Noldus

EthoVision XT 18

Technical specifications
Using EthoVision XT for rodents

SYSTEM CONFIGURATIONS

EthoVision® XT Base allows you to perform complete tests with one animal in one arena. You can extend the functionality with one or more of the eight available modules:

- *Mouse Behavior Recognition Module* – Automatically detect ten mouse behaviors such as grooming, rearing, and digging.

- *Rat Behavior Recognition Module* – Automatically detect ten rat behaviors such as grooming, rearing, and sniffing.

- *External Data Module* – Integrate EthoVision XT data with co-acquired external data.

- *Multiple Arenas Module* – Track animals in up to a hundred arenas simultaneously.

- *Social Interaction Module* – Track multiple animals within each arena.

- *Trial & Hardware Control Module* – Create complete test protocols to initiate and control interaction with external equipment, based on real-time analysis of the animal's behavior.

- *Quality Assurance Module* – Assign different rights to different users and comply with Good Laboratory Practice guidelines.

DATA MANAGEMENT

1. SETUP

Template experiments
EthoVision XT contains a user-guided set-up with templates for several test 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.

DanioVision experiments
If you use the DanioVision system for multi-well plates you can select the DanioVision experiment option which optimizes the arena settings and detection settings for zebrafish larvae in well plates.

Live Mouse Tracker experiments
If you use the Live Mouse Tracker acquisition system (https://livemousetracker.org) and want to analyze these data in EthoVision XT you can select the Live Mouse Tracker experiment option when creating a new experiment. This creates a modified version of a standard, multi-subject, multi-body point experiment, with predefined, permanent settings for Live Mouse Tracker.

Good Laboratory Practice
Additionally, for those conducting Quality Assurance experiments, particularly in environments adhering to Good Laboratory Practice (GLP) standards, EthoVision XT offers specialized options to meet these rigorous requirements. This ensures that your experiments not only yield reliable data but also comply with industry standards for quality and reproducibility.

Flexibility
For researchers seeking maximum flexibility in their experimental design, the "New from scratch" option allows for complete customization of the experiment

setup. This feature is ideal for innovative research that goes beyond standard protocols, offering the freedom to tailor every aspect of the experiment to unique research 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 connect external data acquisition systems to EthoVision XT[1].

- Track in one or multiple arenas, with a max of 100 arenas[2].

- Track one or multiple subjects per arena, with a maximum of 16 subject roles[2].

- For mice, rats and zebrafish you can use center point tracking, or center of gravity, nose and tail base. For mice and rats you have the option between a contour-based technique or a deep learning based technique. You can also track based on color or color marker[2].

- 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
- Score (also) offline, to score at a lower of faster playback speed. You can also edit scored events.

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.

You can define different behaviors, you can score those categories with keyboard keys or with the mouse.

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(s)

- subdivisions of the arena (zones)

- cumulative zones (define individual smaller zones to be taken into account for cumulative analysis)

- hidden zones (used for shelters) and entry zones to analyze entries/exits

- zones organized in layers, so you can define and analyze zones that overlap.

- points of interest

You can define an unlimited number of zones in an arena, and create zones of any shape. Calibration of your arena will allow EthoVision XT to calculate parameters such as velocity and distances to certain points. If you are tracking in multiple arenas, you can copy and paste the settings from one arena to the other.

Multiple arena definition

When your experiment contains multiple arenas – for instance a group of open fields or home cages – you can use the multiple arena set-up functionality. This means that you do not need to draw each arena separately. EthoVision XT allows you to easily rotate, scale, transpose, resize, and reposition the series of arenas simultaneously. In DanioVision experiments automated arena detection detects the individual wells in standard-ized well-plates automatically.

Arena/zone auto-subdivision

This option allows the automated subdivision of circular and rectangular arenas in multiple equal zones, including auto-labeling of these subdivisions. For example, it can be used to divide a water maze into quadrants, simplify-ing the process of analyzing behavior across different sections of the arena.

Multi camera set-up

In EthoVision XT you can combine the input of multiple cameras. This way you can track from multiple arenas (in combination with the Multiple Arenas Module) without the need to place them all under one camera.

Trial control options

You can program EthoVision XT to automatically start and stop tracking, based on any of the parameters

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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.

With the additional module for Trial & Hardware Control, you can define complete test protocols to initiate and control interactions between your animals and external sensor and stimuli devices. Real-time analysis of any of the parameters of EthoVision XT allows you to control complete test protocols based on your animal's behavior. You can use it for complex learning tasks such as the radial arm maze with automatically controlled doors and food delivery using pellet dispensers. Attach and control all standard (TTL-based) hardware.

If you are using multiple arenas, conditions are applied to each arena individually.

Tracking methods (body point estimate) for 1 animal per arena
The tracking method specifies how the body points are estimated, once the subject is detected. Depending on how many subjects and body points you specify in the Experiment Settings, one or more methods for tracking of body points are available in the Method section of the Advanced Detection Settings.

Deep Learning based Body Point Detection Technique:
When using this technique a new algorithm based on a convolutional neural network finds the nose- and tail-base points of rodents with high accuracy and few or no nose-tail swaps. The network was trained to extract features from video images of rodents of various colors and in various backgrounds, with videos in which the nose and the tail-base were annotated. During tracking, the network analyzes a portion of the image that includes the detected subject. It creates a map of probability of occurrence for both the nose- and the tail-base points, and finally makes an estimate of the position of the nose point and the tail-base point, based on the highest probability.

Contour-based Body Point Detection Technique:

- Any species - Default || This detection method analyzes the contour of the area detected as subject at each sample to assign the nose-point and tail-base. Make sure in the detection settings that the tail is fully detected. With this method it may be possible to track 'non-rodent' shapes but the method is not designed for it. This method is automatically activated when you track the center-point in one animal per arena, and you specified Other species in the Automated setup.

- Rodents - Default || This detection method analyzes the varying shape of the contour of the area detected as subject and builds up a 'rodent model'. It is more robust than the Shape-based method because it does not require the nose and tail to be visible: it can 'predict' the position of the nose and the tail based on previous samples. Make sure in the detection settings that the tail is removed from the body contour with Erode and Dilate. Choose this method when you want to track a single rodent without occlusions or without difficult tracking conditions

- Rodents - For occlusions || This method can handle severe shape distortions, for example when the animal's body is occluded or when multiple animal's touch. However, it requires a lot of computer performance. Choose this method for rodents in a social interaction context, or when you track rodents that can be occluded, for example, by bars or other objects in the cage. Make sure in the detection settings that the tail is removed from the body contour with Erode and Dilate.

- Other species - For occlusions || This method does not make any assumption about the shape of the subject. Choose this method to track for example insects, crustaceans and large animals. Also use this method if you track fish from the side view of the water tank, and the Adult Fish method does not give good results. However, with this method you only track the center point.

Contour adjustments can be made in one of two ways or by combining both methods.

- *Erosion* – decreases the animal's surface area with 1-10 pixels.
- *Dilation* – increases the animal's surface area with 1-10 pixels.

Besides the above-mentioned options to optimize the detection and identification of the subject(s) contrast settings can be fine-tuned, subject size can be defined and video smoothing can be applied. There are also some additional detection settings specific for when you are using the Rat or Mouse Behavior Recognition Module.

Tracking methods multiple subjects per arena
Color Marker tracking:

- The color market itself is tracked and used for the subject identification.

Contour based body point detection offers 2 options:

1. Center of gravity tracking only.

2. Center of Gravity and Nose point and Tail-base tracking

In this case 2 Subject identification methods are available:

- Marker-Assisted identification - Identities are distinguished based on a color-marker.
- Unmarked subjects - identities may switch when paths crosse.

Deep Learning based Body Point detection:
This method requires the animals have uniform color but appear somewhat different in the camera image. When 2 identical mice or rats are used, it is recommended to use one of the following methods to make them appear different for the deep learning network:

- Use a tail marker.
- Bleach the fur on the back of one animal.
- Shave part of the fur on the back of one animal.

Hooded rats (2-tone rodents) are not supported.

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 subject.
- *Static subtraction* – Looks at differences between a reference image (without the animal) and the current video image (with the animal in the arena).
- *Dynamic subtraction* – Like the previous, looks at differences between a reference image and the current 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.

The trial list also allows you to import trials from previously acquired experiments, with the aim to analyze data collected in two or more identical experiments as one data set.

2. DATA ACQUISITION

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3. In combination with the Rat Behavior Recognition Module or the Mouse Behavior Recognition Module respectively.

EthoVision XT offers the following 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 72 hours depending on the hardware used.
3. *Longer trials* – To acquire trials longer than mentioned above, you can split your multi-day testing into multiple trials.
4. *Record video and acquire data* – EthoVision XT records a video to an MPEG4 file which you can use for data acquisition later. (This method is recommended if the computer is not fast enough for live data acquisition and video recording simultaneously.)
5. *Acquire data from existing video files* – Track data from video recorded with programs other than EthoVision XT.
6. *Acquire data from a batch of existing video files at once* – Track data from a series of pre-recorded video files.

In options 2 – 5, the video file is available for post-acquisition visualization in combination with the track file and analysis results. If you choose option 3, 4, or 5, 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, MPEG2, MPEG4 and H.264 AVC video formats.

Series of trials from live video feed
You can use a 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, you can add scores live (while the test is running) or offline (by reviewing the video file). 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. In addition, EthoVision XT is able to automatically recognize ten rat or mouse behaviors[3].

Acquire data with DAQ system
With the External Data Module, external data acquired with a separate DAQ system can be synchronized with

the tracking data acquired with EthoVision XT. When you simultaneously acquire tracking data and physiological data, EthoVision XT sends out a synchronization signal to the external DAQ system. After data acquisition, you can import the physiological data into EthoVision XT and the two data streams are synchronized. You can only co-acquire external data when you track data live or use EthoVision XT to record the video (options 1 – 3). EthoVision XT offers import profiles for a number of DAQ systems, such as DSI's Ponemah.

You can import:

- Signals sampled at a constant rate (with equidistant time stamps).
- Signals sampled at a non-constant rate (with non equidistant time stamps).
- Physiological data stored in ASCII or EDF format.
- Ultrasonic vocalization data recorded with UltraVox XT.

Analyzing data — You can:

- Analyze external data after resampling to the EthoVision XT sample rate. For example, you can resample EEG from 250 Hz to 25 Hz, and then calculate the mean of the signal and other statistics.
- Convert a signal, for example cage temperature, to a state variable with possible values 1 and 0, based on the value of the signal. You can use this state variable to analyze specific time intervals. For example, analyze all time intervals when cage temperature was above 25 °C.
- Synchronize the external data with the video and the track data after import.

Exporting data — You can export the original (exported) external data as well as the resampled data.

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 will find samples that match a specific criterion, for instance to find missing points or samples that are separated by a distance greater or smaller than a specific value. With the Track Editor, points can be deleted, moved to another position or interpolated. Furthermore it can be used to swap subjects in case these were assigned incorrectly, or correct the nose-point and the tail-base in the case they were swapped.

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 stays stored.

4. SMOOTH TRACKS

Small (erratic) movements, such as body wobbles or outliers, may lead to an overestimation of certain parameters.

- The Lowess filtering method, which essentially smooths the acquired tracks of the body center point).
- As an alternative 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.
- You can also remove outliers using the 'Maximal distance moved'.

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

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. You can also visualize integrated data: the video, the animal's track, and the values of the selected parameters 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.

All output variables 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.

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. Hiqh quality export options with min-max scale allow for use in publications.

7. ANALYZE DATA

Standard available output variables

- *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).

- *Acceleration* – The rate of change of velocity of the body point over time, indicating how quickly it is speeding up or slowing down.

- *Acceleration state* - A categorization of the acceleration behavior of the body point, typically defined by thresholds to describe different states of motion, such as 'accelerating', 'decelerating', or 'constant speed'.

- *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* – 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* – 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.

Additional variables Social Interaction Module

- *Distance between subjects* – The distance between a body point of one animal and a body point of another animal.
- *Body contact* – Contact between the bodies of two animals (not related to body points, but to the whole detected area of the animal's body).
- *Proximity* – A discrete variable with two possible states, 'in proximity' and 'not in proximity', indicating whether an animal is within the proximity of another animal, with user-definable thresholds.
- *Relative movement* – discrete variable describing the animal's movement relative to another animal with four possible states; 'moving to', 'moving from', 'no relative movement', and 'no interaction'. The maximum interaction distance is user-definable.
- *Net weighted movement* – The signed, distance-weighted change in distance between two subjects from one sample to the next.
- *Weighed movement from* – The distance-weighted change in distance between subjects, when an animal (actor) is moving away from another animal (receiver).
- Weighed movement to – The distance-weighted change in distance between subjects, when an animal (actor) moves towards another animal (receiver).
- *Side by side* – A parameter indicating when two animals are positioned parallel to each other, reflecting a specific type of social interaction or alignment.
- *Train* – A parameter used to describe a behavior where one animal follows directly behind another, forming a 'train'.

Additional parameters Live Mouse Tracker
Note, these behaviors can be analyzed when you are using a Live Mouse Tracker data acquisition system (https://livemousetracker.org) and imported these data into EthoVision XT

- Approach rearing subject
- Drink
- Follow
- Get Away
- Group
- Make and break group
- Make contact
- Move
- Nest
- Nose contact
- Nose contact sequence
- Out of nest
- Rear
- Social approach
- Stretch attend posture
- Social escape
- Stop

Additional parameters Trial & Hardware Control Module

- *Trial Control State* – A time interval specified by two events of Trial & Hardware Control that occurs 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.
- *Trial Control variable* – With this variable you can analyze and visualize the variables defined in the Trial Control Settings. For example, you defined a variable named Counter, which counted the number of correct choices in a conditioning task, and you want to have the maximum value that the variable reached during the various trials.
- *Trial Control Event* – A point event, with no duration, defined by an element of the Trial & Hardware Control (a condition, action, rule, subrule, or reference).
- *Hardware variables* – EthoVision XT allows you to analyze hardware events in the software. Hardware events can either be commands that were sent out from EthoVision to external devices (such as a 'drop pellet' command) and signals that were sent back by hardware devices to the USB-IO box/EthoVision XT computer. You can analyze these signals and answer questions like "How many pellets did the pellet dispenser drop?"

Additional parameters Rat Behavior Recognition Module

- Drinking
- Eating
- Grooming

- Jumping
- Rearing supported
- Rearing unsupported
- Resting
- Sniffing
- Walking
- Twitching
- *Behavior probability* – the probability of the behavior that is detected. You can use this variable to display behavior probability in the integrated visualization
- *Merged behavior* – you can merge automatically detected behaviors into one group, such as rearing (unsupported and supported rearing) and eating and drinking combined.

All automatically scored behaviors have a duration, except for twitching, which has a frequency.

Additional variables Mouse Behavior Recognition Module

- Drinking
- Eating
- Grooming
- Hopping
- Rearing supported
- Rearing unsupported
- Resting
- Sniffing
- Walking
- Digging
- *Behavior probability* – the probability of the behavior that is detected. You can use this variable to display behavior probability in the integrated visualization
- *Merged behavior* – you can merge automatically detected behaviors into one group, such as rearing (unsupported and supported rearing) and eating and drinking combined.

All automatically scored behaviors have a duration, except for twitching, which has a frequency.

Multi-condition variables

You can create new, customized parameters by combining two or more of the ones mentioned above, including manually or automatically scored events, hardware events, and external data.

Other custom-build parameters are available upon request.

JavaScript variables

You can create custom dependent variables with JavaScript code. With JavaScript you have an almost endless range of possibilities to extract information from your track. JavaScript code is handy when the standard dependent variables cannot do what you need. JavaScript variables can be of type continuous, event, or state. You can find JavaScript variables ready to use on the EthoVision XT 18 Installation package and they are also available on GitHub: https://github.com/noldus/EthoVision-JavaScriptCustomAnalysis

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
- Maximum
- Minimum
- Number of samples used to calculate the statistic

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 variables 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 hardware log* – If you are using the Trial & Hardware Control Module, you can also export the hardware log file.

- *Export manual scoring log* – If you have manually scored events, these can be exported as raw data are using the Trial & Hardware Control Module, you can also export the hardware log file.

- *Export Trial Control log* – (i.e. , the instructions of Trial Control and when the events occurred. e.g. when a condition became true).

- *Export the external data log (original 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.

QUALITY ASSURANCE

The Quality Assurance Module offers three tools to safeguard your experiment.

1. The ability to set user management rights

2. An application log that logs:

- Start and stop of trial
- Creation of new experiment
- Start of an experiment
- Stop of an experiment
- Restoration of an experiment

3. An experiment log that contains:

- Snapshots of and changes made in the experiment settings, detection settings, and arena settings (zones)
- Date and time a trial started and ended duration of a trial
- Values of independent variables belonging to that trial
- Hyperlinks to earlier logged arena settings, trial control settings, and detection settings
- Comments made by the user
- Snapshot of data selection and analysis profile

Both the application log and experiment log can be exported and printed.

HELP FEATURES

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

- *Video tutorials* – EthoVision XT incorporates mini video tutorials in certain parts of the software 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.

- *Sample experiments* – Provided with the software. More are available on at my.noldus.com.

HARDWARE COMPONENTS

Minimum set-up:

- EthoVision XT Base software
- Camera (e.g. GigE, USB3, Basler, Analog)
- Computer

Optional components:

- DanioVision™ Observation Chamber
- PhenoTyper®
- Various mazes and open fields
- Backlight unit
- Camera table
- DAQ system
- MediaRecorder (for high resolution recordings e.g.)
- UltraVox XT
- USB-IO-box

SUPPORTED CAMERAS BY ETHOVISION XT 18

EthoVision XT was tested with a selected range of cameras (see table on the next page). The maximum number of cameras that you can use depends on whether EthoVision XT is installed on a desktop or laptop computer.

- Cameras must be of the same type; a combination of analog and digital cameras is not supported.
- Mixing video images from different camera types is not supported.
- The drivers for the supported cameras are available on MyNoldus: my.noldus.com.

Type	Brand/model	Max. number	Interface board (for desktop)	Driver version (a)	Frame rate (fps)
GigE	Basler acA1300-60gm (monochrome)	Desktop: 1-16 Laptop: not tested, not supported	CT1000 Pro (1 camera) Adlink PCIe-GIE74 (1-4 cameras) Basler GigE Interface Card, 4 port PoE (1-4 cameras)	7.5.0.15658	60
GigE	Basler acA1920-40gc (color)	Desktop: 4 Laptop: not tested, not supported	CT1000 Pro (1 camera) Adlink PCIe-GIE74 (1-4 cameras)	7.1.0.25066	40
USB 3.0	Basler acA1920-155µm (monochrome)	Desktop: 1 Laptop: 1	U3-PCIE1XG205-1S (1 camera) U3X4-PCIE4XE111 (1 camera)	7.5.0.15658	60
Analog	PhenoTyper	Desktop: 1-16, Laptop: not tested, not supported	Picolo Alert (1-4 cameras)	Multicam 6.15.1.3573	25 (PAL) 29.97 (NTSC)

SYSTEM REQUIREMENTS

For optimal performance and functionality of EthoVision XT, an up-to-date professional workstation is strongly recommended. EthoVision XT 18 is tested on Windows 11 Pro 64 bit, version 23H2 and Windows 11 Pro 64 bit version 24H2 on a Desktop Dell Precision 3680 and Laptop Dell Precision 3591. For those systems we guarantee compatibility.

The recommended computer requirements for workstations are:

- Processor (CPU) – 14th Gen Intel Core i7-14700, 20 cores, 2,1-5,4 GHz

- Internal memory (RAM) – 32 GB for desktop PCs and 16 GB for laptop PCs.

- Graphics card (GPU) – NVIDIA Quadro T1000, 8 GB GDDR6.

- USB 3.0 ports or an additional Ethernet network interface adapter, depending on the cameras.

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

Performance will depend on the functionality used. Some processes, such as live acquisition or tracking multiple animals within one arena, will require more from the processor of your computer, and may influence the working speed.

REQUIREMENTS DEEP LEARNING

Deep learning relies on the CUDA computing platform. For this reason, the graphics processing unit (GPU) driver must support CUDA version 12,2. For tracking live or from video files, we tested and guarantee compatibility with:

For a Desktop computer Dell Precision 3680:

- NVIDIA GeForce RTX 4060, 8 GB GDDR6, driver version 552.22.

For a laptop computer Dell Precision 3591:

- NVIDIA RTX 1000 Mobile, 6 GB GDDR6, driver version 553.09.

When a driver version is not sufficient for supporting CUDA, EthoVision XT gives the message in the Experiment Settings: *The graphics card driver is not installed or the graphics card does not support CUDA*. We recommend to download and install the latest driver from the card's manufacturer.

The following limitations apply:

- For one subject tracking: Up to four arenas, Live tracking or from video file. No minimal trial duration required. Maximal trial duration tested 72 hours.

- For two-subject tracking: Up to four arenas, only tracking from video file. Tracking of hooded rats not supported. It is recommended to perform trials of at least five minutes. Maximal trial duration tested 1 hour.

- Subject size: The apparent length of the subjects should be at least 10% of the size of arena. We recommend that the apparent length of the subject's body is at least 120 pixels for rats and 50 pixels for mice (nose to tail-base).

Apparatuses
EthoVision XT's neural networks have been trained with videos of:

- One subject per arena, subjects of uniform color: Open field (regular or with round objects in it), PhenoTyper (with or without bedding material), Elevated plus maze, Three-chamber social approach cage, Barnes maze, Fear conditioning cage with floor grid, Y-maze (with no objects), and a maze with multiple chambers and openings.

- One subject per arena, hooded rats: Open field (regular or with round, rectangular or triangular objects in it), PhenoTyper (with or without bedding material), and Elevated plus maze.

- Two subjects per arena: Open field (with no objects), PhenoTyper (with or without bedding material), and home cage.

REQUIREMENTS BEHAVIOR RECOGNITION

When using the Rat or Mouse Behavior Recognition Module, it is recommended that you take the following requirements and preferences into account:

- These modules require the detection of the nose point, center of gravity and tail base.

- The subject's apparent length (nose to tail base in walking position) must be at least 60 pixels for rats, and 55 pixels for mice. In all cases its apparent length must not exceed half the arena size.

- Rats must be older than approximately three weeks. In all cases subjects must be able to walk.

- The animal needs to be uniform in color. (Hooded rats have not been tested with this module.)

- You can track one animal per arena. When tracking live, you can use only one arena.

- The modules are tested in open field and home cage situations without a shelter. Using a shelter or other objects in your arena can influence the results because the animal gets closer to the camera (making its apparent size bigger) if it climbs on top.

- It is preferred that the animal is visible in the arena at all times. If this is not the case there will be missing data around the episodes where the animal is not seen.

- Use a top-view camera position and lighting from the top (instead of backlight).

- Slow changes in the light during the course of a test are not a problem. Moving spotlights can reduce the reliability of the detection.

- Video frame size must be greater than 352 x 288 pixels per arena.

- The frame rate of the recorded videos can only be a multiple of the sample rate. For example, with a sample rate of 25 samples per second, the video frame rate can only be 25, 50, or another multiple of 25 frames per second. The sample rate must be set between 25 and 31 samples per second.

- The video image must not be overexposed. Details of the subject's fur must be visible.

- Video compression should be as low as possible.

For contact details, please check

www.noldus.com

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