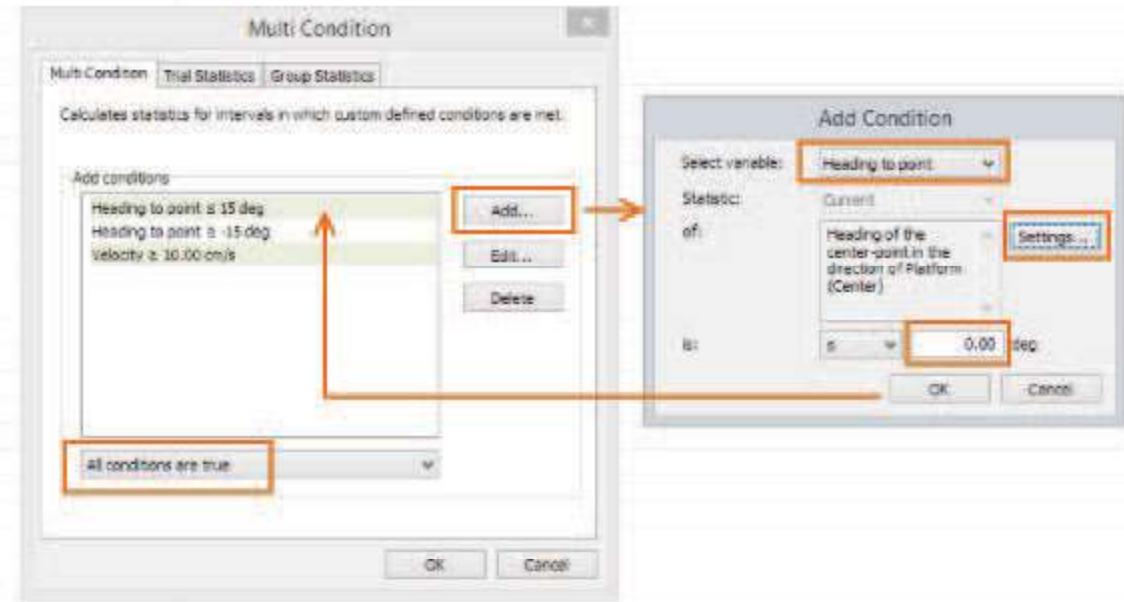
The track segments are defined for each condition independently. Then, the track segments are combined according to the logic specified (All conditions true = AND; Any condition true = OR).

Below: The variable *Multi condition* marks the time when the animal swims towards the platform with a certain speed. Here the multi condition is defined with three conditions: (1) Heading to platform less or equal than +15 degrees; (2) Heading to platform greater than -15 degrees; (3) Velocity greater than 10 cm/s. Conditions are combined with **All conditions true**. The first plot shows *Multi condition*; the second *Heading to point* (center of the platform), with both negative and positive values; the third plot *Velocity*.



To specify a Multi-condition

1. In the Analysis profile, under **Custom Variables**, click the button next to **Multi condition**.
2. Click the **Add** button at the right of **Add conditions**.
3. Choose the variable from the **Select variable** list.
4. To specify further options, click **Settings**. Next, specify which values (or range of) make the condition true.
5. Click **OK**. The condition is added to the list.



6. Repeat the steps 2-5 to add more conditions.
7. Combine the conditions with **All conditions are true** (AND logic) or **Any condition is true** (OR logic).
8. Complete the procedure to add the variable. See Calculate statistics: procedure.

Notes

- To define a Multi condition you can use continuous variables, like *Distance moved*, and state variables, like *In zone* (excluding *Activity state* and *Acceleration state*).
- Variables that are not available are: variables representing points in time, like *Rotation*, *Zone transition*, *Zone alternation*; behaviors of Behavior Recognition, and behaviors scored as point events are not available.
- To edit a condition, select that condition in the list and click **Edit**. To delete a condition, click that condition and click **Delete**.

See also

- For how to use a Multi condition in Trial Control, see the Reference Manual - Trial and Hardware Control in EthoVision XT.
- To analyze the track segments based on the Multi condition, see Nesting over a Multi condition in the Data profile.

