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 seven available modules:

- *Rat Behavior Recognition Module* – Automatically detect ten rat behaviors such as grooming, sniffing, and twitching.
- *Multiple Body Points Module* – Track the nose point and tail base, in addition to center point tracking[1].
- *Multiple Arenas Module* – Track animals in up to a hundred arenas simultaneously.
- *Social Interaction Module* – Track multiple animals within one 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.
- *Physiology Integration Module* – Integrate Etho-Vision XT data with co-acquired external data.
- *Quality Assurance Module* – Allows you to assign different rights to different users and reach Good Laboratory Practice compliance.

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 type of experiment (water maze, plus maze, 96-well plate, etc.). With these templates, many basic settings are pre-set, such as arena definition, detection settings, and parameters for analysis. Fine-tune these settings to fit your specific needs.

Experiment settings
Here you can specify:
- the number of cameras you are using
- video sources and their setttings
- settings for external data acquisition systems connected to EthoVision XT[2]
- the number of arenas (1-99)[3]
- the number of subjects per arena and subject roles (1-16)[3]
- detected body points, (Center of gravity, nose and tail base, color, or color marker)[1,3]
- activity analysis – a non-tracking option analyzing overall activity per arena based on pixel changes
- behavior recognition – for automatic recognition of rat behavior you need to specify if a feeder, drinking bottle, and wall are present in your set-up
- the hardware you want to use for Trial & Hardware Control[3]
- units of distance

Manual scoring settings
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 data acquisition. You can define:
- *Start-stop behaviors* – behaviors that are independent from each other.
- *Mutually exclusive behaviors* – behaviors grouped in such a way that activating one behavior automatically stops the other behavior.

You can define up to 32 different behaviors using the keys from the computer keyboard. If you need to define more behaviors, you can do so without assigning a key code.

Arena settings
The arena is the region in the video image where the animal is tracked. You can define:
- the arena(s)
- different zones within one or multiple zone-groups
- cumulative zones (define individual smaller zones to be taken into account for cumulative analysis)

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[1] Designed for use with rats or mice.
[2] Only available with the Physiology Integration Module
[3] Depending on if you are using one of the modules.

- hidden zones (used for shelters) and entry zones belonging to the hidden zone
- 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 arena settings from one arena to the other.

Multiple arena definition
When your experiment contains multiple arenas – for instance using a multi-well plate or a collection 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.

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 settings
There are two ways of trial control:
1) *Automatic Trial Control (ATC)* is included in Etho-Vision XT Base. With ATC, you can program Etho-Vision XT to automatically start and stop tracking, based on any of the parameters offered by Etho-Vision XT. This feature also includes an external command option, which allows you to start another computer program when a condition becomes true. For example, start present a visual stimulus on a computer screen or play a sound file when the animal enters a particular zone.
2) *The Trial & Hardware Control (THC) Module* allows you to define complete test protocols that include Boolean/logical operators and randomization functions 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. It lets you control external equipment from within EthoVision XT and define certain protocols. For example, you can automatically control the Atlantis Platform in a water maze task. Or you can use it for more 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.

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 to calculate the probability that each pixel is the animal.

If you are using the Multiple Body Points Module, there are three different detection methods for multiple body points.
- *Shape-based* – Analyzes the contour of the animal at each sample point to detect the nose point and tail base.
- *Model-based* – Analyzes the varying shape of the contour of the animal over time and builds a 'rodent model'. It does not require the nose and tail to always be visible; it can predict their position based on previous samples.
- *Advanced model-based* – Learns the animal shape and how it moves in the first 15 frames and continually updates its statistics. Use this method when shapes are distorted, such as when the animal's body is occluded or when multiple animals touch.

The 'scan window' option reduces the processor load and minimizes problems with reflection (sometimes occurring in certain lab cages or aquatic environments), because EthoVision XT only searches for the animal in a specific part of the arena, based on the animal's size and its location in the previous frame.

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 Behavior Recognition Module.

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.
2. *Acquire data live and record video* – Live tracking, while EthoVision XT records a backup video to an MPEG4 video file.
3. *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.)
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 – 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 FireWire cameras, GigE cameras and analog 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, MPEG2, 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 Event Recorder). 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 behaviors[4]: grooming, jumping, unsupported rearing, supported rearing, twitching, sniffing, walking, resting, drinking, and eating.

Acquire data with DAQ system

With the Physiology Integration 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 DataQuest A.R.T. and Polar's Precision Performance.

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4 *In combination with the Rat Behavior Recognition Module.*

3. EDIT DATA

EthoVision XT is a very robust and accurate tracking system. However, if for some reason minor errors do occur, you can easily correct them by simply moving, deleting, or swapping sample points.

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.

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.)
3) *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.

All trial parameters can be presented in tables and graphs. 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 allow for use in publications.

7. ANALYZE DATA

Standard available parameters[5]

- *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 from one zone to another.
- *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. Relative meander takes the direction of turning into account (clockwise or anti-clockwise), whereas absolute meander does not.

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[5] *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.*

- *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.

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.
- *Elongation* – A discrete variable with three possible states, 'stretched', 'normal', and 'contracted', indicating the elongation percentage of the animal's body, with user definable thresholds.
- *Head directed to zone* – The duration the animal's head is directed towards a specific zone or a circular area around a point.

Additional parameters Social Interaction Module5
- *Distance between subjects* – The distance between a body point of one animal and a body point of another animal.

- *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).

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 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 10 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* – behavioral state
- *Eating* – behavioral state
- *Grooming* – behavioral state
- *Jumping* – behavioral state
- *Rearing supported* – behavioral state
- *Rearing unsupported* – behavioral state
- *Resting* – behavioral state
- *Sniffing* – behavioral state
- *Walking* – behavioral state
- *Twitching* – behavioral event
- *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.

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

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.
- *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.

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.

- The ability to set user management rights.
- 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
- 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

- *Additional documentation* – Reference manual, catalog.
- *Online help* – Product leaflets, application notes, short tours, and product news.
- *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. FireWire, GigE)
- computer

Optional components:
- DanioVision™ Observation Chamber
- PhenoTyper®
- various mazes and open fields
- backlight unit
- camera table
- DAQ system

SYSTEM REQUIREMENTS

For optimal performance and functionality of Etho-Vision XT, an up-to-date computer system is strongly recommended. EthoVision XT 10 is designed for Windows 7 and 8 64 bit Professional version and has been tested on a Dell Precision T3600. For that system we guarantee compatibility.

The optimal computer requirements for workstations are:
- Processor (CPU) – Quad core 2.8 GHz.
- Internal memory (RAM) – 8 GB.
- DVD-player.
- Hard disk – 1 TB free space (to store experiments including video files).
- Graphics card – 1920x1080256 MB (512 recommended), needs to support DirectX 9.0c and Video Acceleration.

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 SPECIFIC FOR THE RAT BEHAVIOR RECOGNITION MODULE

When using the Rat Behavior Recognition Module, it is recommended that you take the following require-ments and preferences into account:
- The Rat Behavior Recognition Module needs to be combined with the Multiple Body Points Module.
- The detected rat needs to be at least 60 pixels in length (nose to tail base) in a walking position.
- The rats needs to be uniform in color. The system is optimized for white rats.
- You can track one animal per arena. When tracking live, you can use only one arena.
- The Rat Behavior Recognition Module is 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 if it climbs on top.
- The animal should be visible in the arena at all times.
- Use a top-view camera position and lighting from the top (instead of backlight).

Please check www.noldus.com for contact details.

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