

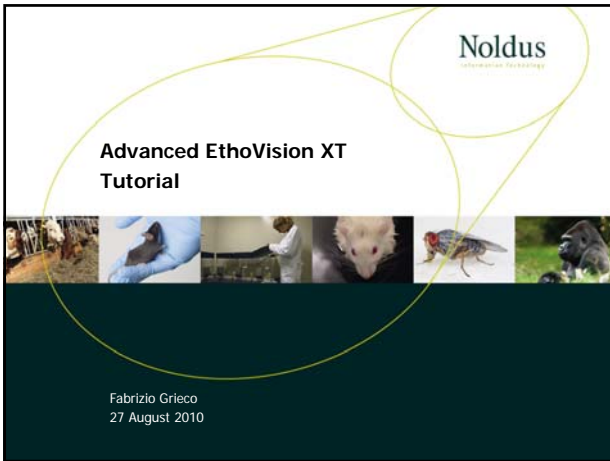
# EthoVision® XT Tutorial

## 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



**Noldus**  
Information Technology



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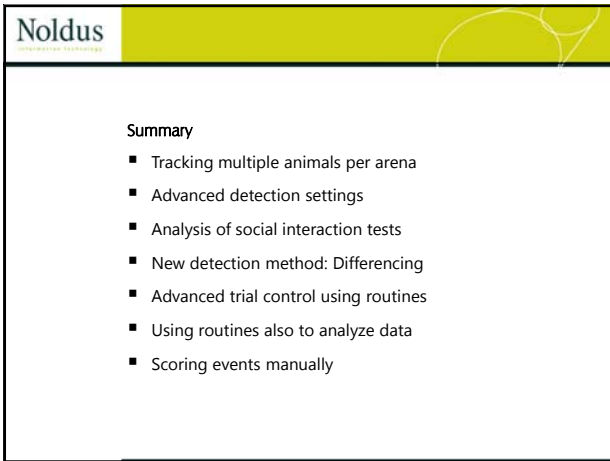
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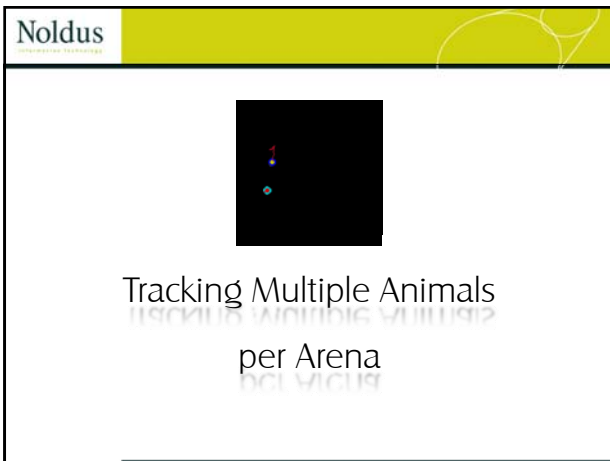
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**Noldus** Tracking multiple animals

The diagram illustrates the experimental setup. On the left, a 'Test environment' is shown as a rectangular tray containing two white mice. A camera is mounted above the tray, connected by a blue line to a 'PC with EthoVision' on the right. The PC setup includes a tower case, a monitor displaying a software interface, a keyboard, and a mouse.

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**Noldus** Tracking multiple animals

**Color marker tracking**

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

A circular arena containing two mice and two small colored markers (one red, one green) on the floor.

**Marker-assisted tracking**

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

Two close-up images of mice. The first shows a mouse with a yellow marker on its back. The second shows a mouse with a green marker on its back.

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**Noldus** 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

Two mice are shown on a dark background. One mouse has a yellow marker on its back, and the other has a green marker on its back.

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**Noldus**  
Color blob and subject shape

**Two main steps**

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



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**Noldus**

Advanced Detection Settings

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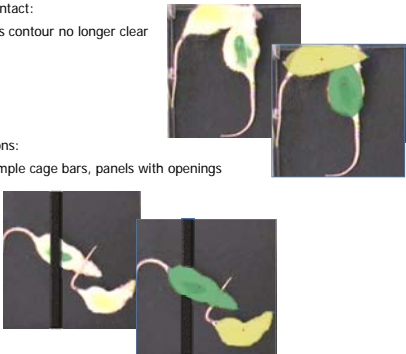
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**Noldus**  
Advanced detection settings: When needed?

- Body contact:  
Subject's contour no longer clear
- Occlusions:  
For example cage bars, panels with openings



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**Noldus** **Advanced detection settings**

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

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**Noldus** **Body contact (Modeling vs. Performance)**

Optimized for **Modeling** **Performance**

Shapes are more consistent through time

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

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**Noldus** **Occlusions (Noise vs. Occlusions)**

Optimized for **Noise** **Occlusions**

Smaller blobs are not part of the animal (noise)

Separate blobs are "forced" to be as one

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## Analysis Functions for Social Interaction Tests

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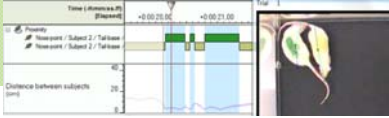
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### Topics

**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

Social Behavior		Location	
Distance between subjects	<input type="checkbox"/>		
Proximity	<input type="checkbox"/>		
Relative movement	<input type="checkbox"/>		
Net weighted movement	<input type="checkbox"/>		
Weighted movement from	<input type="checkbox"/>		
Weighted movement to	<input type="checkbox"/>		

Individual Behavior	
Movement	<input type="checkbox"/>
Body elongation	<input type="checkbox"/>
Mobility	<input type="checkbox"/>
Rotation	<input type="checkbox"/>
Head directed to zone	<input type="checkbox"/>

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### Analysis of social interaction

**Selecting track segments according to the behavior...**

- Per subject Nesting**  
  
Different intervals!
- Across subjects Nesting over subjects**  
  
Same interval!

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**Noldus** Analysis of social interaction

**New dependent variables in the Analysis profile**

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



Distance between subjects

Proximity

Relative movement

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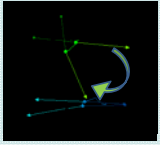
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**Noldus** Analysis of social interaction

**New dependent variables in the Analysis profile**

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

} approach/avoidance



Net weighted movement

Weighted movement to

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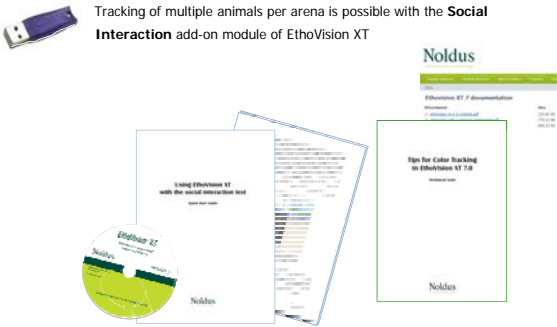
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**Noldus** Tracking multiple animals

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



Using EthoVision XT with the social interaction tool

Tips for Color Tracking in EthoVision XT 7.0

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Detection Methods:  
Differencing

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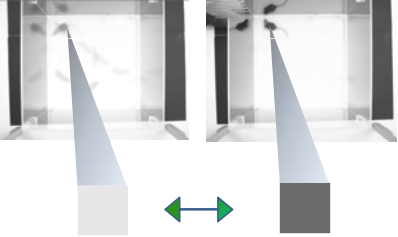
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Noldus Differencing: How it works

Method  
Differencing  
Gray scaling  
Static subtraction  
Dynamic subtraction

- Differencing uses a reference image just like Dynamic subtraction.



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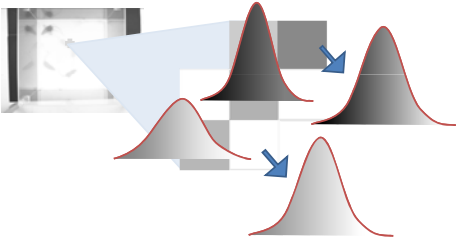
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Noldus Differencing: How it works

- 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.



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**Noldus** **Differencing: How it works**

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

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**Noldus** **Differencing: How it works**

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

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**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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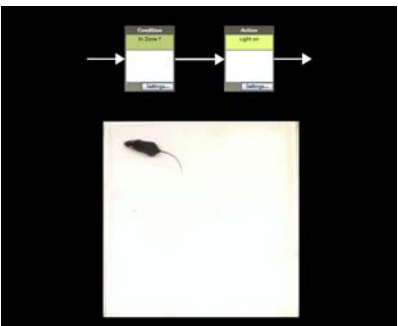
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### Conditions and actions

A Condition is followed by an Action



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**Noldus** **Calling a subrule: the Reference box**

A Reference box can call a subrule one or more times

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**Noldus** **Trial control routines to analyze data**

An example from trial and hardware control

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**Noldus** **A subrule can be called from different locations**

Start-Stop

Subrule

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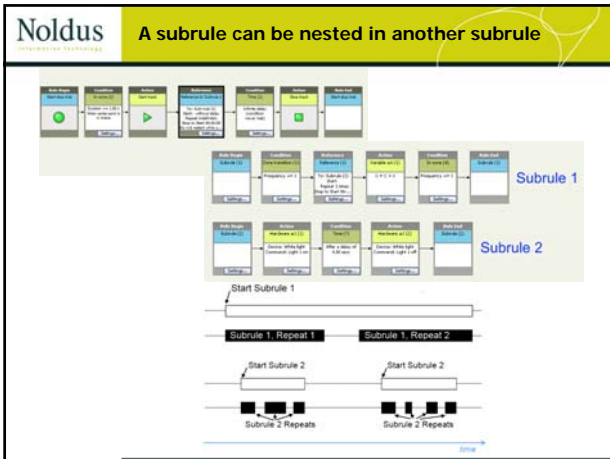
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The slide includes a USB drive icon and the text: "Subrules can only be defined with the **Trial and Hardware Control** add-on module of EthoVision XT". Below the text are images of a CD labeled 'EthoVision XT', a manual titled 'Trial and Hardware Control in EthoVision XT', and another manual titled 'Using EthoVision XT with a learning experiment'.

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Using Trial Control Routines  
Also to Analyze Data

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**Noldus** Trial control routines to analyze data

An example from tracking in a three-dimensional arena

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**Noldus** Trial control routines to analyze data

EthoVision must "see" the bird landing on the platform...

... not when flying over!

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**Noldus** Trial control routines to analyze data

Use Trial Control to describe the landing event

Rule Begin	Condition	Operator	Condition	Rule End
Tree 1	In Zone Tree 1 Current in tree when sensor goes in Tree 1	In Tree 1 AND Still	Not in Tree 1	Tree 2
	Condition Not Moving Current in tree Moving of Centered with Responder 1 and L7 only			

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

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## Scoring Behaviors Manually

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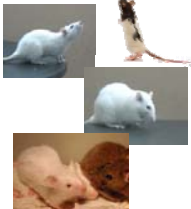
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### Scoring behaviors manually

**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.

Behavior Name	Type	Description	Initially Active	Subject 1
Grooming				
Unilateral stroke	Mutually exclusive		<input type="checkbox"/>	u
Bilateral stroke	Mutually exclusive		<input type="checkbox"/>	b
No grooming	Mutually exclusive		<input checked="" type="checkbox"/>	0



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

Behavior Name	Type	Description	Initially Active	Subject 1	Subject 2
Rearing	Start - Stop		<input type="checkbox"/>	r	r

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**Noldus** Scoring behaviors manually

**Tips**

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



The screenshot shows a window titled "Acquisition Control" with a close button (X) in the top right corner. Below the title bar are several icons: a play button, a stop button, a refresh button, a play button with a right-pointing arrow, and a checkbox labeled "Detection determines speed". A blue arrow points to the checkbox, which is currently unchecked. Below the icons are standard video playback controls: a double left arrow (rewind), a single left arrow (previous), a vertical bar (play/pause), a single right arrow (next), and a double right arrow (fast forward).

- 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.

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