EthoVision® XT Tutorial

Summary

- Tracking multiple animals per arena
- Advanced detection settings
- Analysis of social interaction tests
- New detection method: Differencing
- Advanced trial control using routines
- Using routines also to analyze data
- Scoring events manually

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Advanced EthoVision XT Tutorial

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27 August 2010

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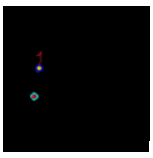
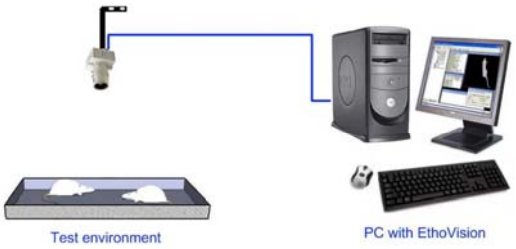


Tracking Multiple Animals per Arena

Tracking multiple animals



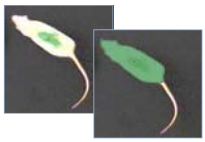
Tracking multiple animals

Color marker tracking

- Only the marking spot is tracked
- Subject size = size of the marking spot
- For any species

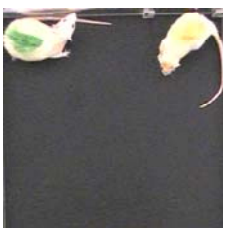
Marker-assisted tracking

- EthoVision identifies colors, and recognizes the shape
- Only for tracking rodents



Using color markers

- Markers as round as possible
- Markers not too large – should not cover the animal's flanks
- Colors easily recognizable by EthoVision
- Use a full-spectrum light source



Color blob and subject shape

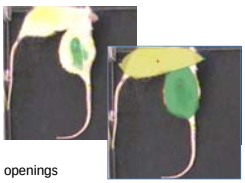
Two main steps

- Color blob → Individual recognition
- Shape → By the detection method (e.g. Dynamic subtraction)

Advanced Detection Settings

Advanced detection settings: When needed?

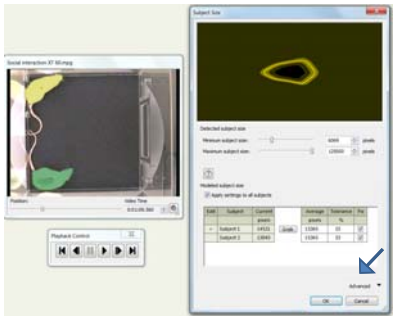
- Body contact:
  Subject's contour no longer clear



- Occlusions:
  For example cage bars, panels with openings

Advanced detection settings

Click the **Advanced** button in the **Subject Size** window



Body contact (Modeling vs. Performance)

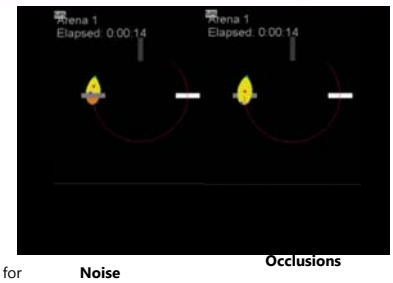
Optimized for **Modeling**

Shapes are more consistent through time

Performance

The shape is broken in parts (smaller parts are considered noise)

Occlusions (Noise vs. Occlusions)



Optimized for **Noise**

Smaller blobs are not part of the animal (noise)

Occlusions

Separate blobs are "forced" to be as one

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Analysis Functions for Social Interaction Tests

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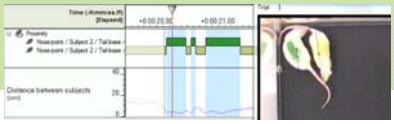
Topics

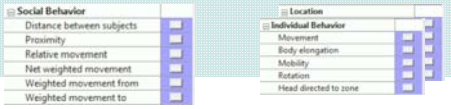
Selecting data based on the behavior

- Define intervals with the **Nesting** functions in the Data Profile



Analyzing behaviors

- Choose the **dependent variables** in the Analysis Profile



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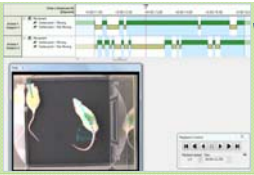
Analysis of social interaction

Selecting track segments according to the behavior…

- Per subject
  Nesting

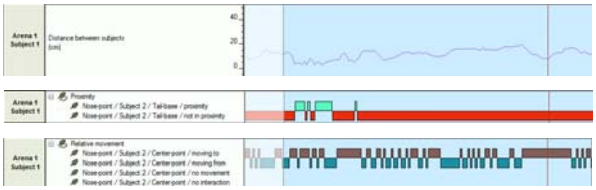


- Across subjects
  Nesting over subjects

Analysis of social interaction

New dependent variables in the Analysis profile

- Distance between subjects
- Proximity
- Relative movement
- Net weighted movement
- Weighted movement from/to

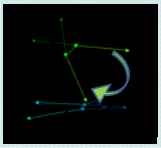


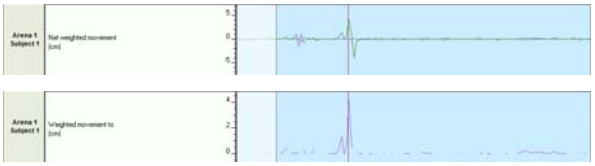
Analysis of social interaction

New dependent variables in the Analysis profile

- Distance between subjects
- Proximity
- Relative movement
- Net weighted movement
- Weighted movement from/to

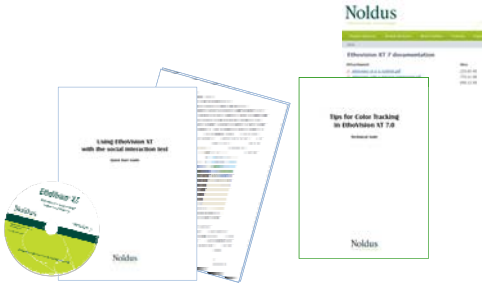
} approach/avoidance





Tracking multiple animals

Tracking of multiple animals per arena is possible with the **Social Interaction** add-on module of EthoVision XT



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Detection Methods: Differencing

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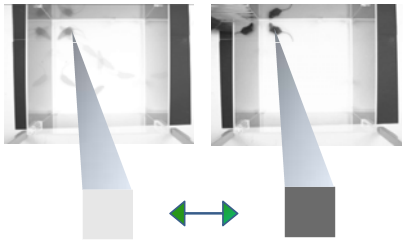
Differencing: How it works

- Differencing uses a reference image just like Dynamic subtraction.



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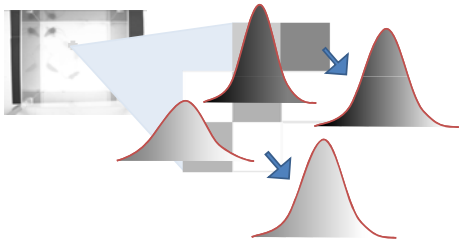
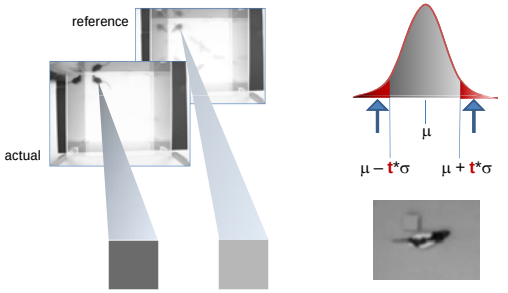
Differencing: How it works

- Instead of considering a fixed range of contrast between subject and reference image, Differencing calculates the probability distribution of pixel values in the reference image.



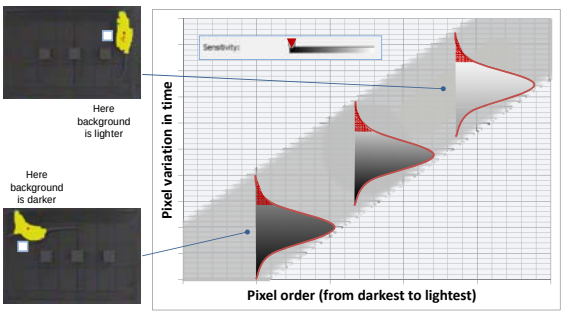
Noldus — Differencing: How it works

- Each pixel in the current image is compared with the grey value probability distribution.



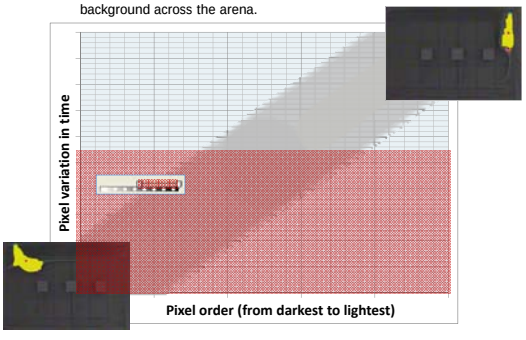
Noldus — Differencing: How it works

- Differencing is controlling for local variotion in pixel gray value.



Noldus — Subtraction compared with Differencing

- Subtraction uses a constant range of contrast between animal and background across the arena.



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Advanced Trial Control Using Routines

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The problem: Repeating tasks

Behavior → Action

- When the rat enter the maze arm, open the arm door

Conditioning experiment

- When the mouse dwells in a trigger zone, provide a reward
- When the mouse enters the shelter, provide an aversive stimulus

Longer-term recurrent tasks

- For 6 days at midnight, start the conditioning procedure

In all these situations, conditions and actions must be repeated

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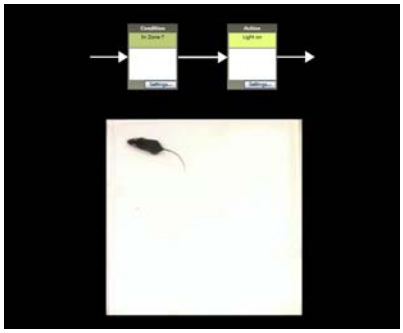
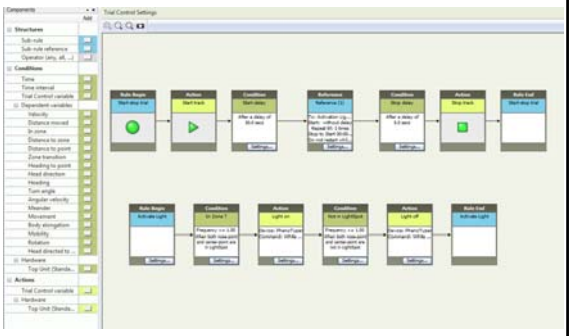
Conditions and actions

A Condition is followed by an Action



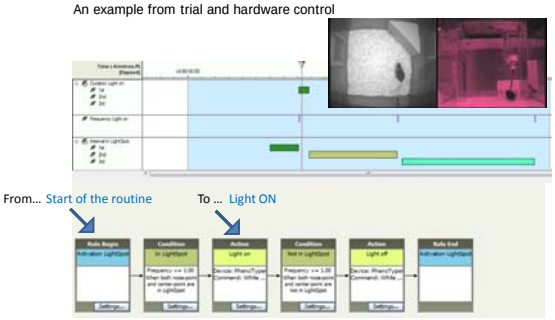
Calling a subrule: the Reference box

A Reference box can call a subrule one or more times

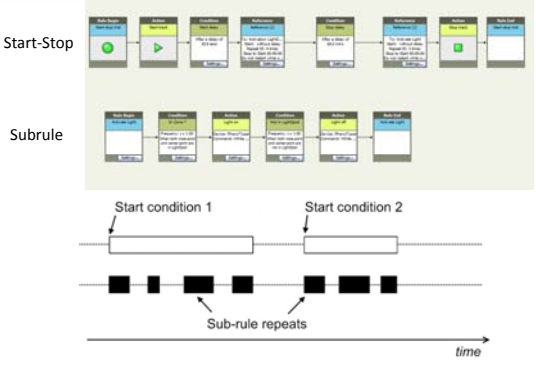


Trial control routines to analyze data

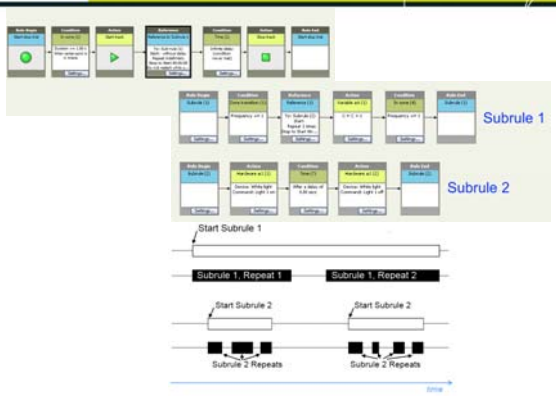
An example from trial and hardware control



A subrule can be called from different locations

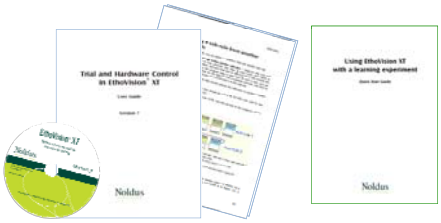


A subrule can be nested in another subrule



Trial and hardware control

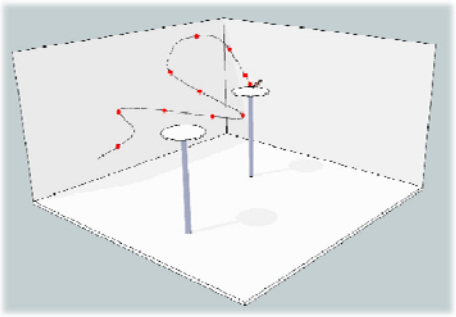
Subrules can only be defined with the **Trial and Hardware Control** add-on module of EthoVision XT



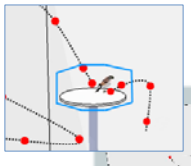
Using Trial Control Routines Also to Analyze Data

Trial control routines to analyze data

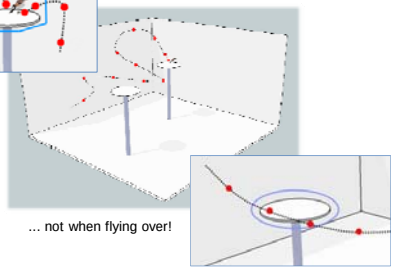
An example from tracking in a three-dimensional arena



Trial control routines to analyze data

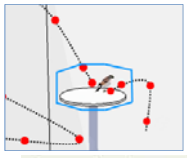


EthoVision must “see” the bird landing on the platform...

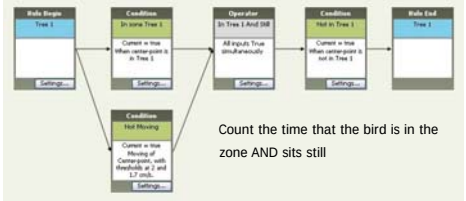


... not when flying over!

Trial control routines to analyze data



Use Trial Control to describe the landing event



Count the time that the bird is in the zone AND sits still

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Scoring Behaviors Manually

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Scoring behaviors manually

Use the Manual Scoring Settings:

To define behaviors that EthoVision cannot detect automatically for example:

- Rearing
- Sniffing
- Grooming
- Social interactions/ parental care

To detect *Stretching*: use the Elongation variable.

To detect *Head dips* in a plus maze, define zones at the end of the open arms and analyze when the nose points enters the zone.

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Defining behaviors

Click **Manual Scoring Settings** in the Experiment Explorer.

- Define behaviors in a **Mutually exclusive** group if they are related to one another

  Example: Grooming Bilateral, Grooming Unilateral, etc.

Behaviors				Keys
Behavior Name	Type	Description	Initially Active	Subject 1
Grooming				
Unilateral stroke	Mutually exclusive		☐	u
Bilateral stroke	Mutually exclusive		☐	b
No grooming	Mutually exclusive		☑	0

- Define a behavior as **Start-Stop** if it is independent of the others.

  Example: Rearing

Rearing	Start - Stop		☐	r	r

Scoring behaviors manually

Tips

- If you track from video files, clear the **Detection Determines Speed** option, otherwise the video may play faster than 1x.

- You cannot rewind the video during a trial, so the behaviors must be scored in one run. Make the *coding scheme* as simple as possible.
- To score behaviors from multiple subjects and/or arenas more easily, or with a complex coding scheme consider using The Observer XT.