

## SYSTEM CONFIGURATIONS

EthoVision® XT Base allows you to perform complete tests with one animal in one arena. With the Multiple Body Points Module added to the Base, you can also track the nose point and tail base of rodents or adult zebrafish.

## DATA MANAGEMENT

### 1. SETUP

#### Template experiments

EthoVision XT contains a user-guided set-up with templates for several tests paradigms. At the start of the program you can either start a brand new experiment, re-use the settings from a previous experiment, or use the user-guided set-up to open an experiment template suited to a specific test (water maze, plus maze, etc.). With these templates, many basic settings are pre-set, such as arena definition, detection settings, and parameters for analysis. You can fine-tune these settings to fit your specific needs.

#### Experiment options

- Tracking can be done live or from a video file.
- You can use digital cameras or analog cameras (but not a combination of the two).
- You can use center point tracking, or center of gravity, nose and tail base for rodents or adult zebrafish.
- Activity analysis – a non-tracking option analyzing overall activity per arena based on pixel changes. This is very useful for freezing detection.
- Distance can be measured in millimeters, centimeters, meters, or inches.
- Time can be measured in milliseconds, seconds, minutes, or hours.
- Rotation can be measured in degrees, radians, gradians, or rotations.

#### Manual scoring

The Manual Event Recorder is a built-in coding system which allows you to define a list of behaviors that you can score manually during and after data acquisition (live or offline).

- Score live solely or in addition to offline scoring to use scoring to trigger trial events.
- Score (also) offline, to score at a lower or faster playback speed. You can also edit scored events.

You can define different types of behaviors:

- *Start-stop behaviors* – behaviors that are independent from each other.
- *Point events* – behaviors without a duration.
- *Mutually exclusive behaviors* – behaviors grouped in such a way that activating one behavior automatically stops the other behavior.

Optionally, you can define up to 32 different behaviors using the keys from the computer keyboard. Without key codes defined, you can manually score events using the mouse.

#### Arena settings

The arena is the region in the video image where the animal is tracked. You can define:

- the arena
- different zones
- cumulative zones (define individual smaller zones to be taken into account for cumulative analysis)
- hidden zones (used for shelters) and entry zones belonging to the hidden zone
- multiple zone-groups (if you want to define overlapping zones in an arena, you should define them in separate zone groups or use cumulative zones)
- points of interest

You can define an unlimited number of zones in an arena, and create zones of any shape. Calibration of your arena

<sup>1</sup> Depending on if you are using one of the modules.

will allow EthoVision XT to calculate parameters such as velocity and distances to certain points.

### **Arena/zone auto-subdivision**

This option allows the automated subdivision of a circular and rectangular arena in multiple equal zones. It includes auto-labeling of the subdivisions.

### **Trial control options**

You can program EthoVision XT to automatically start and stop tracking, based on any of the parameters offered by EthoVision XT. This feature also includes an external command option, which allows you to start another computer program when a condition becomes true. For example, present a visual stimulus on a computer screen or play a sound file when the animal enters a particular zone.

### **Detection settings**

EthoVision XT offers four different detection methods:

- *Gray scaling* – Defines all connecting pixels with a gray value between two threshold values as a possible object.
- *Static subtraction* – Looks at differences between pixels in a reference image (without the animal) and the new digitized image (with the animal).
- *Dynamic subtraction* – Looks at the absolute contrast between pixels in a reference image and the new digitized image, while updating the reference image on each sample.
- *Differencing* – Makes a statistical comparison between pixels in a reference image and the current image. The statistical comparison uses the variance in contrast between the current and reference image and determines whether each pixel has changed enough to be considered part of the subject.

### **Trial list**

The trial list consists of trials that are planned, carried out, or skipped, including system variables and user-defined independent variables. All are organized in a cross table and data can be copied to and from Excel sheets. In EthoVision XT you can now schedule a list of trials based on pre-recorded video files. This list can be automatically acquired as a batch in data acquisition.

## **2. DATA ACQUISITION**

EthoVision XT offers five acquisition methods.

1. *Acquire data live* – Live tracking requires no disk space for video storage, but creates no video backup either.

The maximum trial duration without video recording (as tested) is 72 hours.

2. *Acquire data live and record video* – Live tracking, while EthoVision XT records a backup video to an MPEG4 video file. The maximum trial duration with video recording (as tested) is 24 hours. To acquire trials longer than mentioned above, you can split your multi-day testing into multiple trials.
3. *Record video and acquire data* – EthoVision XT records a video to an MPEG4 file which you can be used for data acquisition later. (This method is recommended if the computer is not fast enough for live data acquisition and video recording simultaneously.)
4. *Acquire data from existing video files* – Track data from video recorded with programs other than EthoVision XT.
5. *Acquire data from a batch of existing video files at once* – Track data from a series of pre-recorded video files.

In options 2 – 4, the video file is available for post-acquisition visualization in combination with the track file and analysis results. If you choose option 3, or 4, it is possible to analyze the samples at a rate faster than the actual sample rate, depending on the processor speed.

EthoVision XT supports a number of cameras. While tracking is done live it is possible to create video files as a backup and for integrated visualization (acquisition method 2). EthoVision XT is designed to work with MPEG1 and MPEG4 video formats.

### **Series of trials from live video feed**

You can use one live video feed to acquire a series of trials from. You can predefine start & stop conditions and an inter-trial interval.

### **Acquire additional behavioral data**

You can score behaviors manually while acquiring video track data. For this you need to predefine a list of behaviors. (See 1. Set-up - Manual scoring). This list of behaviors is visible in the scoring panel of the acquisition screen. You can score behaviors by directly clicking them in the panel or by pressing the predefined keys.

## **3. EDIT DATA**

In the track editor the stored video file can be used to evaluate the tracking result. This allows you to quickly and easily find wrong data points and correct them. Furthermore it is possible to select samples automatically. In the latter case EthoVision XT will find samples that match a specific criterion, for instance to find missing points or samples that are separated by a dis-

tance greater or smaller than was to be expected. With the track editor, points can be deleted, moved to another place or interpolated. Furthermore it can be used to exchange nose-point and the tail-base.

You can save a copy of the track with the edits when you are satisfied, so you always go back to that last copy when you make a mistake in the edits. Furthermore the original track data also stay stored.

#### 4. SMOOTH TRACKS

Small (erratic) movements, such as body wobbles or outliers, may lead to an overestimation of certain parameters. You can eliminate this by using the Lowess filtering method, which essentially smooths the acquired tracks of the body center point. You can also remove outliers using the Maximal distance moved.

As an alternative to the Lowess smoothing method, you can apply 'Minimal distance moved' smoothing. This allows you to specify the minimal distance moved between samples that is taken into account as actual movement. This allows you to exclude micro movements.

#### 5. SELECT DATA

EthoVision XT offers four methods for data selection or separation.

- 1) *Filtering* – Picking out entire tracks, based on the value of system variables, user-defined variables, or the presence of co-acquired external data.
- 2) *Nesting* – Picking out parts of tracks, according to the zone the animal (or one of its body points) was in, the behavioral state the animal was in, or during a certain time interval.
- 3) *Time bins* – Time intervals of constant duration that you can define to analyze data as if the tracks were split into segments. (Length can vary between 1 second and 24 hours.)
- 4) *Results per zone* – An easy way to calculate the results of individual analysis parameters per zone.

#### 6. VISUALIZE DATA

- 1) With track visualization, you can plot all the selected tracks, or partial tracks, in one matrix. In this view you can sort rows and columns according to one or more independent variables.
- 2) Using integrated visualization, the video, the subject's track, and the values of the selected parameters will be presented in one dynamic, synchronized view. With the built-in screen recording tool you can make a screen recording of either full screen or the module specific area (tracks and plots) of the integrated visualization.
- 3) All trial parameters can be presented in tables and graphs. You can customize the layout of the statistics results table, making data export to other applications easier. You can view the results per trial or for groups, such as treatment groups.
- 4) You can also visualize your data in heatmaps. For this you can select data and variables of interest, and present heatmaps per individual of group. High quality export options allow for use in publications.

#### 7. ANALYZE DATA

##### Standard available parameters<sup>2</sup>

- *Velocity* – Distance traveled by the body point per unit of time.
- *Distance moved* – The distance traveled by a body point from the previous sample to the current one.
- *In zone* – A discrete variable with two possible states, 'in zone' and 'not in zone', depending on whether the body point is within a specific zone (or group of zones).
- *Distance to zone* – The shortest distance between a body point and the border of a specific zone (or group of zones).
- *Distance to point* – The shortest distance between a body point and a particular point of interest. (Centers of arenas and zones are automatically defined as points.)
- *Zone transitions* – The number of times an animal moves between a user-defined sequence of zones (2 or more).

- *Zone alternations* – The number of times an animal visits a user-defined number of zones consecutively (typically used in y-maze experiments).
- *Target visits and errors* – Number of errors and successes in e.g. Barnes maze target zone or baited arm in radial maze.
- *Heading to point* – Direction of movement of a body point between one sample and the previous, relative to a user-defined point. This indicates to what degree the animal moves towards the point, regardless of whether the animal faces the point.
- *Heading* – Direction of movement of a body point between one sample and the previous, relative to a line parallel to the x axis in the coordinate system.
- *Turn angle (relative and absolute)* – The change in moving direction of a body point between one sample and the previous. Relative turn angle takes the direction of turning into account (clockwise or anti-clockwise), whereas absolute turn angle does not.
- *Angular velocity (relative and absolute)* – The change in moving direction of a body point per unit of time (speed of change in direction). Relative angular velocity takes the direction of turning into account (clockwise or anti-clockwise), whereas absolute angular velocity does not.
- *Meander* – The change in moving direction of a body point, relative to the distance moved. It is a measure of the tortuosity of a trajectory. Relative meander takes the direction of turning into account (clockwise or anti-clockwise), whereas absolute meander does not.
- *Movement* – A discrete variable with two possible states, 'moving' and 'not moving', indicating whether the spatial movement of a body point is within user-definable speed thresholds.
- *Mobility state* – A discrete variable with three possible states, 'highly mobile', 'mobile', and 'immobile', indicating the percentage of change in the animal's surface area between consecutive samples, with user-definable thresholds.
- *Mobility continuous* – Calculates the mobility for which the complete area detected as animal is changing, even if the center point remains at the same place.
- *Rotation* – The number of clockwise and/or counter-clockwise rotations made by the animal, in which the definition of a rotation can be freely chosen (for instance turning 360°, 180°, or 90°).
- *Activity state* – The duration of levels of activity within the arena boundaries. It calculates four levels of activity (high, active, moderately active and inactive)

based on user definable thresholds for activity and duration.

- *Activity continuous* – The percentage of activity within the arena boundaries.
- *Manually scored events* – Behaviors that were scored manually.
- *Free Interval* – for custom analysis of interval duration, cumulative durations, etc.

#### **Additional parameters Multiple Body Points Module**

- *Head direction* – The smallest angle formed by the direction that the nose point is pointing towards in a certain sample, relative to a line parallel to the x-axis in the coordinate system.
- *Body elongation state* – A discrete variable with three possible states, 'stretched', 'normal', and 'contracted', indicating the elongation percentage of the animal's body, with user definable thresholds.
- *Body elongation* – This parameter indicates the elongation percentage of the animal's body per user defined averaging interval.
- *Head directed to zone* – The duration the animal's head is directed towards a specific zone or a circular area around a point.
- *Body angle state* – The body angle of the animal over a user-defined interval and user-defined thresholds with the output in four states: 'Straight', 'Bent', 'Bent clockwise' and 'Bent counterclockwise'.
- *Body angle* – The body angle of the animal (relative or absolute) per sample point.

#### **Multi-condition parameters**

You can create new, customized parameters by combining two or more of the ones mentioned above, including manually scored events. Other custom-build parameters are available upon request.

#### **Statistics available**

Depending on the parameter, the following statistics are available:

- frequency
- latency to first occurrence
- latency to last occurrence
- total duration
- variance
- standard deviation
- standard error
- number
- mean

<sup>2</sup> Parameters are calculated for the center point of your animal. If you are using the Multiple Body Points Module, you can also calculate most parameters for the nose point and tail base.

- maximum
- minimum
- number of samples

The following group statistics are available:

- group mean and standard deviation
- group mean and standard error
- 5-number summary (Minimum, First Quartile, Median, Third Quartile)

For grouped statistics charts are automatically created. By default, this is a bar chart. When time bins are used a line chart is created. When specifying a 5-number summary a box-whiskers chart is created. Charts can also be exported as image files.

## OUTPUT AND EXPORT DATA

Track data, values of dependent variables, and all statistics can be exported as \*.xls or \*.txt file:

- *Export track data and dependent variable values* – Export the X,Y coordinates together with the value of the parameters chosen. Each track is exported as a separate file and the arena name and track number are attached.
- *Export statistics* – Export the spreadsheet-like table with all statistic results. They layout of the statistics results table can be customized, making data export to other applications easier.
- *Export manual scoring log* – If you have manually scored events, these can be exported as raw data.

Arena definition, graphs and heatmaps can be exported as \*.jpg, \*.png, \*.bmp, and \*.gif. The heatmaps can be exported in a high resolution (up to 3072 \* 2304 px), making it suitable for publications.

## HELP FEATURES

- *Browser based (HTML), highly searchable and responsive Help* – Can be opened on tablets.

- *Video tutorials* – EthoVision XT incorporates mini video tutorials at certain parts of the program to assist you making optimal settings. A complete (from start to finish) tutorial is available via the Help menu.
- *Help desk* – Professional personal help, available in many different time zones via phone, Skype, or e-mail.
- *Demonstration projects* – Provided with software.

## HARDWARE COMPONENTS

Minimum set-up:

- EthoVision XT Base software
- camera (e.g. GigE, USB3, IP)
- computer

## CAMERAS

USB web cams (like the Microsoft Lifecam Studio or the Logitech Brio) may be used with EthoVision XT Base plus Multiple Body Points Module, however note that these consumer range cameras change quickly and are not developed for video tracking purposes. Automated settings, image quality, image resolution and the processing power of the computer may affect the quality of tracking. In addition, cable length is limiting. When using a USB camera, keep in mind that a higher resolution might seem better initially, but it requires more processing without adding more value to the video for the actual video tracking. When you use USB web cameras, make sure that the option 'dropped frames correction' is selected in the Detection Settings.

EthoVision XT was tested with a wide range of cameras (see table below). Here are some points to keep in mind when selecting a camera for your setup:

- We recommend using the GigE cameras from Basler since they deliver stable frame rates, are sensitive under low light conditions, and can be used with long cables.

- IP cameras can be used as well, though they are not recommended when accurate timing of video frames is required. When you use IP cameras, make sure that in the Detection Settings the option Dropped frames correction is selected. Also, note that IP cameras must support the ONVIF communication standard. You do not need to install drivers for these cameras.
- Analog camera can also be used in combination with our encoder boards (see table below). We tested EthoVision XT with Ikegami, Panasonic and JVC analog cameras.
- The drivers for the supported cameras are available on the EthoVision XT installation USB stick, or on the EthoVision XT downloads web page.

- Hard disk* – 1 TB free space (to store experiments including video files).

Performance will depend on the functionality used.

For contact details, please check

[www.noldus.com](http://www.noldus.com)

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Due to our policy of continuous product improvement, information in this document is subject to change without notice.

## SYSTEM REQUIREMENTS

For optimal performance and functionality of EthoVision XT, an up-to-date professional workstation is strongly recommended. EthoVision XT 14 is tested on Windows 7 Service Pack 1, and Windows 10, 64 bit Professional version and on a Dell Precision T3620 and a Dell Precision T5810 desktop, and a Dell Precision 7520 laptop. For those systems we guarantee compatibility.

The optimal computer requirements for workstations are:

- Processor (CPU)* – Intel Xeon or i7 CPU, Quad core 3.5 GHz.
- Internal memory (RAM)* – 8 GB.
- USB 3.0 ports.*

Type	Brand/model	Interface board (for Desktop)	Driver version (a)	Frame rate (fps)
GigE	Basler acA1300-60gm	CT1000 Pro	5.0.9.10388	60
GigE	Basler acA1300-30gm	CT1000 Pro (1 camera)	5.0.9.10388	30
USB 3.0	Basler acA1920-155µm	U3-PCIe1XG205-1S (1 camera)	5.0.9.10388	60
IP (b)	Axis M1054, Axis M1065L, Axis P5525	CT1000 Pro	-	25
Analog	Various brands/models (c)	Piccolo Alert Piccolo U4 H.264 Piccolo U8 H.264	6.13.0.3180 (Piccolo Alert) 2.5.0.139 (U4/U8 H.264)	25 (PAL) 29.97 (NTSC)