

**Reference Manual**

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**PhenoTyper®**

**Noldus**  
Information Technology

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For EthoVision XT version 18

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# Contents

## **1 Introduction to PhenoTyper..... 6**

WHAT IS PHENOTYPER? —	6
THIS AND OTHER MANUALS —	7
MAIN FEATURES OF PHENOTYPER —	8
HOW CAN I USE PHENOTYPER? —	11
WHAT'S NEW IN PHENOTYPER? —	12
ADVANTAGES OF PHENOTYPER COMPARED WITH ORDINARY TEST SETUPS —	14
APPLICATIONS OF PHENOTYPER —	16
PHENOTYPER CONFIGURATIONS —	18
USING MULTIPLE PHENOTYPERS —	20

## **2 Assemble PhenoTyper..... 21**

BEFORE YOU START —	21
ASSEMBLE THE CAGE —	23

## **3 Set up PhenoTyper 1..... 35**

AIM —	35
TOPICS —	35
CABLES AND ADAPTERS —	36
CONNECT ONE PHENOTYPER TO THE PC —	37
CONNECT MULTIPLE PHENOTYPERS TO THE PC —	40
WORKING WITH GROUPS OF PHENOTYPERS —	46
CONNECT ADDITIONAL DEVICES —	50
LIGHTING CONDITIONS —	51
TEST THE PHENOTYPER'S FUNCTIONS —	51

ADJUST LIGHT INTENSITY —	53
COPY LIGHT INTENSITY VALUES FROM ONE PHENOTYPER TO ANOTHER —	55
THE PHENOTYPER CAMERA —	58

## **4 Set up an experiment with PhenoTyper 1... 60**

CREATE AN EXPERIMENT —	60
EXPERIMENT SETTINGS —	60
ARENA SETTINGS —	63
ASSIGN THE PHENOTYPER DEVICES TO THE ARENAS —	65
TRIAL CONTROL SETTINGS —	68
DETECTION SETTINGS —	68

## **5 Set up PhenoTyper 2 ..... 69**

AIM —	69
TOPICS —	69
CABLES AND ADAPTERS —	70
CONNECT ONE PHENOTYPER TO THE PC —	71
CONNECT MULTIPLE PHENOTYPERS TO THE PC —	74
8-PHENOTYPER CONFIGURATION —	77
16-PHENOTYPER CONFIGURATION —	81
WORKING WITH GROUPS OF PHENOTYPERS —	83
CONNECT ADDITIONAL DEVICES —	83
LIGHTING CONDITIONS —	83
TEST THE PHENOTYPER'S FUNCTIONS —	83
ADJUST LIGHT INTENSITY —	84
THE PHENOTYPER CAMERA —	85
ADD A TTL-CONTROLLED MODULE —	85

## **6 Set up an experiment with PhenoTyper 2 .. 88**

CREATE AN EXPERIMENT —	88
EXPERIMENT SETTINGS —	88
ARENA SETTINGS —	90
ASSIGN THE PHENOTYPER DEVICES TO THE ARENAS —	91

TRIAL CONTROL SETTINGS —	93
DETECTION SETTINGS —	93

## **7 Common tests with PhenoTyper..... 94**

GENERAL BEHAVIOR MONITORING —	94
CREATE ROUTINES —	97
FOOD RECEIVING TASK —	98
ILLUMINATED SHELTER TASK —	101
COGNITIONWALL™ TASK —	104
ANXIETY TESTS —	105
OTHER REFERENCES —	107

## **8 Maintenance ..... 108**

CLEAN THE CAGE —	108
ACCEPTABLE CLEANING TECHNIQUES AND SUBSTANCES —	110
SUBSTANCES THAT CAN DAMAGE PHENOTYPER —	112
CLEAN THE DRINKING BOTTLE —	112
CLEAN THE TOP UNIT —	113
RECOMMENDED MATERIALS —	113

## **A Technical Support ..... 115**

HELP DESK —	115
REPLACEMENT OF COMPONENTS —	115
PARTS LIST —	116

## **B Technical Specifications .....121**

PHENOTYPER 1 —	122
PHENOTYPER 2 —	124

## **C Declaration of Conformity ..... 126**

PHENOTYPER 1 —	126
PHENOTYPER 2 —	128

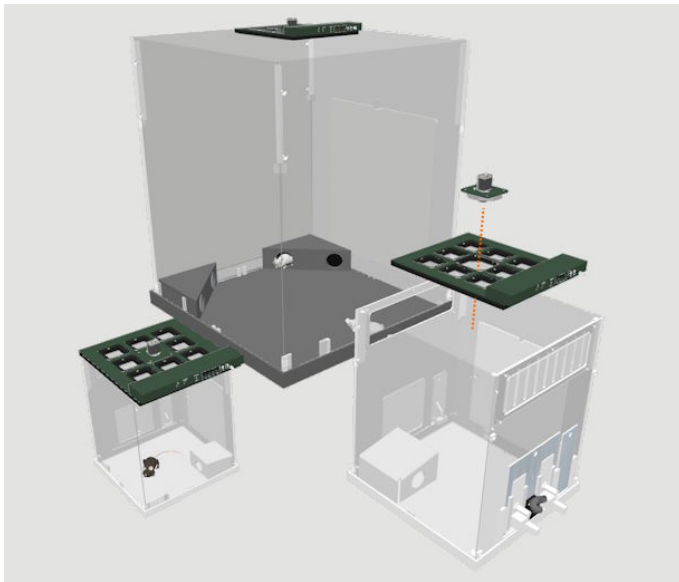
## **Index .....131**

# 1 Introduction to PhenoTyper

## WHAT IS PHENOTYPER?

PhenoTyper combines an open field and video-based monitoring equipment in a complete observation system that you can use to monitor rodent behavior automatically. The infrared lighting system ensures robust monitoring regardless of ambient light conditions in the lab.

You can also use PhenoTyper to administer visual and acoustical stimuli to your subjects, or to monitor the subjects using specific sensors. To this purpose, PhenoTyper 2 can easily integrate additional devices.



**Figure 1** *PhenoTyper 2 for different applications. Left: PhenoTyper for mouse. Middle: PhenoTyper for rats in a social interaction context. Right: PhenoTyper for single rats. The Top Unit hosts a GigE Vision digital camera and offers space for sensors and stimulus devices.*

The walls of PhenoTyper can be configured in different ways, making it suitable for a variety of applications.



**Figure 2** *Examples of the components of the PhenoTyper cage.*

PhenoTyper can be fully controlled by the EthoVision XT video tracking software. Activity, movement and specific behaviors (e.g. feeding, grooming) of the subjects can be continuously measured and analyzed afterwards.

## **THIS AND OTHER MANUALS**

To open the manuals, manual, from the Windows **Start** menu choose **All Apps > Noldus > EthoVision XT 18 Other Documentation**. You can also find all the manuals on the my.noldus portal:

[my.noldus.com](https://my.noldus.com)

### ***PhenoTyper - EthoVision XT 18 - Reference Manual.pdf***

(this manual). Includes information on how to install and configure PhenoTyper and control its devices to automate experiments in EthoVision XT. It also includes examples of PhenoTyper applications.

### ***PhenoTyper - EthoVision XT 18 - Service Manual.pdf***

See this manual for specific information on the PhenoTyper camera, and the devices that can be used together with PhenoTyper, for example the Pellet dispenser and PhenoWheel.

### ***EthoVision XT 18 - Trial and Hardware Control - Reference Manual.pdf***

This manual contains extensive information on the control of hardware devices through EthoVision XT. Learn how to combine conditions, actions, subrules to create complex task protocols for your subjects.

### ***EthoVision XT 18 Help***

Consult the Help for extensive information about EthoVision XT, for example how to draw arenas and zones, select tracks for analysis and create charts. To open the EthoVision XT Help, press **F1** in EthoVision XT.

## **MAIN FEATURES OF PHENOTYPER**

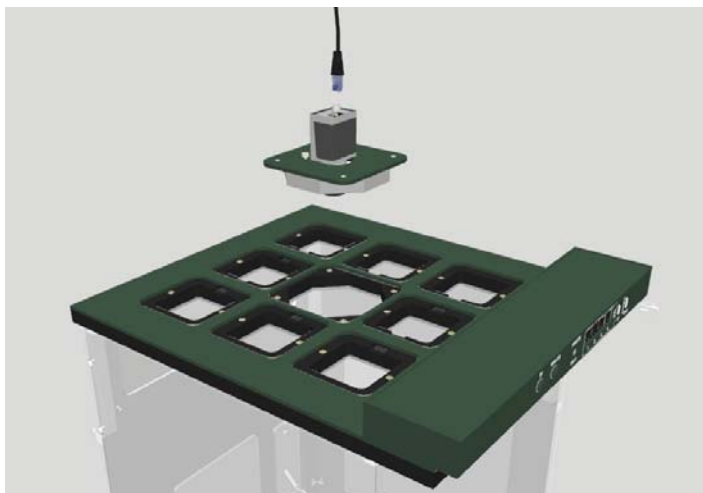
### ***Home cage environment***

Observation and tracking of subjects in a home cage environment has the main advantage that the behavior is more natural and less influenced by human intervention. The cages can be equipped with a shelter, a feeder, a water bottle and a rotating wheel, and can be enriched with bedding material and other objects. For details, see **PHENOTYPER CONFIGURATIONS** on page 18.

### ***Top Unit***

The Top Unit placed over the cage is the “brain” of PhenoTyper. It holds the camera and additional sensors and actuators and contains all the electronic equipment necessary to exchange information with the tracking software (Figure 3). It also includes infrared and white light arrays (see below).





**Figure 3** *The Top Unit of PhenoTyper 2 with the camera module. Additional modules (currently under development) can be added around the camera.*

### ***Lighting***

- An array of Infrared (IR) LEDs is activated as long as PhenoTyper is switched on. This allows continuous monitoring and video-tracking independent of the light conditions in the test room.
- An array of white LEDs (in PhenoTyper 2 only) provides visible white light. You can switch on this light and use it as a visual stimulus or when you need to track color-marked animals.
- You can adjust the intensity of the IR and white light using the controls on the Top Unit front panel.

### ***Video camera***

PhenoTyper is equipped with a monochrome analog camera (in PhenoTyper 1) or a monochrome GigE Vision digital camera (in PhenoTyper 2). Both cameras detect infrared light and exclude most of the light in the visible range. For optimal image quality a built-in infrared array ensures illumination; this way your recordings are

independent of visible light. You can run tests throughout the light-dark cycle because the image of the subject and the background processed in EthoVision XT won't change.

- **IMPORTANT** Do not use incandescent light bulbs as visible light source in the test room, because they produce a lot of infrared light which can interfere with video tracking. If you need extra (visible) light in your test room, use tube lamps, LED lamps or the LED arrays provided with PhenoTyper 2, which produce far less infrared light. See **LIGHTING CONDITIONS** on page 51.
- The camera can see the subjects at night but does not detect the heat produced by animals.
- The lens focus and orientation of the camera is optimized by Noldus. In the case you need to make adjustments, see the PhenoTyper - EthoVision XT 18 - Service Manual (see page 7).

### ***Visible light and sound***

The following stimulus devices are available:

- In PhenoTyper 1: the white light spot and the yellow light spot. Each of these illuminate approximately a quarter of the cage bottom. Furthermore, PhenoTyper 1 has a buzzer module can be used to administer a sound stimulus.
- In PhenoTyper 2: the white light (not a light spot) is currently available as a visual stimulus. Contact Noldus if you need additional stimulus devices.

The stimuli can be administered automatically using the Trial Control function in EthoVision XT.

### ***PhenoTyper - EthoVision XT communication***

PhenoTyper is the ideal cage for use in combination with the tracking and analysis software EthoVision XT, no matter how long and complex the trials are. PhenoTyper receives commands from and sends feedback to EthoVision XT. When working with multiple PhenoTyper's simultaneously, you can administer stimuli in different cages independently. See **Set up PhenoTyper 1** on page 35.

The course of events during a trial is logged in EthoVision XT. The events are readily available for analysis. See examples in **Common tests with PhenoTyper** on page 94. For extensive information, see **Analysis of Trial Control data** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual (see page 7).

## HOW CAN I USE PHENOTYPER?

### *For monitoring and video tracking*

Connect the PhenoTyper camera to the EthoVision XT computer (page 37). The software tracks the subject's position and calculate a wealth of statistics of the subjects' behavior (distance moved, time spent in specific zones etc.). With Behavior Recognition you can analyze behaviors like grooming and sniffing. This setup does not perform actions on any device.

### *For recording video*

Connect the PhenoTyper camera to a computer with EthoVision XT or MediaRecorder (see page 43). MediaRecorder is especially useful when you want separate high-resolution video files for each cage. You can use the video later on to carry out offline video-tracking with EthoVision XT.

### *For video tracking and administration of stimuli*

This advanced setup is meant for controlling the PhenoTyper devices during a trial, for example in a conditioning experiment where the subject is given a reward when it performs a specific behavior. You can have EthoVision XT carry out actions like switching on a light when a condition is met. PhenoTyper is connected to the EthoVision XT computer through the USB-IO box. In the software you can program the **Trial Control** with a sequence of conditions (e.g. check that the mouse has reached a zone) and subsequent actions (e.g. switch on the white light) that will be executed during a trial.

- You can also combine PhenoTyper with other devices like the Noldus Pellet dispenser or the Noldus Lickometer.

- EthoVision XT also receives feedback from PhenoTyper. For example, you can track in your data how often a stimulus was given, and when a pellet was dropped.
- For extensive information about how to control hardware devices, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

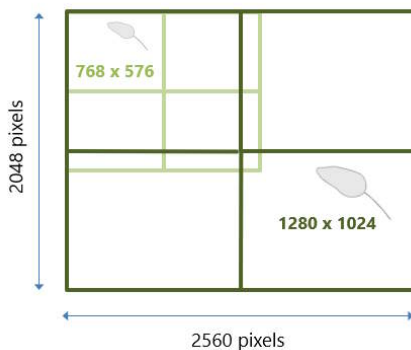
## WHAT'S NEW IN PHENOTYPER?

### *Digital video quality*

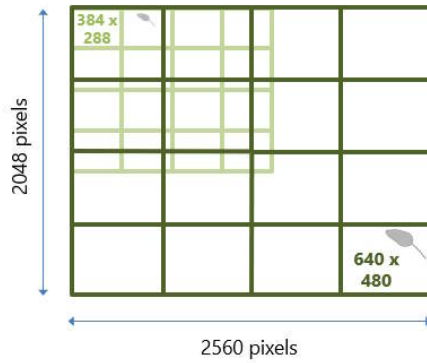
*Digital video quality.* PhenoTyper 2 hosts a Basler acA1300-60gm monochrome digital camera. The main advantage of a digital camera is that its image is less sensitive to noise and allows to record video at a resolution greater than that attainable with PhenoTyper 1. A GigE Vision camera such as that of PhenoTyper can be easily connected to a PC through an Ethernet interface board, so you no longer need an expensive frame-grabber board to record video.

Here we compare the video resolution of PhenoTyper 2 (1280 x 1024) with that of PhenoTyper 1 (PAL video, 768 x 576).

- in a four- PhenoTyper configuration:

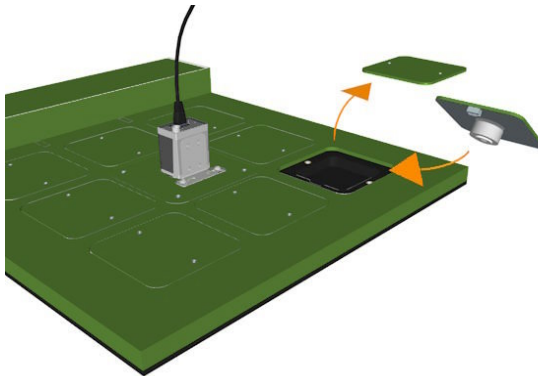


- In a 16-PhenoTyper configuration:



### *Modularity*

Another great improvement in PhenoTyper 2 resides in its modularity. With PhenoTyper 1 adding a custom device to the Top Unit was relatively cumbersome. In contrast, PhenoTyper 2 has a modular structure. Besides the central module that hosts the video camera, additional devices can be attached using the eight slots available. When needed, you can replace them with other devices for a new protocol.



### ***Improved lighting***

PhenoTyper 2 is equipped with an internal white light ring that ensures consistent illumination across your experiments. Better lighting means better detection of the subjects, and more reliable and reproducible results.

### ***Backward compatibility***

PhenoTyper 2 is backward-compatible with PhenoTyper 1, allowing for an effortless upgrade without changing cables and cage elements.

### ***Current development of PhenoTyper 2***

The Top Unit of PhenoTyper 2 is subject to continuous development. Some functions of PhenoTyper 1 are not yet available in that Top Unit. Contact Noldus if you wish to use a specific device with PhenoTyper 2.

## **ADVANTAGES OF PHENOTYPER COMPARED WITH ORDINARY TEST SETUPS**

### ***Robustness***

The video camera blocks out visible light, therefore EthoVision XT works on infrared light only. This makes tracking independent of ambient light conditions in the lab, and even allows to carry out studies of circadian rhythms without having to change settings in EthoVision XT when the light conditions change.

### ***Ease of use***

You no longer have to worry about choosing, mounting and positioning a camera, nor how to set up the ambient light conditions in the lab. PhenoTyper is also a complete monitoring system: camera, lights, open field, and even visual and acoustical stimuli are all integrated in one system.

### ***Flexibility***

PhenoTyper can be configured in different ways and extended with a number of accessories. Not only you can combine cage elements, but

you can also easily customize the cage walls so that the cage is tailored to your research. This makes it possible to apply PhenoTyper to a wide range of studies and research questions, from a short open field test to multi-day circadian rhythm monitoring, from toxicology trials to operant tests.

### ***Scalability and reproducibility***

You can easily extend your study by adding cages and replicating experiments with the same settings in EthoVision XT. Furthermore, because the cages can be easily standardized using the same components, you can improve reproducibility by applying the same setup in different studies and across labs.

### ***Minimal human interference***

PhenoTyper is a home-cage environment. The subject can live in the cage for days when provided with food and water. Tests can be performed in a series, without moving the subject. This way the subject's behavior is more natural and handling by the operator is minimized.

### ***Variable observation periods***

Because PhenoTyper is a home cage and the video image is independent of the external lighting conditions, it can be used in short-term tests as well as in multi-day recordings. For example, it is sometimes necessary to observe animals for long time in order to detect phenotypic differences between strains or experimental treatments.

## APPLICATIONS OF PHENOTYPER

There is a wide range of applications of PhenoTyper as a home-cage monitoring system, and the following list is not exhaustive. Check [noldus.com](https://noldus.com) regularly for more examples of use of PhenoTyper with the relevant literature.

For an extensive review of studies conducted with PhenoTyper, see the following article:

Grieco, F. *et al.* (2021) Measuring behavior in the home cage: study design, applications, challenges, and perspectives. *Front. Behav. Neurosci.* 15:735387. doi: 10.3389/fnbeh.2021.735387

<https://www.frontiersin.org/articles/10.3389/fnbeh.2021.735387/full>

Test paradigm/behavior to be measured	PhenoTyper add-on or device needed	EthoVision XT module needed
<b><i>Spontaneous behaviors</i></b>		
Activity-related measures	-	-
Activity-related measures (> 12 hrs)	Drinking bottle, Feeder, Shelter	-
Circadian rhythm	Drinking bottle, Feeder, Shelter	-
Habituation, novelty response	Drinking bottle, Feeder, Shelter	-
Running wheel activity	Running wheel	Trial and Hardware Control
Feeding behavior	Feeding monitor	Trial and Hardware Control
Drinking behavior	Lickometer	Trial and Hardware Control
Rearing, grooming, sniffing, jumping, twitching (rat)	-	Rat Behavior Recognition



Rearing, grooming, sniffing, hopping, digging (mouse)	-	Mouse Behavior Recognition
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### ***Cognition***

Discrimination learning	e.g. CognitionWall	Trial and Hardware Control
Reversal learning	e.g. CognitionWall	Trial and Hardware Control
Avoidance learning	Illuminated shelter	Trial and Hardware Control
Appetitive operant conditioning	Shelter, Pellet dispenser	Trial and Hardware Control

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### ***Anxiety***

Light avoidance	Shelter	Trial and Hardware Control
Sheltered feeding	Open and close feeding platforms	-

---

### ***Social behavior***

Dyadic interaction	-	Social Interaction
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### ***Optogenetics***

Single or dual channel stimulation	Single/Dual channel commutator in top unit, dual channel LED illuminator, pulse train generator	Trial and Hardware Control
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### ***Biotelemetry***

Physiology (third party product needed)	Telemetry receiver placed under PhenoTyper	Trial and Hardware Control, External Data
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## PHENOTYPER CONFIGURATIONS

### *Cage size*

- **Model 3000.** Suitable for mouse studies.
- **Model 4500.** Suitable for both rat and mouse studies.
- **Model 9000.** Suitable for multi-subject rat studies.

See the cage sizes in **Technical Specifications** on page 122.

### *Cage types*

You can make three main cage types depending on the walls and accessories chosen:

#### **Basic cage**

Cage with one plain wall, one wall with door, and two ventilation walls.

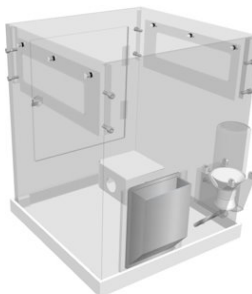
Suitable for short-term studies.



#### **Home cage**

Cage provided with door, a feeder, a drinking bottle, and a shelter. Two walls have ventilation holes.

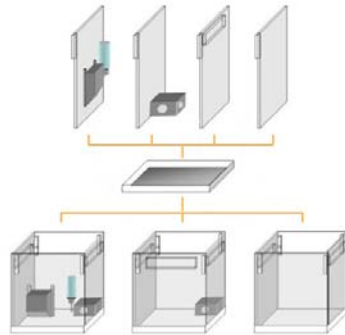
Suitable for long-term observations.



### Custom cage

Any combination of walls and accessories that differs from the Basic and the Home cage.

You can build, for example, a custom cage with feeder and bottle but no shelter, or a cage with three or four ventilation walls if necessary.



Note:

- The Model 4500 includes two drinking bottles and one feeder larger than that of Model 3000.
- The Model 9000 home cage includes two drinking bottles and two large feeders.
- The Model 4500 and 9000 are provided with a shelter bigger than that of the Model 4500.

### ***Additional wall types***

Beside the standard walls in clear perspex, several wall types are also available in a matte version.

Wall types are available for the following systems:

- The PhenoWheel for mice. See the section **PhenoWheel** in the PhenoTyper - EthoVision XT 18 - Service Manual.
- The Activity Wheel for rats.
- The Pellet dispenser and the Pellet receptacle. See the section **The Pellet Dispenser and the Pellet Receptacle** in the PhenoTyper - EthoVision XT 18 - Service Manual.
- The Mouse Feeding Monitor.
- The CognitionWall. See page 104.

- For connecting third party hardware to the PhenoTyper cage, contact Noldus to see what the possibilities are for your specific devices.

## USING MULTIPLE PHENOTYPERS

When using multiple PhenoTypers, consider the following:

### *Maximum number of PhenoTyper cameras*

The following configuration have been tested:

- **PhenoTyper 1** (analog camera): 16. See page 40.
- **PhenoTyper 2** (digital camera): 16. See page 74.

See also page 35 for how to set up PhenoTyper.

You can also record video using MediaRecorder and then do tracking offline (see page 43). MediaRecorder is especially useful when you need to record high-resolution video for each cage.

### *Maximum number of PhenoTypers that can be controlled*

You can have EthoVision XT control the PhenoTyper devices. The EthoVision XT Trial Control Settings are applied to each PhenoTyper independently, provided that each PhenoTyper is defined as a separate arena (page 60).

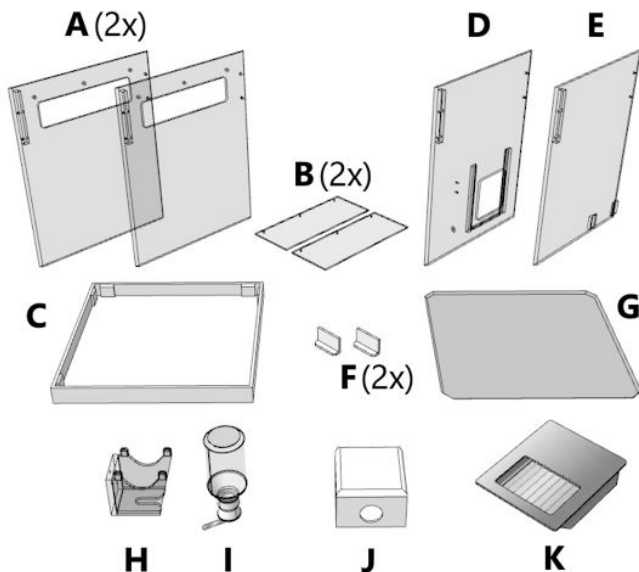
- **PhenoTyper 1:** You can connect four PhenoTypers to one Top Unit Interface and up to 13 Top Unit interfaces to one EthoVision XT computer through the Noldus USB-IO box. You could in principle control up to 52 PhenoTypers simultaneously. However, the resulting video resolution would be very low. Therefore, we recommend to connect up to 16 PhenoTyper to one computer.
- **PhenoTyper 2:** You can connect PhenoTypers directly to the USB-IO box or the Nortio TTL Terminal Hub. A maximum of eight PhenoTypers connected through TTL ports were tested.

## 2 Assemble PhenoTyper

### BEFORE YOU START

Assembling PhenoTyper involves the same basic steps no matter what the came model and configuration is. The instructions below are based on the home cage configuration for PhenoTyper model 3000.

#### *Home cage components*



**Figure 4** Wall elements for the home cage. F applies for model 4500 only. The model 4500 comes with two drinking bottles (H+I). The letters are used throughout the instructions.

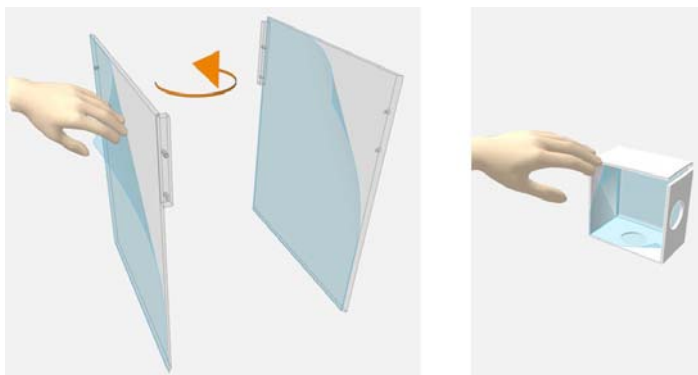
**NOTE** The PhenoTyper components you have purchased may not match exactly the illustrations in this manual. For example, walls may be matte instead of being translucent.

<b>A</b>	Walls with airgap (2x per cage). See page 23.
<b>B</b>	Airgap shields (2x per cage). See page 23.
<b>C</b>	Cage bottom. See page 24.
<b>D</b>	Wall prepared for feeder and bottle (replaced by a plain wall* in the basic cage). See page 24.
<b>E</b>	Wall prepared for shelter (replaced by a plain wall in the basic cage). See page 25
<b>F</b>	L pieces for fixing the walls (2x per cage). See page 27
<b>G</b>	Cover plate. See page 34.
<b>H</b>	Bottle support. See page 28.
<b>I</b>	Drinking bottle. page 29.
<b>J</b>	Shelter. See page 30.
<b>K</b>	Feeder. See page 31.

\*) The plain wall is part PTC3-W000 for cage model 3000 and PTC4-W000 for cage model 4000. See part serial numbers, see page 116.

### ***Remove the protecting foil from the walls***

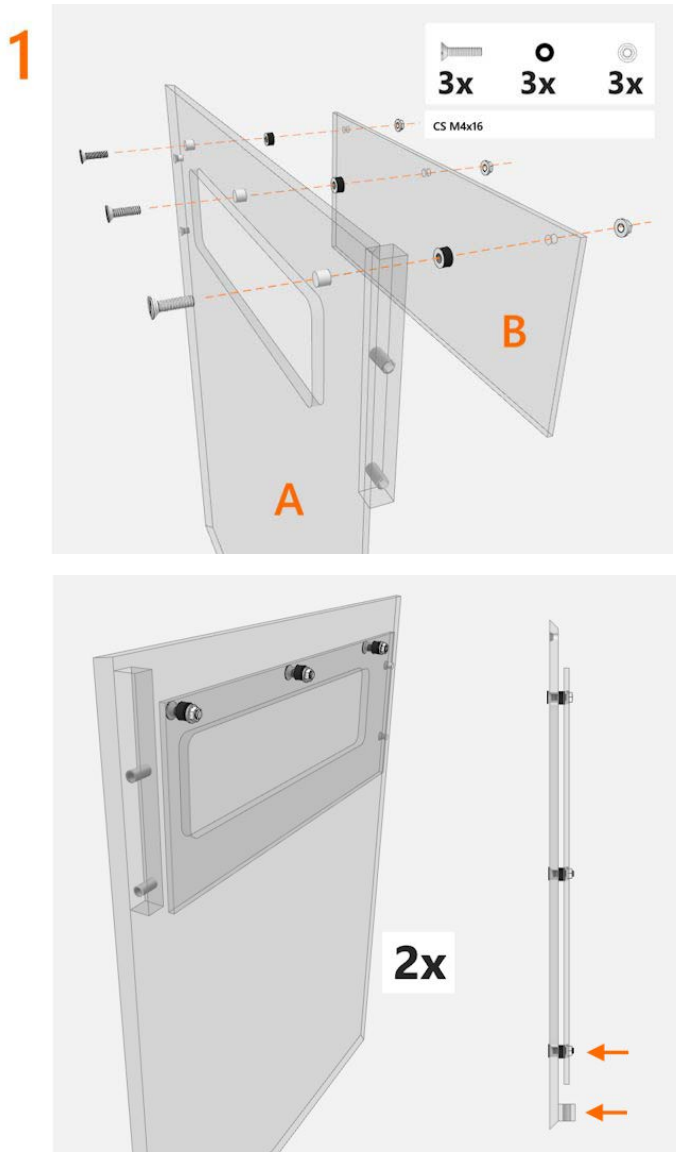
All cage elements A-E, G and J in Figure 4 are provided with a protecting plastic foil on both sides. Remove the foil before assembling the cage.



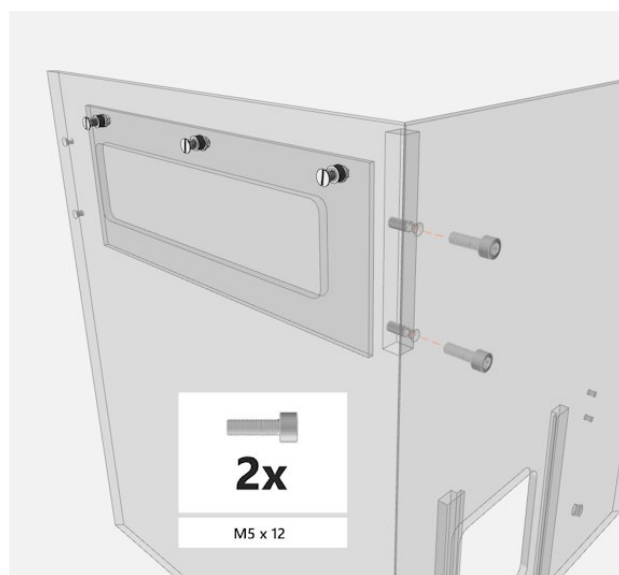
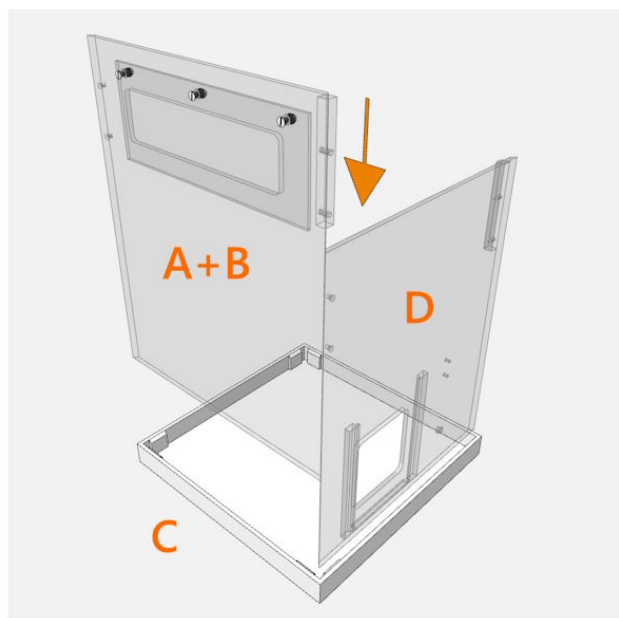
### ***Mounting set***

- For cage model 3000: page 117. For cage model 4500: page 119.

**ASSEMBLE THE CAGE**

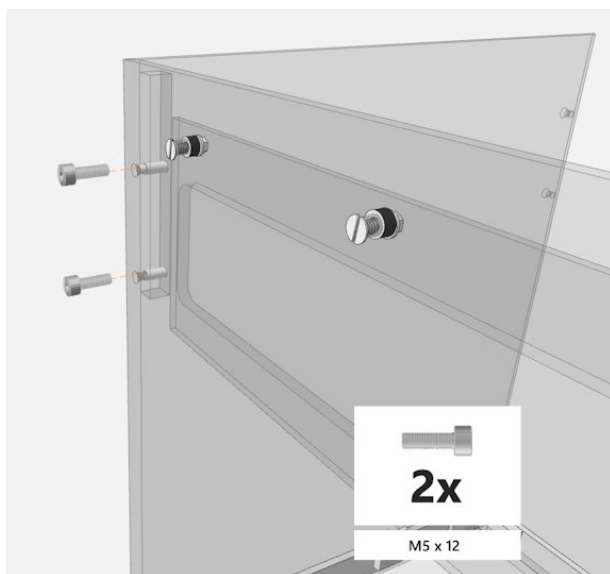
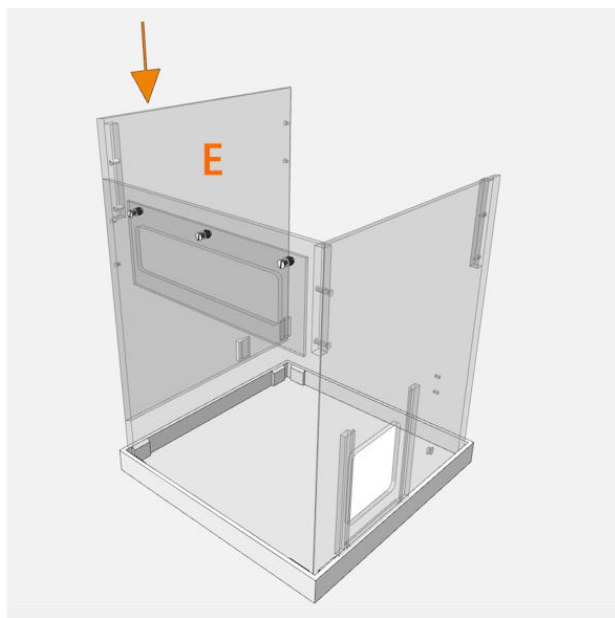


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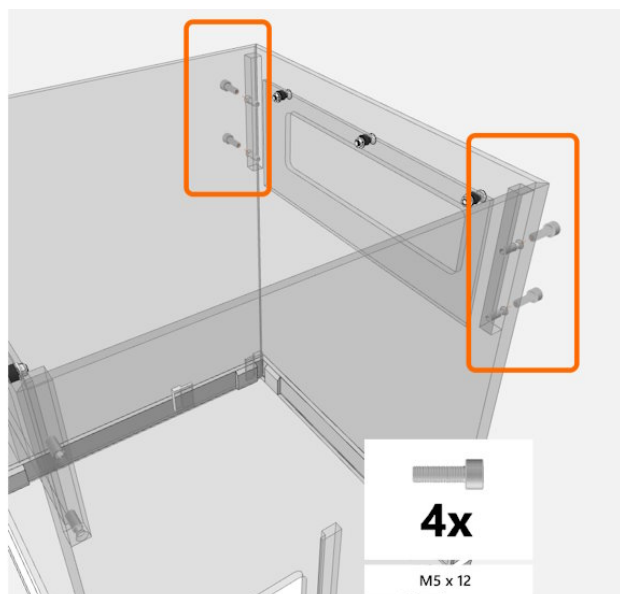
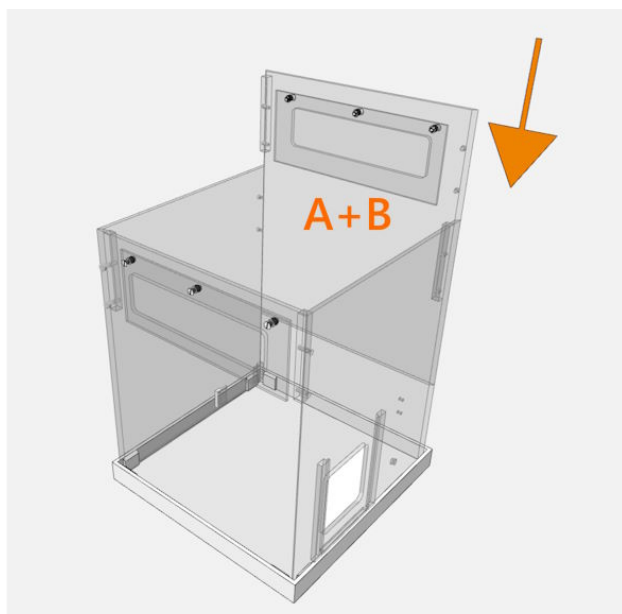




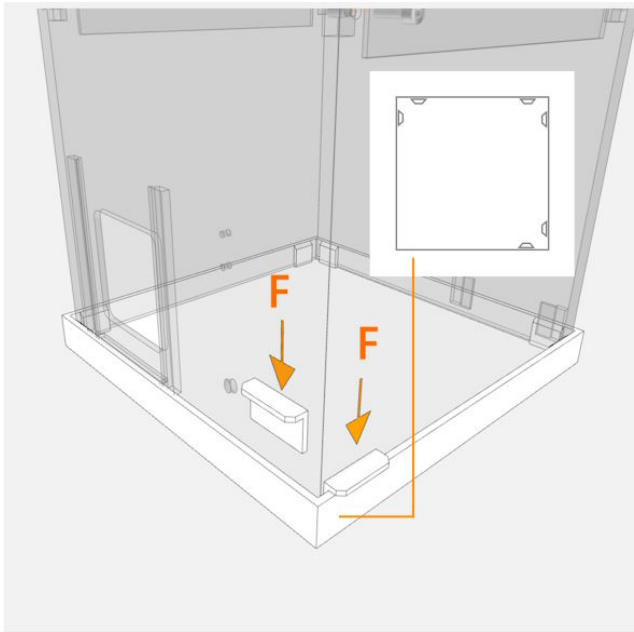
3



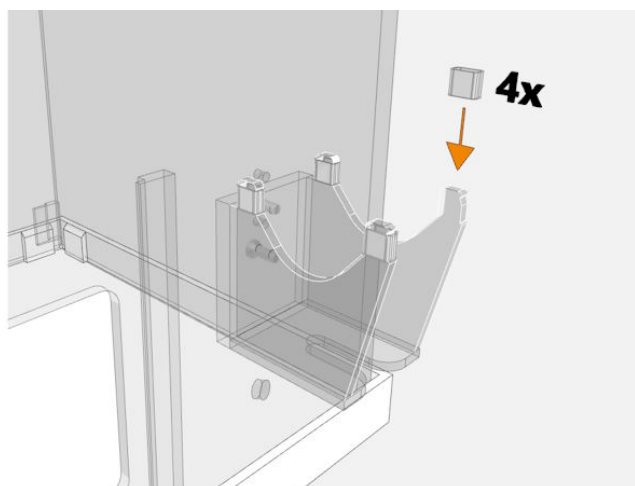
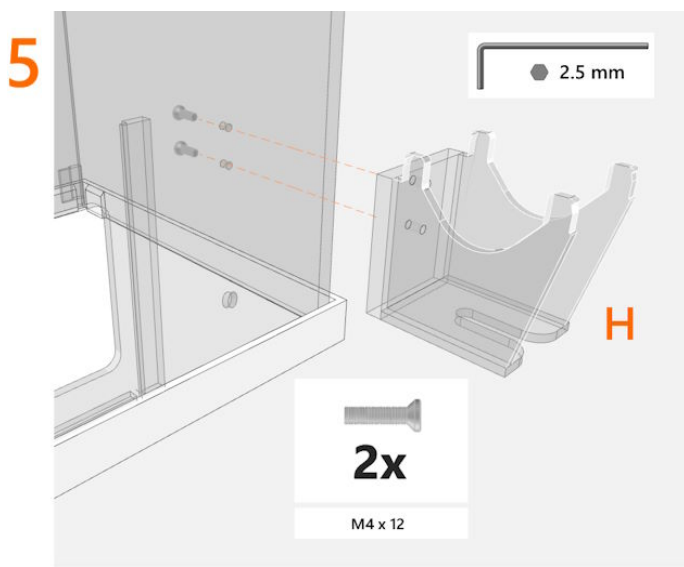
4



*optional for cage 4500*

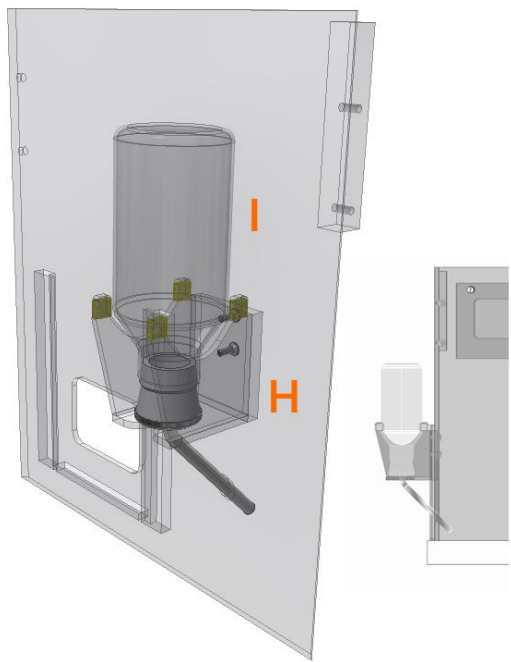


### Add the drinking bottle



If you have PhenoTyper walls with one hole only for the bottle support, attach the bottle support with one M4 12 mm screw.

The correct position of the drinking bottle is as follows:

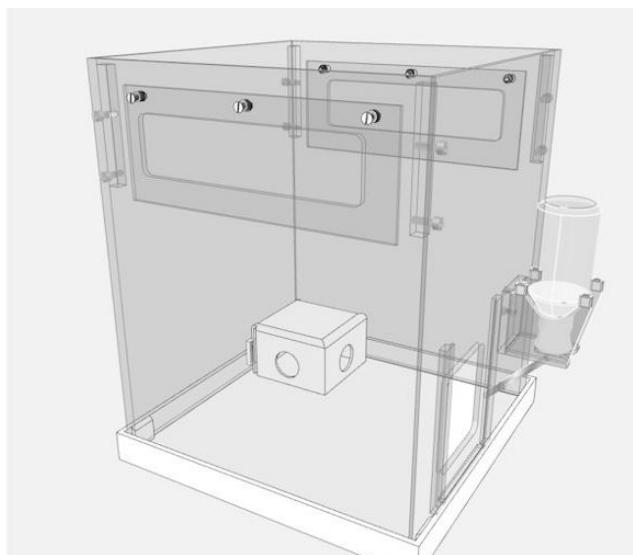
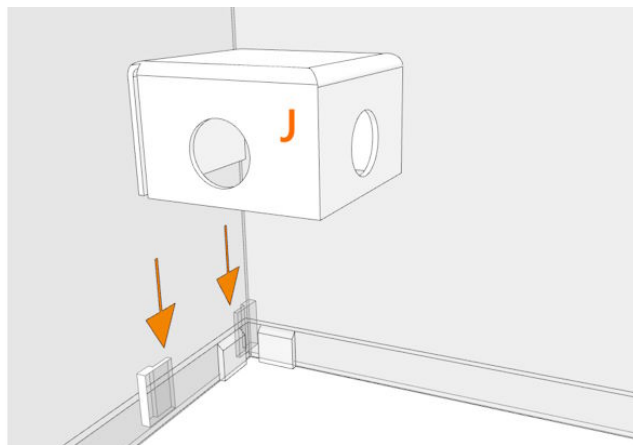


### ***Add the shelter***

Attach the shelter to cage walls PTC3(4)-Woo5 (cage wall prepared for shelter). See **PARTS LIST** on page 116.

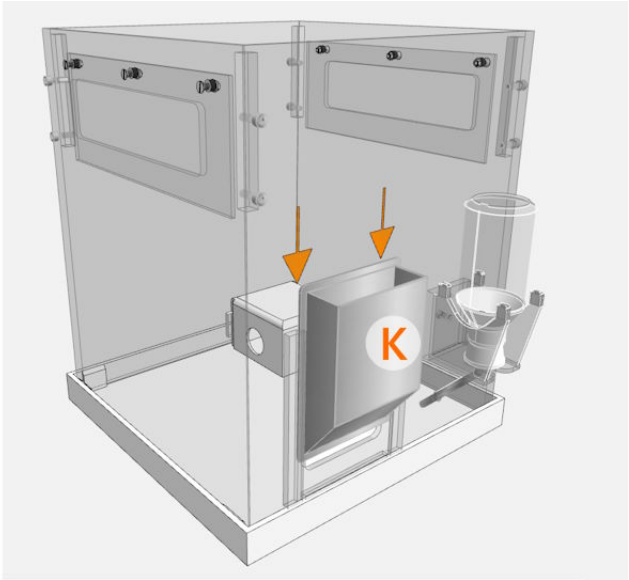
Before placing the shelter, remove the protecting sheet from the inside!

6



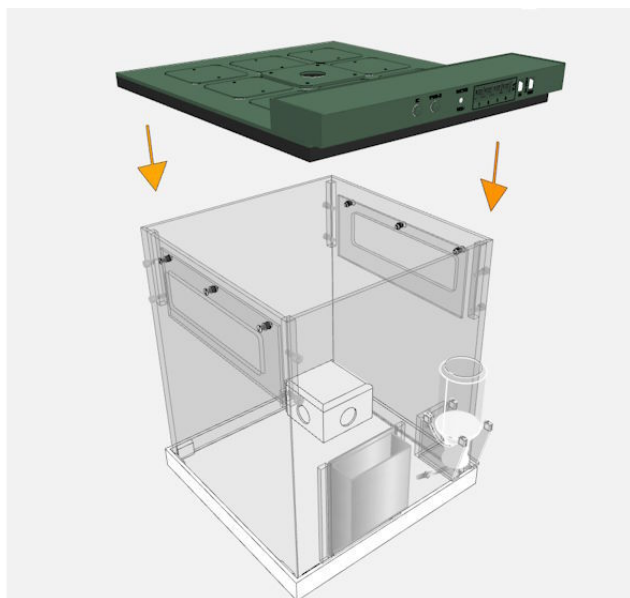
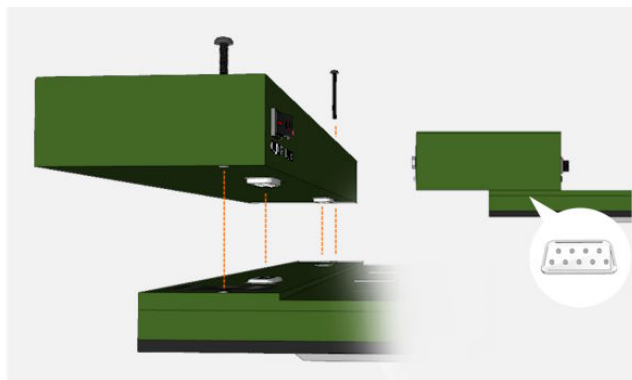
*Add the feeder*

7



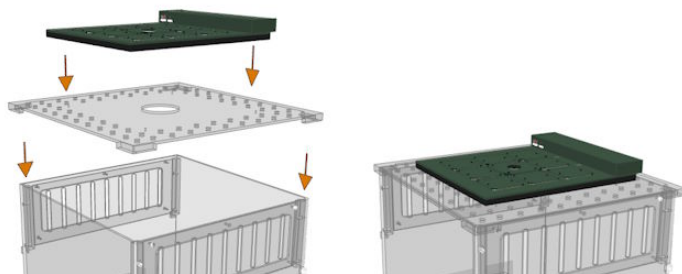
## ***The Top Unit - PhenoTyper 2***

**8**



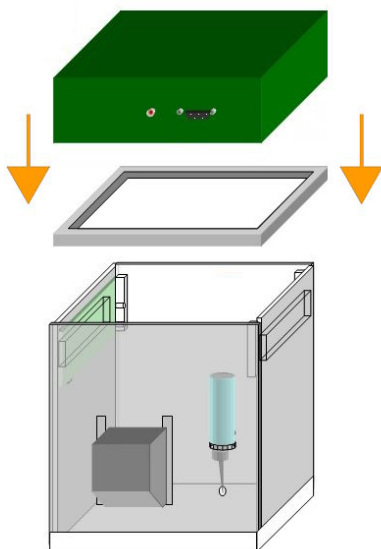
For models 4500 and 9000:





### ***The Top Unit - PhenoTyper 1***

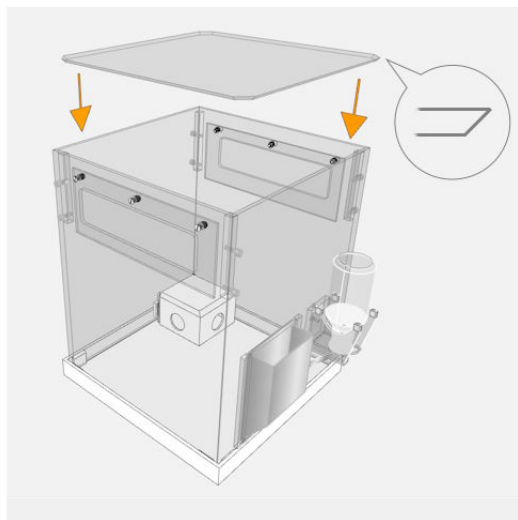
Place the metal frame and the Top Unit on top of the cage.



### ***The cover plate - PhenoTyper 1***

When you do not use the Top Unit, place the cover plate (G in Figure 4) on top of the cage. This prevents the animal from escaping.

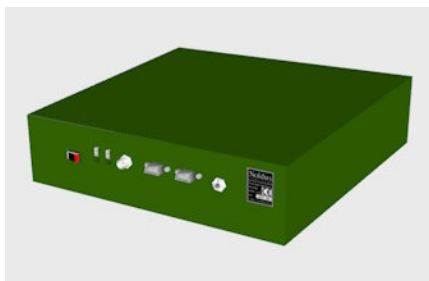
**IMPORTANT** Do not leave the cover plate in between the Top Unit and the cage during the experiments. It may decrease image quality.



# 3 Set up PhenoTyper 1

## AIM

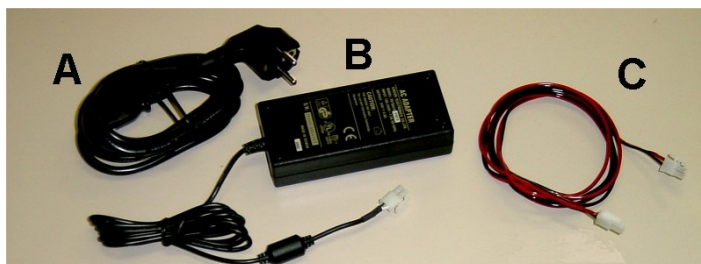
This section applies to PhenoTyper version 1, with the Top Unit hosting an analog camera.



## TOPICS

- **CABLES AND ADAPTERS** - page 36
- **CONNECT ONE PHENOTYPER TO THE PC** - page 37
- **CONNECT MULTIPLE PHENOTYPERS TO THE PC** - page 40
- **WORKING WITH GROUPS OF PHENOTYPERS** - page 46
- **CONNECT ADDITIONAL DEVICES** - page 50
- **LIGHTING CONDITIONS** - page 51
- **TEST THE PHENOTYPER'S FUNCTIONS** - page 51
- **ADJUST LIGHT INTENSITY** - page 53
- **COPY LIGHT INTENSITY VALUES FROM ONE PHENOTYPER TO ANOTHER** - page 55
- **THE PHENOTYPER CAMERA** - page 58

## CABLES AND ADAPTERS



**Figure 5** *The PhenoTyper's cable set.*

- **A, B** – Power cord and transformer.
  - For PhenoTyper 1: code PT-PS1.
- **C** – Power feed-through cable (code PT-PSC) for connecting multiple PhenoTypers.
- **D** – Composite video cable of variable length with BNC connectors (code PT-BNCxx; see Figure 6).



**Figure 6** *Video cable with BNC connectors.*

For the complete list of components and their codes, see page 116.

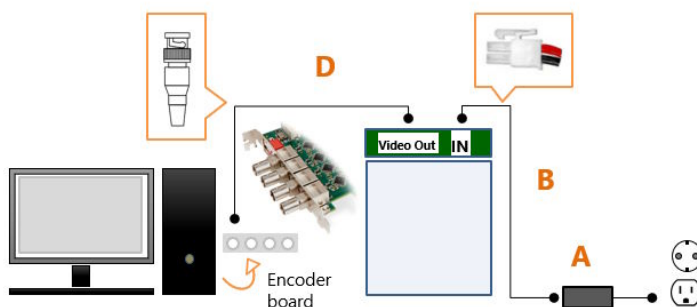
If you purchased the USB-IO box and the Top Unit Interface, you also have the following cables:

- 1x Network cable, 1m (green).
- 4x Network cable, 2 m (green).

- USB cable with connectors of type A and B.
- Power cord and DC adapter 24 V 3A.

## CONNECT ONE PHENOTYPER TO THE PC

### *Power and video*



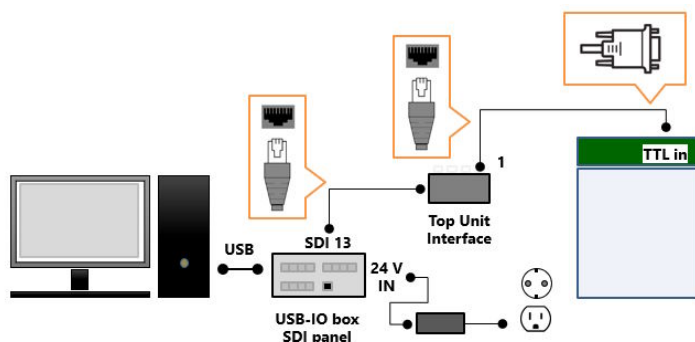
1. Connect the PhenoTyper's **Supply In** socket to the power outlet using the elements A and B (see Figure 5 on page 36).
2. Connect the PhenoTyper's **Video Out** socket to one of the BNC connectors of the Euresys Picolo Alert encoder board installed on your computer, using a video cable with BNC connectors (D). Connect the video cables directly to the BNC connector on the board.

Below: 1. Power plug. 2. Video cable from the PC's encoder board.



For installing the Picolo Alert encoder board, see the EthoVision XT Help under **Camera Installation > Install analog cameras**.

### *Control of devices using the USB-IO box*



1. Connect the **USB** port of the USB-IO box (or the Mini USB-IO box) to one of your PC's USB ports using the USB cable that comes with the (Mini) USB-IO box.
2. Connect the **24 V IN** port of the (Mini) USB-IO box to the power outlet using its adapter.
3. Connect the **SDI Control** port number **13** of the USB-IO box (or the Mini USB-IO box) to the **USB IO-box** port of the Top Unit Interface, using the 1 m green network cable with RJ-45 connectors at its ends.
4. Connect the port **1** of the Top Unit Interface to the **TTL In** port of PhenoTyper, using the 2 m green network cable with one RJ-45 connector and a SubD-9 connector at its ends.

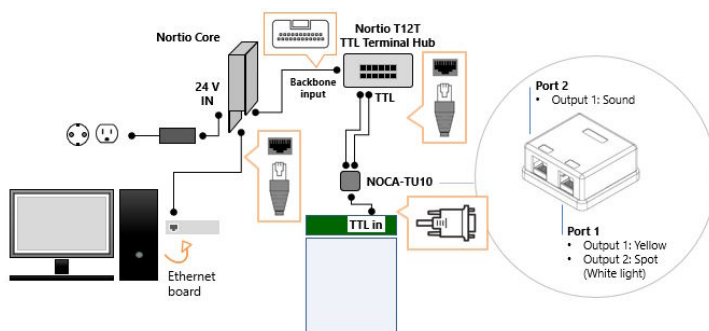
### *Control of devices using the Nortio system*

For this configuration you need:

- The Nortio Core.
- The Nortio T12T TTL Terminal Hub.
- The interface cable NOCA-TU10 (see the figure below). This cable is provided at one side with a Sub-D connector (for PhenoTyper) and at the other side with a box with two RJ-45 network ports.
- One or two network cables, depending on how many PhenoTyper devices you want to control.



Connect PhenoTyper as shown here.



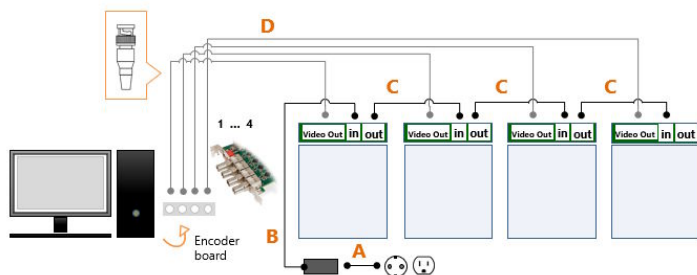
Which PhenoTyper devices you can control depends on which ports on the NOCA-TU10 box you use and which **Output** line you specify in EthoVision XT.

For example, to control the white light:

- Connect **Port 1** of the NOCA-TU10 box to a TTL port of the Nortio T12T TTL Terminal Hub.
- In the EthoVision XT Experiment Settings, locate the TTL port number and from that list select **Top Unit Light (NOCA-TU1x)**.
- In the Arena Settings, map the TTL port on the corresponding PhenoTyper and assign a name to the device (e.g. PT 1 Port 1).
- In the Trial Control Settings, specify to activate **Output line 2** for the device PT1 Port 1.

## CONNECT MULTIPLE PHENOTYPERs TO THE PC

### Power and video



1. Connect the first PhenoTyper's **Power Supply In** socket to the power outlet using the elements A and B (page 36).
2. Using three power feed-through cables (C), connect the **Power Supply out** socket of the first, second and third PhenoTyper to the **Power Supply in** socket of the next one, respectively. So, **1 out** to **2 in**, **2 out** to **3 in**, **3 out** to **4 in**.
3. Connect each PhenoTyper's **Video Out** socket to one of the BNC connectors of the Euresys Pico Alert encoder board installed on your computer, using a video cable with BNC connectors (D). Connect the video cables directly to the BNC connector on the board.

#### Note:

- Resolution of each camera image: 768 x 576 (PAL Top Unit camera) or 640 x 480 (NTSC Top Unit camera). The resulting picture-by-picture video file created by EthoVision XT is 1536 x 1152 (PAL) or 1280 x 960 (NTSC).
- For mix the video image of four to sixteen PhenoTyper's using Quad Units, see page 41.
- Furthermore, if you have the MediaRecorder software you can record full resolution video file from each PhenoTyper (page 43).
- For more information, in the EthoVision XT Help see **Camera Installation > Install analog cameras**.



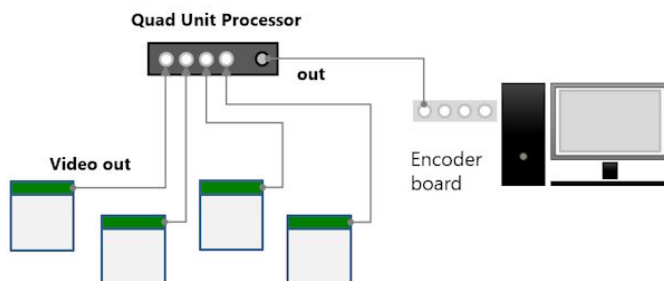
- When using more than 4 PhenoTypers, connect each group of 4 PhenoTypers to a separate power outlet.

### ***Mix the camera images with a Quad Unit***

This section only applies to PhenoTyper 1 (analog camera).

Use a video processor (Quad Unit) to mix video of 4 or 16 PhenoTypers. EthoVision XT will process that mixed image. You can work with this setup when you track live or from previously-recorded video files (offline).

**IMPORTANT** If you track the subject's center-point, nose-point and tail-base, mix maximally four video images. Otherwise the individual images become too small for accurate tracking.



To mix four PhenoTyper images, you need one Quad Unit.

1. Connect the **Video Out** socket of each PhenoTyper to one of the quad unit's **Video In** sockets, using composite cables with BNC connectors.
2. Connect the quad unit's **Video Out** socket one of the BNC connectors of the of the encoder board installed on the PC.

To mix sixteen PhenoTyper images, you need four Quad Units.

1. Connect the **Video out** from of each 4-Phenotypers group to the video inputs of a quad unit.
2. Connect the four video outputs of the quad units to the inputs of the PicoLo Alert encoder board on the EthoVision XT computer.

## Notes

- In your EthoVision XT experiment, define as many arenas as cages.
- When using Quad Unit VM-Q401A: When it does not display video, chances are that it is set to the incorrect TV standard. First, unplug the device from the power supply. Next, to select the PAL mode, hold down the Left arrow button on the front of the device while plugging in the power supply. To select the NTSC mode, hold down the Right arrow button while plugging in the power supply.
- **IMPORTANT** When you mix the camera images with a Quad Unit, the resolution of each camera view is one quarter of the original. This is because both the width and the height of the image are reduced to half of the original. Especially when you use the Behavior Recognition function, check that the apparent size of the subjects in pixels is still acceptable. You find more information in the topic **Behavior Recognition: Prerequisites** in the EthoVision XT Help.
- When mixing four PhenoTyper images with a Quad Unit, the video resolution of each camera image is 384 x 288 (PAL Top Unit camera) or 320 x 240 (NTSC Top Unit camera). The resulting resolution of the picture-by-picture video file created by EthoVision XT is 768 x 576 (PAL) or 640 x 480 (NTSC).
- When mixing four camera images with the Pico Alert encoder board (see the scheme on page 40) the original resolution is preserved. The resulting picture-by-picture video file created by EthoVision XT is 1536 x 1152 (PAL) or 1280 x 960 (NTSC).
- When mixing 16 PhenoTyper video images with Quad Units, the resolution of each camera image is 384 x 288 (PAL) or 320 x 240 (NTSC). Because the output resolution of each Quad Unit is preserved in the Pico Alert encoder board, the resulting resolution of the picture-by-picture video file created by EthoVision XT is 1536 x 1152 (PAL) or 1280 x 960 (NTSC).
- Do not mix 16 camera images if you want to track the nose-point and the tail-base point of the rodents, because the image will be too small for accurate tracking. Mix maximally four images.

- For a complete overview of the video resolutions and frame rates supported by EthoVision XT, see the EthoVision XT Help.

### ***Record video to separate video files***

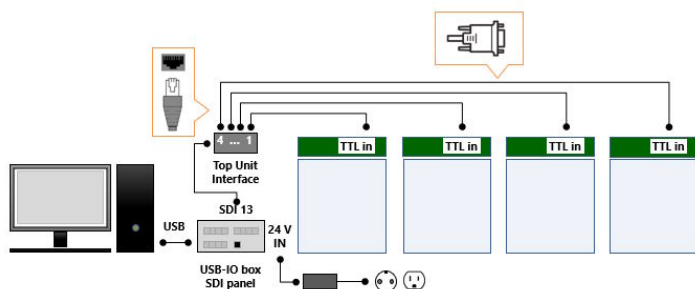
This section applies to both PhenoTyper 1 and PhenoTyper 2.

You can use the MediaRecorder software to record video from each PhenoTyper to a separate video file. This setup is possible only if you track offline and you have MediaRecorder. Each PhenoTyper sends the video signal directly to one input of the Picolo Alert encoder board (for PhenoTyper 1) or to the Ethernet interface board (for PhenoTyper 2). For details, see the MediaRecorder Help.

1. Once you have recorded video to separate video files, create a new EthoVision XT experiment. Choose **Setup > Experiment Settings** and under **Video source** select **From video file**.
2. Because each video file contains the image of one arena, in your Experiment Settings, under **Number of Arenas**, select 1.
3. You can now load the video files in EthoVision XT and perform *offline* tracking.

If you choose this setup, mind that you cannot control hardware devices during offline tracking. In an alternative setup, you can do live tracking with hardware control, and let EthoVision XT trigger video recording with MediaRecorder (see **Trial Control** in the EthoVision XT Help).

## Control of devices using the USB-IO box



1. Connect the **USB** port of the USB-IO box (or the Mini USB-IO box) to one of your PC's USB ports using the USB cable that comes with the IO box.
2. Connect the **24 V IN** port of the (Mini) USB-IO box to the power outlet using its adapter.
3. Connect the **SDI Control** port number **13** of the USB-IO box (or the Mini USB-IO box) to the **USB IO-box** port of the Top Unit Interface, using the 1 m green network cable with RJ-45 connectors at its ends.
4. Connect the port **1**, **2**, **3** and **4** of the Top Unit Interface to the **TTL In** port of the first, second etc. PhenoTyper, respectively, using 2-m green network cables with one RJ-45 connector and a SubD-9 connector at their ends.

### Note

- By default, the USB IO-box is set to be connected with one Top Unit Interface only (and therefore four PhenoTyper) through the **SDI Control** port 13. If you work with 8 to 16 PhenoTyper, you must use additional SDI Control ports. Connect the **USB-IO box** port of each Top Unit Interface to one of the **SDI Control port 1 to 4** of the USB-IO box. For example, the Top Unit Interface of group 1 to **SDI control** port number **1**, etc. Make sure that the IO-box is set correctly for communicating with multiple Top Unit Interfaces (see **Jumper Settings** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual. To open this manual, from the Windows **Start**

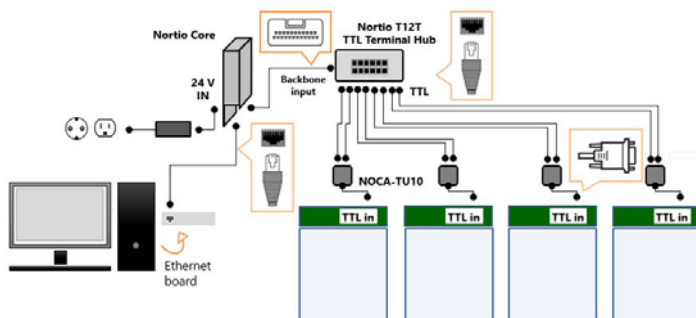
menu choose **All Apps > Noldus > EthoVision XT 18 Other Documentation.**

### ***Control of devices with the Nortio system***

Follow this section if you have the Nortio Core and the Nortio T12T TTL Terminal Hub. You also need:

- For each PhenoTyper, one interface cable NOCA-TU10 (see the figure below). This cable is provided at one side with a Sub-D connector (for PhenoTyper) and at the other side with a box with two RJ-45 network ports.
- For each PhenoTyper, one or two network cables, depending on how many PhenoTyper devices you want to control.

Connect the Nortio T12T TTL Terminal Hub to the PhenoTyper as shown here.



For more details, see page 38.

## WORKING WITH GROUPS OF PHENOTYPERS

You can set one to 16 PhenoTypers controlled by one EthoVision XT computer. When working with more than 4-16 PhenoTypers, it is advisable to use multiple computers. A common configuration includes groups of four PhenoTypers, each controlled by one PC. This optimizes level of throughput and video resolution, which ultimately affect data quality.

### *Experiments*

Create one EthoVision XT experiment in each PC controlling a group of PhenoTypers. However, if you plan to conduct the same test with two or more groups of PhenoTypers, it is wise to create a template experiment:

1. Choose **File > New from Template**.
2. Select the PhenoTyper configuration, whether it includes zones of interest etc.
3. Draw the arenas, zones and adjust the detection settings (page 60).
4. Choose **File > Make backup**. Copy the backup file to the other computers controlling a group of PhenoTypers.
5. On each computer, choose **File > Restore backup**. Save the experiment with a unique name for example PhenoTyper Group A, B, C, etc.

### *Assign arenas to the PhenoTypers*

When you mix the image of 16 PhenoTypers (PhenoTyper 1) or 8 PhenoTypers (PhenoTyper 1 and 2), each group of four cages will be displayed in a quarter of the monitor window (A to D in the figure below). In the mixed video image, group A with PhenoTypers 1 to 4 will be shown in the top-left corner.

PhenoTyper	1	2	5	6
	Group A		Group B	
	3	4	7	8
	9	10	13	14
	Group C		Group D	
	11	12	15	16

After creating an EthoVision XT experiment, define the number of arenas in the Experiment Settings (8 or 16). When opening the Arena Settings, the arenas are sorted in rows, that is, the first row contains Arenas 1 to 4, corresponding to PhenoTypes 1 and 2 of Group A and PhenoTypes 5 and 6 of Group B. For example, the label Arena 3 is placed over the image of the first PhenoTyper in Group B, while the label Arena 5 is placed over the third PhenoTyper in Group A.

	1	2	5	6
Arena 5	3	4	7	8
	9	10	13	14
	11	12	15	16

This could cause some confusion.

To create a more logical correspondence between PhenoTyper number and Arena number, do one of the following:

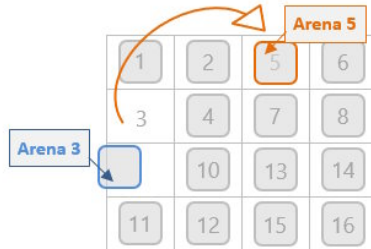
- Exchange the cables from the quad unit, in such a way that phenotypers of one group are displayed on the same row on the monitor.
- Alternatively, in EthoVision XT, re-order the arenas, for example exchange Arena 3 with Arena 5.
  - First, de-select **Arena 9** or other arenas in the Arena Settings panel to have more space where to place object temporarily.



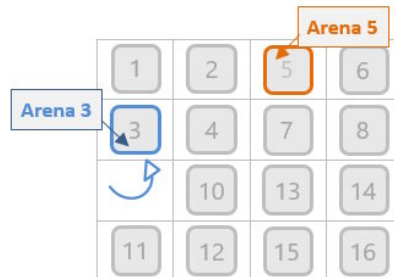
- Drag with the mouse to select a rectangle around the first arena that you want to move, including its zones and labels. Make sure that you do not select the zones of neighboring arenas. Next, drag the objects to the temporary position.



- Repeat the step above to move the second arena to the new position.



- Finally, move the first arena to the new position.





Repeat the steps for the other arenas that need to be swapped.

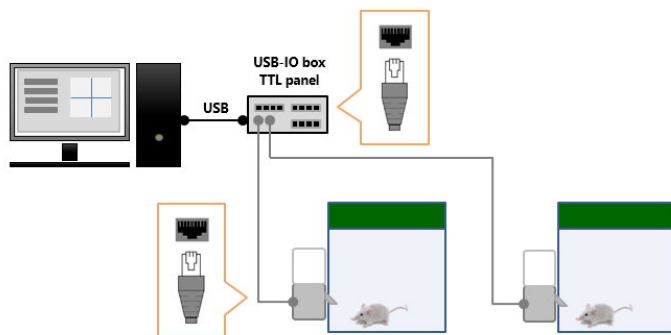
### ***Analyze data from multiple experiments***

If you want to analyze the data of PhenoTypers of multiple groups, you can open a master experiment and import the trials from the original experiments associated with those groups. Note that in order to import trials from multiple computers, the settings in the experiments must be identical. For example, the zone names. For information on importing trials from other experiments, see the topic **Import trials from other experiments** in the EthoVision XT Help.

## CONNECT ADDITIONAL DEVICES

### *TTL devices*

Use the USB-IO box (**TTL Control** panel) or the Nortio Core and the Nortio T12T TTL Terminal Hub to connect any other TTL compatible device. For example, the Noldus Pellet dispenser.



### *SDI devices*

Use the USB-IO box (**SDI Control** panel) to connect SDI devices like the Lickometer and PhenoWheel.

**NOTE** The Nortio system does not work with SDI devices. However, you can connect the Lickometer. See the PhenoTyper - EthoVision XT 18 - Service Manual.

### *For more information*

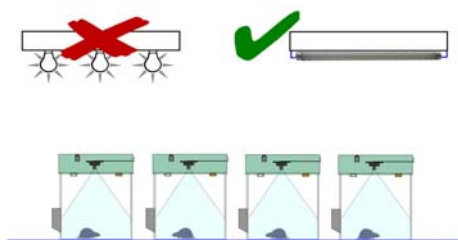
See the following manuals:

- For devices that work with PhenoTyper, the PhenoTyper - EthoVision XT 18 - Service Manual.
- For the USB-IO box and the Nortio system, the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.
- For defining and mapping devices in your EthoVision XT experiment, the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

## LIGHTING CONDITIONS

PhenoTyper uses infrared as light source, and since the camera is infrared sensitive and is provided with an IR-pass filter, EthoVision XT works independent of external light conditions (day vs. night). However, the light we see also contains a variable amount of infrared frequencies, which is seen by the camera and therefore may compromise detection by EthoVision XT. For example, direct sunlight or light produced by bulb lamps contain a lot of infrared light.

If you need extra visible light in your test room, use tube lamps and LED lamps, which produce little or no infrared light. This minimizes the amount of infrared light that comes from outside the PhenoTypers.

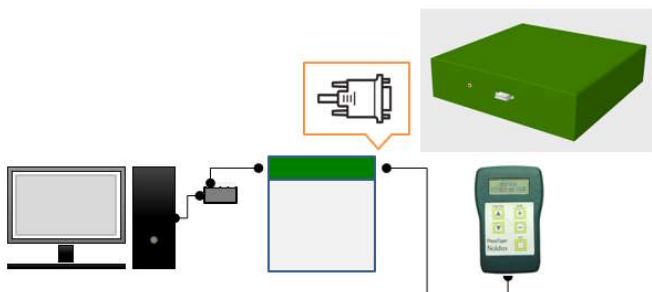


## TEST THE PHENOTYPER'S FUNCTIONS

1. If you want to test the infrared light source, follow this step. Otherwise, go to step 2.

Connect the video cable from PhenoTyper (or the quad unit if you work with multiple PhenoTypers) to a monitor or to the encoder board on your EthoVision XT computer. In the latter case, create a new experiment, next in the Experiment Settings select Live tracking, select the camera input and click the camera preview button. There you should see the live camera image.

2. Connect the Control Unit to the PhenoTyper's Top Unit by attaching the Control Unit cable to the PhenoTyper's 15-pin Sub-D socket located on the front panel, next to the red LED.



3. Switch on PhenoTyper.



**Result** – The Control Unit's LCD display switches on.

Some of the lamps may burn less intensely than others when they are switched on. Wait a few seconds until all lamps burn equally.

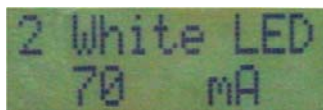
4. Press one of the Control Unit **FUNCTION** buttons one or more times to select the device (1 to 6).



Each time you press a button, the Control Unit display changes:

1 BUZZER → 2 White LED → 3 Yellow LED → 4 IR array-1 → 5 IR array-2 → 6 IR array-3 → READ FROM PT → WRITE TO PT → 1 BUZZER

In the following example, we have selected the white light:



**Result** – The corresponding light turns on (see below for infrared lights).

Do not press the **TEST** button at this point! If you do, light intensity may change because the value read from another cage is copied to that cage.

5. To test the buzzer, select **1 BUZZER** and press the **TEST** button. Press **TEST** again to stop the buzzer.

If sound does not work when you connect multiple PhenoTypers, it is likely that the jumpers inside the Top Units are not set correctly for EthoVision XT.

For information on jumper settings, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

## ADJUST LIGHT INTENSITY

Use the instructions below if you want to change the intensity of light produced by PhenoTyper. If you need to customize light intensity of two or more PhenoTyper cages, copy the light settings from one cage to the next (see page 55).

1. Connect the Control Unit to PhenoTyper (see page 52).
2. Switch on PhenoTyper.

**Result**– The Control Unit's LCD display switches on.

Some of the lamps may burn less intensely than others when switched on. Wait a few seconds until all lamps burn equally.

3. Press one of the Control Unit's **FUNCTION** buttons one or more times to select the device. Each time you press the **UP** button, the display changes in the following way:



1 BUZZER → 2 White LED → 3 Yellow LED → 4 IR array-1 → 5 IR array-2 → 6 IR array-3 → 1 BUZZER → READ FROM PT → WRITE TO PT → 1 BUZZER

Check the light intensity:

- Directly (for the yellow and white light spots).
  - Through the camera image in EthoVision XT (for the IR light). If you have not done this yet, connect the video cable from the PhenoTyper to one of the video inputs of the encoder board on the EthoVision XT computer. Next, start EthoVision XT, create a new experiment, next in the Experiment Settings select **Live tracking**, select the camera input and click the camera preview button. There you should see the live camera image.
4. Press the **LEVEL** buttons on the Control Unit to increase or decrease light intensity.



### Notes

- The values shown on the Control Unit screen can range from 0 to 200 (this indicates the current in mA) for the yellow and white lights, and from 0 to 100 for the infrared lights. Please note that even if you set the value to the minimum (that is, 0) the lights are still switched on, although the light intensity is very low.
- Intensity values are stored in the non volatile EEPROM memory of the PhenoTyper's Top Unit. Therefore, once you set the values you want, you do not need to check them the next time you work with PhenoTyper, even if power was turned off.

- Measure light intensity with a light meter or a light meter app for smartphones that you can download from the internet. Make sure that the white/yellow light intensity is equal between cages, and that it does not change with time (unless for reasons related to the experimental treatment).
- You cannot change the intensity of the sound produced by PhenoTyper.
- The duration of the signals cannot be set from the Control Unit. You set this in EthoVision XT, using **Time** conditions in the Trial Control Settings. For details, see **Trial Control Settings** in the EthoVision XT Help, and the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

## **COPY LIGHT INTENSITY VALUES FROM ONE PHENOTYPER TO ANOTHER**

This section only applies to PhenoTyper 1 (analog camera).

You can copy the values of light intensity (infrared LED arrays and visible light spots) from one cage to other cages. If you want to copy one value only (for example, the intensity of the yellow light), first copy all values from one cage (see below) and then write the value you require to the other cages (see page 56).

### ***Read the values from PhenoTyper***

1. Connect the Control Unit to the PhenoTyper cage you want to copy the values from (see **CONNECT ONE PHENOTYPER TO THE PC** on page 37) and switch on that cage.
2. Press the **FUNCTION** buttons until **READ FROM PT** is shown on the Control Unit display.



3. Press **TEST**.



**Result** – The Control Unit reads the light intensity values from all sources (IR, yellow and white). The yellow and white light switch on and the Control Unit display shows '**READING...**' until the reading process is completed.

4. Follow one of the procedures below to copy the values to the other cages.

***Write all values to PhenoTyper***

1. Connect the Control Unit to the PhenoTyper cage you want to copy the values to (see **CONNECT ONE PHENOTYPER TO THE PC** on page 37) and switch on that cage.
2. Press the **FUNCTION** buttons until **WRITE TO PT** is shown on the Control Unit display.



3. Press **TEST**.



**Result** – The Control Unit writes the light intensity values for all sources (IR, yellow and white) to the cage. The yellow and white light switch on and the Control Unit display shows '**WRITING...**' until the writing process is completed.

If a value copied differs from that currently selected in the cage, the Control Unit display shows **Increase...** or **Decrease...** while the intensity



is increased or reduced to match the value copied, respectively. When the display shows **WRITE TO PT** again, all values are updated.

Repeat the procedure to copy the values to other cages.

### ***Write a single value to PhenoTyper***

1. Make sure that you have read the light intensity values from a cage (see above).
2. Connect the Control Unit to the PhenoTyper cage you want to copy the value to (see **CONNECT ONE PHENOTYPER TO THE PC**) and switch on that cage.
3. Press the **FUNCTION** buttons until the light device you want to update is selected.
4. Press **TEST**.

*Result* – The Control Unit writes the light intensity value to the cage. If you have selected the yellow or the white light, these switch on for a short time. The display shows '**WRITING...**' until the writing process is completed.

If the value copied differs from that currently selected in the cage, the Control Unit display shows **Increase...** or **Decrease...** while the intensity is increased or reduced to match the value copied, respectively. When the display shows **WRITE TO PT** again, the value is updated.

Repeat the procedure to copy the values to the other cages.

*Example:* Copy the light intensity value of **IR array-1** value from cage 1 to cage 2:

1. Connect the Control Unit to cage 1 and read the values (see above)
2. Connect the Control Unit to cage 2 and press the **FUNCTION** buttons until you see **4 IR array-1**.
3. Press **TEST**.
4. When the display shows **4 IR array-1** again, the value is updated.

## THE PHENOTYPER CAMERA

### *Camera orientation*

If the camera does not point to the center of the cage bottom, and some parts of the cage like the feeder are not visible enough, you can change the orientation of the camera. For more information, see the PhenoTyper - EthoVision XT 18 - Service Manual.

### *Lens focus*

If the camera image is out of focus:

1. The camera is in the center of the Top Unit's covering plate. Remove the ring around the lens, and rotate the lens ring.



2. Put the test animal (or an object of approximately the same height) in the cage.
3. Put the Top Unit back on the cage. Check in EthoVision that the test animal/object is on focus.

If you have a NTSC Top Unit with serial number 01000 or below, you have to remove the covering plate. Turn off PhenoTyper, unscrew the covering plate, and loosen a small screw on the lens, then rotate the focus ring.

When closing the Top Unit, do not over-tighten the first screws! Doing so may make it difficult to put the remaining ones in the right place. Instead, insert first two or three screws on different sides of the plate, and turn them 2-3 times. The plate should be still free to move at this stage. Move the plate so that the remaining screws can fit in the corresponding holes.

### ***Additional settings for PhenoTyper 1***

Although the PhenoTyper camera is optimized for video tracking, there may be cases when you need to adjust some camera settings. For example, when the image of the animal is too bright, you can switch off the Automatic Gain Control and adjust the Shutter speed.

- PhenoTyper Top Units with a serial number between 01051 and 01412 are equipped with OSD functionality. For details, see the PhenoTyper - EthoVision XT 18 - Service Manual, under **The PhenoTyper Top Unit Camera > Camera Settings**.

For Top Units with serial number above 1470, contact Noldus to order an OSD controller.

## 4 Set up an experiment with PhenoTyper 1

For this section you need:

- PhenoTyper 1 and the USB-IO box, or
- PhenoTyper 1, the Nortio Core and the Nortio TTL Terminal Hub.

### CREATE AN EXPERIMENT

1. Choose **File > New from Template**. In the **Select a template option** window, select **Apply a pre-defined template**.
2. Follow the instructions in the guided setup. Under **How is the arena configured**, choose **PhenoTyper** (for one cage) or **PhenoTyper, quad** (four cages).

After you have created the new experiment, you must still check/adjust Experiment Settings, Arena Settings and Detection Settings before you can track any animal correctly.

### EXPERIMENT SETTINGS

#### *Select the cameras*

1. In the Experiment Explorer, click **Experiment Settings**.
2. Under **Video source**, select the number of cameras from the list next to **Number of sources**.

For PhenoTyper 1 (analog camera): If you use a quad unit to mix four camera images, select **1**. Select your cameras under **Sources**.

For more information, see the EthoVision XT Help.

### ***Configure the devices of PhenoTyper 1 (USB-IO box)***

Follow this procedure if you have connected PhenoTyper to EthoVision XT through the USB-IO box.

1. In the Experiment Settings, next to **Trial Control Hardware**, select **Use of Trial Control Hardware** and click **Settings**. In the window that pops up, select **Noldus USB-IO Box** and click **OK**.
2. In the **Device Configuration** window, locate the row **SDI Port 13** and check that under **Device Type**, **Top Unit Interface PTTI-0010** is selected. **Top Unit (Standard)** should be selected for each PhenoTyper that you connected to the Top Unit Interface.

SDI Port 13	Top Unit Interface (PTTI-0010)	
Interface Port 1	Top Unit (Standard)	Top Unit (Standard) 1
Interface Port 2	Top Unit (Standard)	Top Unit (Standard) 2
Interface Port 3	Top Unit (Standard)	Top Unit (Standard) 3
Interface Port 4	Top Unit (Standard)	Top Unit (Standard) 4

In the same way, assign a Pellet Dispenser or custom hardware like an Illuminated shelter to a TTL Port. Assign a Noldus Lickometer, or a PhenoWheel to an SDI port.

For the procedure to test the connected hardware, See **ARENA SETTINGS** (page 63).

### ***Configure the devices of PhenoTyper 1 (Nortio Core + Nortio TTL Hub)***

Follow this procedure if you have connected PhenoTyper to EthoVision XT through the Nortio Core + Nortio TTL Hub.

1. In the Experiment Settings, next to **Trial Control Hardware**, select **Use of Trial Control Hardware** and click **Settings**. In the window that pops up, select **Nortio Core** and click **OK**.
2. In the **Nortio Configuration** window, locate the row that corresponds to the TTL port connected to PhenoTyper through the NOCA-TU1x cable. Select one of the two:
  - **Top Unit Light (NOCA-TU1x)** for yellow and/or white light.
  - **Top Unit Sound (NOCA-TU1x)** for the buzzer.

Repeat the step for each PhenoTyper.

In this example, the port for the white and yellow light is selected in four PhenoTypers. When programming Trial Control, you can specify which device to activate at which point in the trial.

Nortio Core			
Name	Port	Device	State
Nortio Core 01x-0108			✓
	1	<No Device Selected>	

Nortio Hub ( Connected to backbone 1 )			
Name	Port	Device	State
01 NOHU-T12T (06008)			✓
	1	Top Unit Light (NOCA-TU1x)	
	2	Top Unit Light (NOCA-TU1x)	
	3	Top Unit Light (NOCA-TU1x)	
	4	Top Unit Light (NOCA-TU1x)	
	5	<No Device Selected>	

In the following example we are going to use the white light and/or the yellow light, and the sound for each PhenoTyper. Note that port 1 and 2 of the Nortio Hub are connected to the NOCA-TU10 box of the first PhenoTyper; port 3 and 4 to the second PhenoTyper, etc.

Nortio Core			
Name	Port	Device	State
Nortio Core 01x-0108			✓
	1	<No Device Selected>	

Nortio Hub ( Connected to backbone 1 )			
Name	Port	Device	State
01 NOHU-T12T (06008)			✓
	1	Top Unit Light (NOCA-TU1x)	
	2	Top Unit Sound (NOCA-TU1x)	
	3	Top Unit Light (NOCA-TU1x)	
	4	Top Unit Sound (NOCA-TU1x)	
	5	Top Unit Light (NOCA-TU1x)	
	6	Top Unit Sound (NOCA-TU1x)	
	7	Top Unit Light (NOCA-TU1x)	
	8	Top Unit Sound (NOCA-TU1x)	
	9	<No Device Selected>	

See also **The Nortio system** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

### ***Additional options***

- To detect freezing behavior, under **Analysis options** select **Activity analysis**. Click the video button next to it and watch the video tutorial to learn about Activity analysis.
- If you work with rats, you can let EthoVision XT automatically detect behaviors like grooming, drinking, eating and rearing. Select **Behavior recognition** under **Analysis Options**. You need the Rat Behavior Recognition Module for this automatic behavior recognition.

## **ARENA SETTINGS**

Choose **Setup > Arena Settings** and open **Arena Settings 1**. For details, see **Arena Settings** in the EthoVision XT Help.

### ***Calibration***

Click **Draw Scale to Calibrate** and draw a line between two opposite cage walls or along an object of known length. Do this for both dimensions. Enter this length after drawing the line. For reference, the PhenoTyper cage width is 30 cm for model 3000, 45 cm for model 4500 and 90 cm for model 9000.

### ***Arena***

Click **Select Shape and Draw Arena**. Move the outline of the arena over the cage floor in the video image. Check that the arena covers the whole area in which you want to track the animal. Include enough of the walls so that the animal is still tracked when it rears, but exclude any bright reflective rims that might interfere with tracking. Make sure the pointing tip of the label stays within the arena.

Repeat this step for all the cages in the video image.

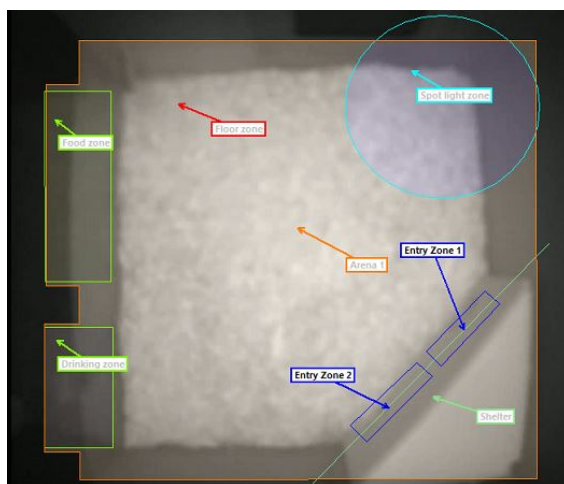
### ***Zones***

Check that the Spotlight zone, Drinking zone, Feeding zone and Shelter zone with its Entry zones are positioned where the respective zones are

on the video image, and that all have the correct shape. If necessary, adjust their size and position.

If necessary, remove zones and/or draw additional zone groups/zones. If you want to know how often the animal is on top of the shelter, you have to create an extra shelter zone. If you use a Pellet Dispenser for learning tasks, create a zone where the pellet drops. Check that the pellets always fall within this zone.

For testing anxiety-like behaviors, draw an additional zone in the center of each PhenoTyper. This way you can analyze the time that the subject spends in the center relative to the total time. This proves useful when comparing data obtained with PhenoTyper and those obtained with classic Open field or Elevated plus maze tests.



**Figure 7** *PhenoTyper with Spotlight zone, Food zone, Drinking zone and the hidden Shelter zone with two Entry zones.*

### **Shelters**

If you put a shelter in PhenoTyper, we advise you to define it as a **Hidden zone**. This way, from the time the animal enters the shelter to the time it is detected again, EthoVision XT records the animal as being



in that zone. To define a hidden zone, draw a zone corresponding to the shelter and specify it as hidden zone. For more information, see **Hidden zones** in the EthoVision XT Help.

### ***Arena settings for Rat behavior or Mouse behavior recognition***

When you selected **Behavior recognition** (only available when you have the Rat Behavior Recognition Module or Mouse Behavior Recognition Module of EthoVision XT), in the Experiment Settings, defining a few special zones and points significantly increases behavior recognition accuracy.

See **Zones and points for Behavior recognition** in the EthoVision XT Help.

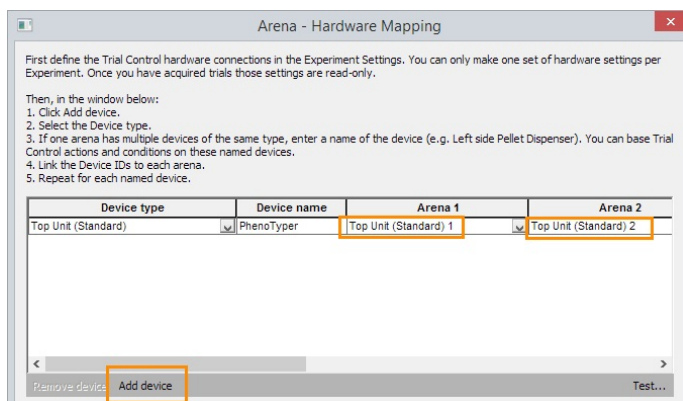
## **ASSIGN THE PHENOTYPER DEVICES TO THE ARENAS**

For this procedure you must have the Trial and Hardware Control Module.

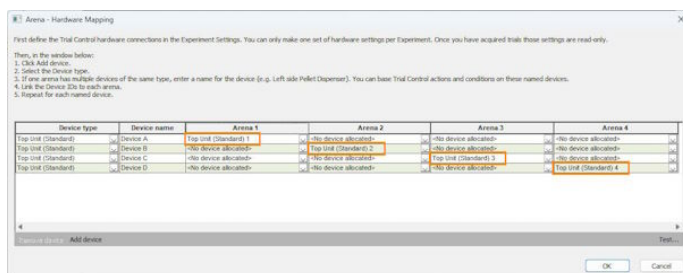
Open the Arena Settings and choose the section that applies depending on the interface device (either USB-IO box or Nortio Core + Nortio TTL Hub).

### ***Assign the devices of PhenoTyper 1 (USB-IO box)***

1. In the **Arena - Hardware Mapping** window, you should see as many rows as device added in the Experiment Settings. If not, click **Add device** for each device you want to add. For example, for four PhenoTypers, click **Add device** four times.
2. Under **Device Type**, select **Top Unit (Standard)** if it is not selected already. For four PhenoTypers, you should see four rows, each with **Top Unit Standard** selected.
3. Under **Device Name**, enter a name (e.g. PhenoTyper Top Unit) or accept the default name (A, B, C...).
4. For each Arena, select the corresponding **Top Unit (Standard) #**. Scroll the table to the right when needed.



For multiple PhenoTypers: Make sure that each Top Unit is assigned to only one Arena (PhenoTyper cage), and that each Arena hosts only one Top Unit.



## 5. For other devices, click **Add device**.

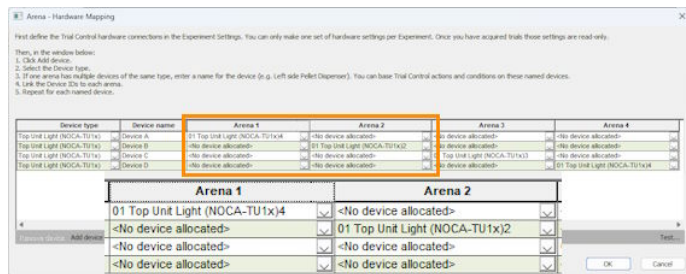
- Repeat the steps above for the Noldus Lickometer, Pellet Dispenser, and PhenoWheel.
- For a third-party hardware device, under **Device Type**, select **Custom Hardware**. Enter a name under **Device name** and select one of the physical devices from the list for each Arena.

For more information about this step, see the *EthoVision XT 18 - Trial and Hardware Control - Reference Manual*.

### Assign the devices of PhenoTyper 1 (Nortio Core + Nortio TTL Hub)

1. In the **Arena - Hardware Mapping** window, you should see as many rows as device added in the Experiment Settings. If not, click **Add device** for each device you want to add. For example, for four PhenoTypers, click **Add device** four times.
2. Under **Device Type**, select **Top Unit Light** or **Top Unit Sound** if it is not selected already. For four PhenoTypers, you have four rows for **Top Unit Light** and four rows for **Top Unit Sound**, depending on what you selected in the previous step (page 61).
3. Under **Device Name**, enter a name (e.g. PhenoTyper 1 Light, etc. ) or accept the default name (A, B, C,...).
4. For each Arena, select the corresponding **Top Unit Light #** or **Top Unit Sound #**. Scroll the table to the right when needed.

In the example below, we have selected the Top Unit Light for each PhenoTyper. Make sure that a each Top Unit device is assigned to only one Arena (PhenoTyper cage), and that each Arena hosts only one Top Unit.



**NOTE** The term **Top Unit Light** indicates both white light and yellow light. You can choose which to activate in the Trial Control Settings.

5. For other devices, click **Add device**.
  - Repeat the steps above for the Noldus Lickometer, Pellet Dispenser, and PhenoWheel.
  - For a third-party TTL hardware device, under **Device Type**, select **Custom Hardware**. Enter a name under **Device name** and select one of the physical devices from the list for each Arena.

For more information about this step, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

### ***To test a device***

Click the name of the device you want to test, and click **Test**. Select the action you want to test and click **Test**. For more information on a device, see the PhenoTyper 2 - Service Manual.

## **TRIAL CONTROL SETTINGS**

For more information on Trial Control, see **Trial Control Settings** in the EthoVision XT Help, and the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

See also the chapter **Common tests with PhenoTyper**.

## **DETECTION SETTINGS**

For more information on Detection Settings, see **Configure Detection Settings** in the EthoVision XT Help.

# 5 Set up PhenoTyper 2

## AIM

This section is about connecting and adjusting the settings of PhenoTyper 2. This is the version with the Top Unit hosting a digital camera.



## TOPICS

- **CABLES AND ADAPTERS** - page 70
- **CONNECT ONE PHENOTYPER TO THE PC** - page 71
- **CONNECT MULTIPLE PHENOTYPERS TO THE PC** - page 74
- **WORKING WITH GROUPS OF PHENOTYPERS** - page 83
- **CONNECT ADDITIONAL DEVICES** - page 83
- **LIGHTING CONDITIONS** - page 83
- **TEST THE PHENOTYPER'S FUNCTIONS** - page 83
- **ADJUST LIGHT INTENSITY** - page 84
- **THE PHENOTYPER CAMERA** - page 85
- **ADD A TTL-CONTROLLED MODULE** - page 85

## CABLES AND ADAPTERS

- **A, B** – Power cord and transformer (see Figure 5).
  - For PhenoTyper 2: code MAPS-0014.
- **C** – Power feed-through cable (see Figure 5) for connecting two PhenoTyper.
  - For PhenoTyper 2: code PT-PSC.
- **D** – Cross network cable with RJ45 connectors for connecting the digital camera to the EthoVision XT PC.

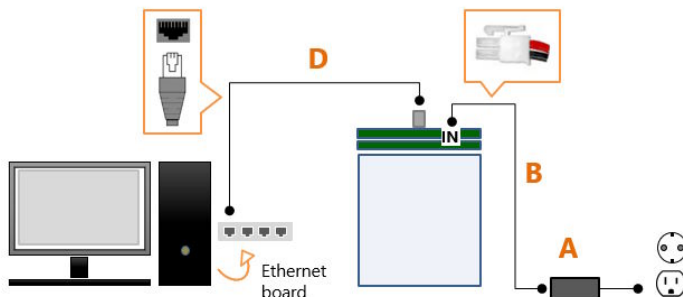


If you purchased the USB-IO box, you also have the following cables:

- Network cables (as many as PhenoTyper Top Units).
- USB cable with connectors of type A and B.
- Power cord and DC adapter 24 V 3A for the USB-IO box.

## CONNECT ONE PHENOTYPER TO THE PC

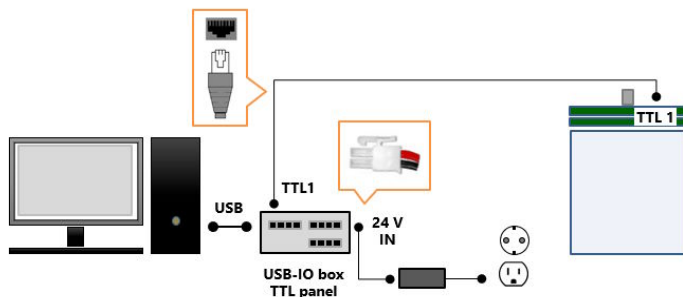
### *Power and video*



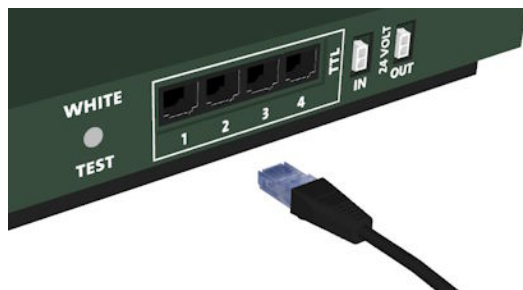
1. Connect the PhenoTyper's IN socket to the power outlet using the elements A and B (see Figure 5, page 36).
2. Connect the camera's Ethernet socket to one of the ports of the Ethernet interface board on your computer, using a cross-network cable with RJ-45 connectors.
3. Furthermore, you must set the IP address in the digital camera and the port on the Ethernet interface board. For how to do so, see **Configure the digital camera** the EthoVision XT Help or watch the video tutorial **Set up your cameras** in EthoVision XT.

### *Control of the white light with the USB-IO box*

Follow this section if you have the USB-IO box.



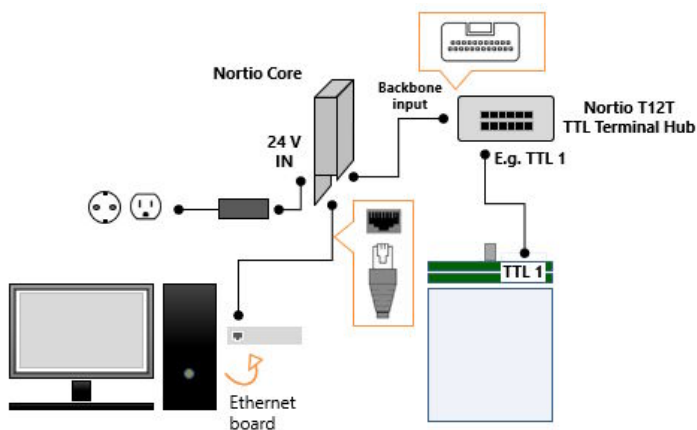
1. Connect the **USB** port of the USB-IO box to one of your PC's USB ports using the USB cable that comes with the IO box.
2. Connect the **TTL 1** port (or another one available) of the USB-IO box to the **TTL 1** port of the Control Unit of the PhenoTyper Top Unit.



3. Make sure that the USB-IO box is connected to the power outlet as shown in the figure above.

### *Control of the white light with the Nortio system*

Follow this section if you have the Nortio Core and the Nortio TTL Terminal Hub.



1. Connect the **Nortio Core** Ethernet port to the Ethernet port of your PC using a network cable. Note that the IP address of the Ethernet



port on your PC must be configured in order to communicate with the Nortio Core. See the chapter **The Nortio System** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

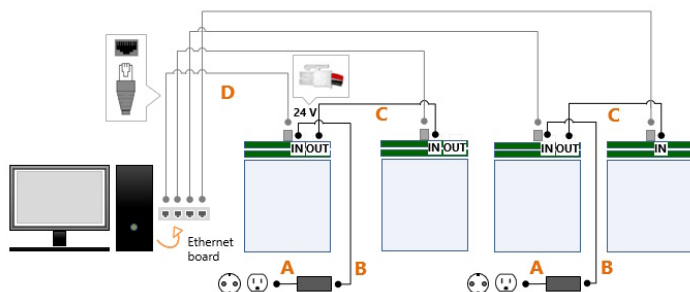
2. Connect the **24 V IN** port of the NortIO Core to the power outlet using its adapter.
3. Connect the NortIO Core to the Nortio T12T TTL Terminal Hub using the matching Backbone input cable.
4. Connect a **TTL** port on the Nortio T12T TTL Terminal Hub to the **TTL 1** port of the PhenoTyper Control Unit, using a network cable. The **TTL 1** port is configured to control the white light.
5. If you have additional devices like Pellet dispensers, connect those devices to available TTL ports of the Nortio T12t TTL Terminal Hub.

#### **Notes**

- For information about the USB-IO box, see the - EthoVision XT 18 - Trial and Hardware Control - Reference Manual.
- At the time of release of PhenoTyper 2, control is limited to the PhenoTyper white light. Contact Noldus to discuss possible extensions.
- See also **ADD A TTL-CONTROLLED MODULE** on page 85

## CONNECT MULTIPLE PHENOTYPERS TO THE PC

### Power and video



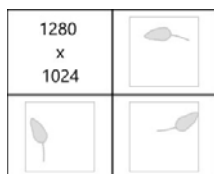
1. Connect the first PhenoTyper's **24 VOLT IN** socket to the power outlet using the elements A (MAPS-0014) and B (page 36). Repeat this step for every other PhenoTyper in the group, that is, the first of each pair.

**IMPORTANT** Connect a maximum of two PhenoTyper Top Units to the same power supply!

2. Using power feed-through cables (C), connect the **24 VOLT OUT** socket of the first PhenoTyper to the **24 VOLT IN** socket of the second. Connect the **24 VOLT OUT** socket of the third PhenoTyper to the **24 VOLT IN** socket of the fourth, and so on.
3. Connect each PhenoTyper's camera to one of the ports of the Ethernet interface board on your computer, using a cross-network cable with RJ-45 connectors (D).

### Notes

- Resolution of each camera image: 1280 x 1024. When using four PhenoTyper's, the resolution of the resulting video is 2560 x 2048.



- When using more than two PhenoTypers, connect each pair of PhenoTypers to a separate power outlet.

### ***Video resolutions that work in PhenoTyper 2***

Note that the maximal resolution attainable in your system is also depending on the frame rate and the tracking methods. Open the EthoVision XT Help (press **F1** in the software) and see the topic **GigE cameras** for a list of tested configurations.

\*\*\*See if this is actually tested

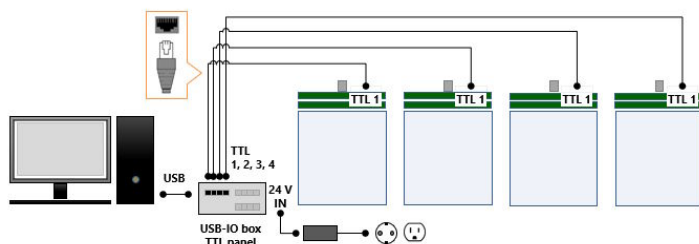
Nr. cameras	Single camera	Merged view	Connections
1	1280 x 1024	-	page 71
2	1280 x 1024	2560 x 1024	page 74
4	1280 x 1024	2560 x 2048	page 74
8	640 x 480	2560 x 960	page 77
16 (*)	640 x 480	2560 x 1920	page 81

(\*) Set frame rate 25. Higher frame rates may result in camera connection being lost.

### ***Record video to separate files***

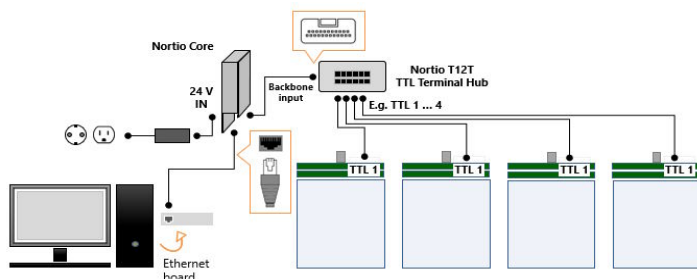
You can use MediaRecorder to save video from each cage to a separate video file, at the maximal resolution. See **Record video to separate video files** on page 43.

### ***Control of devices with the USB-IO box***



1. Connect the **USB** port of the USB-IO box to one of your PC's USB ports using the USB cable that comes with the IO box.
2. Connect the **24 V IN** port of the USB-IO box to the power outlet using its adapter.
3. Connect a **TTL** port on the USB-IO box to the **TTL 1** port of each PhenoTyper, using the network cables with RJ-45 connectors at their ends. This line controls the white light.
4. If your PhenoTyper have additional modules that can be controlled through TTL, connect the TTL ports of the USB-IO box to TTL ports **2** to **4** of each PhenoTyper.

### *Control of devices with the Nortio system*



1. Connect the Nortio Core's Ethernet port to the Ethernet port of your PC using a network cable. Note that the IP address of the Ethernet port on your PC must be configured in order to communicate with the Nortio Core. See the chapter **The NortIO System** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.
2. Connect the **24 V IN** port of the NortIO Core to the power outlet using its adapter.
3. Connect the Nortio Core to the Nortio T12T TTL Terminal Hub using the matching Backbone input cable.
4. Connect a **TTL** port on the Nortio T12T TTL Terminal Hub to the **TTL 1** port of each PhenoTyper, using network cables. The **TTL 1** port is configured to control the white light.

5. If your PhenoTyper have additional devices like Pellet dispensers, connect those devices to the available TTL ports of the Nortio T12T TTL Terminal Hub.

## 8-PHENOTYPER CONFIGURATION

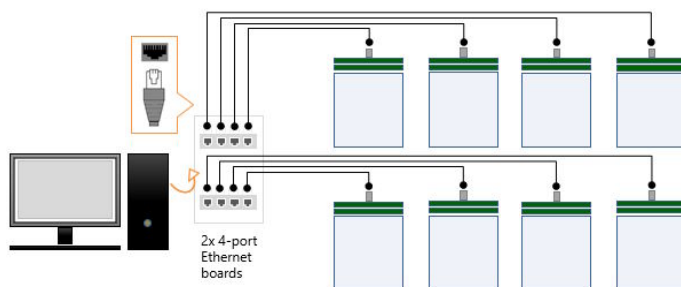
To connect your EthoVision XT computer to eight PhenoTyper cameras, make the video connections in one of the following ways.

- Video connections with two 4-port Ethernet boards (see below)
- Video connections with one 4-port Ethernet board and two network switches (page 78).

### *Video connections with two 4-port Ethernet boards*

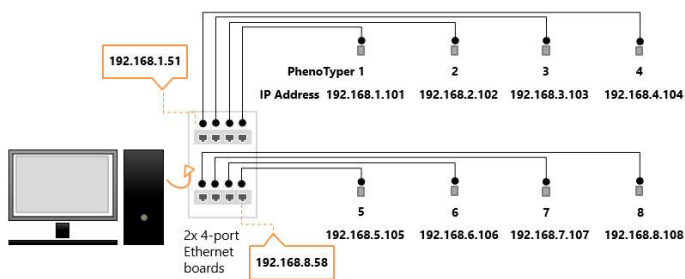
#### Prerequisites

- Only use Ethernet network interface boards with at least 1Gb Ethernet ports. See the suggestions in the section **Camera Installation** in the EthoVision XT Help.
- The Ethernet network interface boards must provide power over Ethernet (PoE) to the cameras. Alternatively, you must connect each camera to the board through a separate PoE injector with its own power plug. This increases the complexity of cabling.



#### Procedure

1. Install the two boards on the PC and assign a unique **IP address** to each port. For example, 192.168.1.51 to 192.168.8.58 with **Subnet mask** 255.255.255.0 for all ports.
2. Connect one group of four PhenoTypers to one board, and the other group to the second board.
3. Assign IP addresses to the cameras that match the IP address of the ports of the Ethernet board (see the figures below). For example, 192.168.1.101 to 192.168.8.108 with Subnet mask 255.255.255.0 for all ports



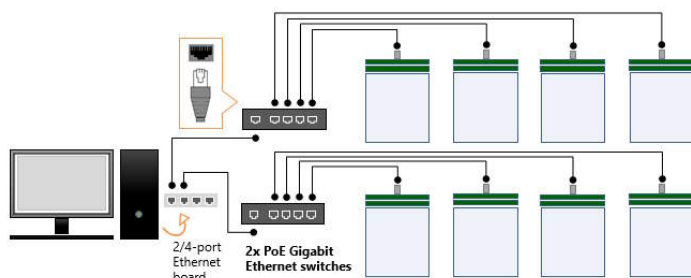
**IMPORTANT** Keep the first two numbers 192.168 of the IP address for all ports and cameras. The third number of the IP address must be unique for each camera-Ethernet port combination.

### ***Video connections with one 4-port Ethernet board and two network switches***

#### **Prerequisites**

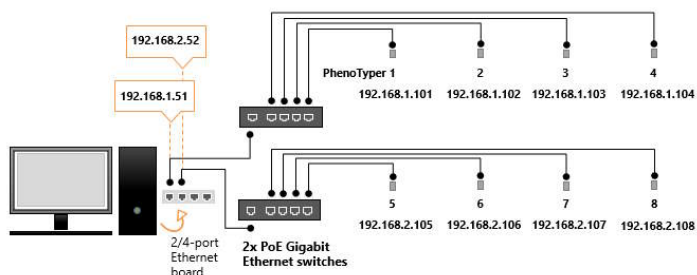
- Only use network switches with at least 1Gb Ethernet ports. Also the Ethernet network interface board must have 1Gb ports. See the suggestions in the section **Camera Installation** in the EthoVision XT Help.
- The network switches must have at least  $n+1$  Ethernet ports, where  $n$  is the number of PhenoTypers connected to the switch. In the example below, we used 2x 5-port switches.
- The network switches must provide power over Ethernet (PoE) to the cameras. Alternatively, you must connect each camera to the

switch through a separate PoE injector with its own power plug. This increases the complexity of cabling.



### Procedure

1. Install the Ethernet network interface board on the PC and the network switches.
2. Connect each PhenoTyper camera to a Ethernet port of the network switch (four cameras per switch).
3. Connect the network switches to two ports of the Ethernet board.
4. Assign an unique IP address to each port on the network switch that is connected to a camera.
5. Assign an IP address to each camera that matches the **first three numbers** of the IP address of the port on the network switch that camera is connected to. See the figure below for an example.



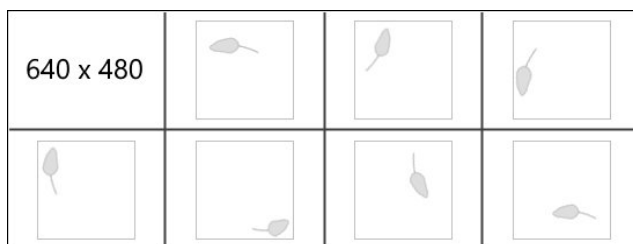
- **IMPORTANT** Keep the first two numbers 192.168 of the IP address for all ports and cameras.

- The network switches must be of type **PoE** (Power over Ethernet), that is, they must provide power to the cameras. Mind that the maximum power provided by a switch, measured in Watt (W), must be greater than that the total requirements of the cameras (one Basler camera requires about 2.5 W) and the other devices connected to the switch.
- The network switches must be able to handle large packets (known as "jumbo packets" or "jumbo frames"). With jumbo frame-capable devices, the maximum packet size can be up to 16 kB.
- For maximum network efficiency, your camera should be configured to use the largest packet size that your network adapters and switches can handle.
- For information on how to assign IP addresses, see **Install the GigE camera** on the EthoVision XT Help.
- Refer to the user guide of the network switch for how to set it up.

### **Video resolution**

Single camera view: 640 x 480.

Merged 8-camera image image: 2560 x 960.



To set the resolution, open the Experiment Settings, under **Video Source** select **Live tracking** and choose 8 as **Number of sources**. Select all the cameras and make sure that the resolution of the single cameras is 640 x 480 and that of the **Merged camera view** is 2560 x 960.



## 16-PHENOTYPER CONFIGURATION

Fos this configuration you need a high-end workstation (see page 82).

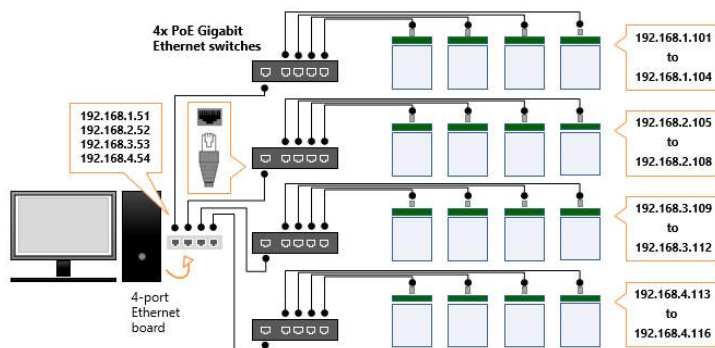
### ***Video connections with with one 4-port Ethernet board and four network switches***

#### Prerequisites

- You have four network switches with at least 5 network ports, and one 4-port Ethernet interface board installed on the EthoVision XT computer.
- See the prerequisites of the network switches on page 78.

#### Procedure

1. Connect the PhenoTyper cameras to the network switches and the latter to the PC as shown in the figure below. See also the procedure on page 78.
2. When assigning the IP addresses, make sure that the third number of the IP address of the cameras and the network switch is the same within each group of four PhenoTyper, and unique.



### ***Video resolution***

Single camera view: 640 x 480.

Merged 8-camera video image: 2560 x 1920.



To set the resolution, open the Experiment Settings, under **Video Source** select **Live tracking** and choose 16 as **Number of sources**. Select all the cameras and make sure that the resolution of the single cameras is 640 x 480 and that of the **Merged camera view** is 2560 x 1920.

**TIP** Put a sheet with a number in each PhenoTyper cage. That way you easily see which PhenoTyper camera has been selected. Select the first four cameras for group 1, then the fifth to the eighth for group 2, etc.

### ***Tested configuration***

- Dell Precision 3660 with processor 12th Gen Intel(R) Core(TM) i7-12700K, 3.6 GHz, 12 Core(s), 20 Logical Processor(s), and 8 GB RAM memory, Windows 11 Pro.
- Video resolution: as specified above.
- Camera frame rate: 25 fps.
- Trial duration: 48 hours.
- This configuration resulted in no missing samples. The test setup included Increasing the resolution may lead to a high percentage of missing samples and/or connection with the camera being lost during the trial.

## WORKING WITH GROUPS OF PHENOTYPERS

See the section on **WORKING WITH GROUPS OF PHENOTYPERS** on page 46.

Note that with PhenoTyper 2 you can have up to eight cages (and cameras) connected to one PC.

## CONNECT ADDITIONAL DEVICES

You can have EthoVision XT control PhenoTyper and additional devices. See the section **CONNECT ADDITIONAL DEVICES** on page 50.

## LIGHTING CONDITIONS

The general recommendations about lighting in the test room for PhenoTyper 1 are also valid for PhenoTyper 2. See **LIGHTING CONDITIONS** on page 51.

## TEST THE PHENOTYPER'S FUNCTIONS

### *Camera image and the infrared light source:*

1. Connect the network cable from the PhenoTyper camera to one of the ports of the Ethernet interface board on the EthoVision XT computer. For information on how to set up the IP addresses of the camera and of the Ethernet interface board, see the EthoVision XT Help.
2. Start EthoVision XT, create a new experiment, next in the Experiment Settings select **Live tracking**, select the camera input and click the camera preview button. There you should see the live camera image.
3. Switch off all the light in the room. If the infrared light source works properly, you should see a clear image of the cage bottom.

### ***White light***

1. Press the **WHITE - TEST** button on the front panel of the Control box.



2. Check that the light inside the cage switches on.

## **ADJUST LIGHT INTENSITY**

### ***PhenoTyper 2 (digital camera)***

For this you need the trimmer adjustment tool that is provided with the PhenoTyper Top Unit, or a small screwdriver.

To adjust the intensity of the infrared light:

1. Connect the network cable from the PhenoTyper camera to one of the ports of the Ethernet interface board on the EthoVision XT computer. For information on how to set up the IP addresses of the digital camera and of the Ethernet interface board, see the EthoVision XT Help.
2. Start EthoVision XT, create a new experiment, and in the Experiment Settings select **Live tracking**, then select the camera input from the list and click the camera preview button. There you should see the live camera image.
3. Turn the **IR** control on the Control box.



4. Check the image in the EthoVision XT screen.

To adjust the intensity of the white light:

1. Turn the **WHITE** control on the Control box.



2. Check the intensity of the white light in the cage. Make sure that the white light intensity is equal between cages, and that it does not change with time (unless for reasons related to the experimental treatment).

**TIP** Measure light intensity with a light meter or a light meter app for smartphones that you can download from the internet.

Repeat the procedure for the other cages.

## THE PHENOTYPER CAMERA

### *Lens focus*

The camera focus is optimized at production. For more information on the camera and lens, see the PhenoTyper 2 - Service Manual.

### *Advanced camera settings*

The Basler acA1300-60gm is the default camera of PhenoTyper 2. For extensive information on camera settings (resolution, frame rate, exposure, gain etc.) and for how to install and configure the camera in EthoVision XT, see **Install GigE cameras** in the EthoVision XT Help.

## ADD A TTL-CONTROLLED MODULE

### *Aim*

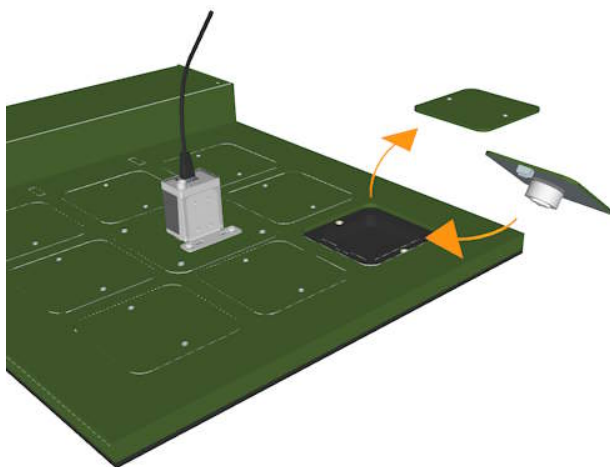
To control an additional TTL device, like a spotlight. The type of control is currently limited to On/Off. You can have EthoVision XT switch on

and off the spotlight, however you cannot adjust the light intensity through the software.

### ***Fit the new module on the Top Plate***

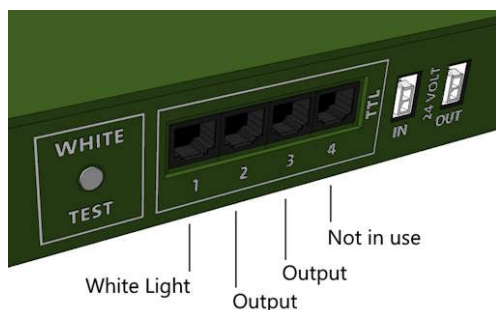
Remove the cover plate from one of the slots of the Top Plate.

Insert the module and gently press it in such a way the connectors come in contact. Fix the module with the two screws.



### ***Current TTL ports configuration***

- **TTL 1** is reserved for the PhenoTyper White light. It is not available for use with other modules.
- **TTL 2** and **TTL 3** are set to output by default. Through these ports you can have EthoVision XT send out On/Off commands to the device you have added. That is, the signal always goes from EthoVision XT to the device through the (mini) USB-IO box or the Nortio TTL Hub. In EthoVision XT Trial Control Settings, choose **Output Line 1** for that device.
- **TTL 4** is currently not available.



**NOTE** The name **Output line** refers to the internal lines that you can set for each port of the interface device, the (Mini) USB-IO box and the Nortio TTL Hub. In general, for each port of those interface devices two output lines (**Output Line 1** and **Output Line 2**) and two input lines (**Input Line 1** and **Input Line 2**) are available. You can specify them in the EthoVision XT Trial Control Settings. For PhenoTyper 2, only **Output Line 1** applies currently. For more details, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual (see page 7).

### ***Advanced solutions***

If you need additional configurations, contact your nearest Noldus representative.

## 6 Set up an experiment with PhenoTyper 2

For this section you need:

- PhenoTyper 2 and the USB-IO box, or
- PhenoTyper 2, the Nortio Core and the Nortio TTL Terminal Hub.

### CREATE AN EXPERIMENT

#### *Prerequisites*

You have followed the section **Set up PhenoTyper 2** on page 69

#### *Procedure*

1. Choose **File > New from Template**. In the **Select a template option** window, select **Apply a pre-defined template**.
2. Follow the instructions in the guided setup. Under **How is the arena configured**, choose **PhenoTyper** (for one cage) or **PhenoTyper, quad** (four cages).

After you have created the new experiment, you must still check/adjust Experiment Settings, Arena Settings and Detection Settings before you can track any animal correctly. Please follow the steps below.

### EXPERIMENT SETTINGS

#### *Select the cameras*

1. In the Experiment Explorer, click **Experiment Settings**.
2. Under **Video source**, select the number of cameras from the list next to **Number of sources**. Select your cameras under **Sources**.

For more information, see the EthoVision XT Help.



### ***Configure the devices of PhenoTyper 2 (USB-IO box)***

Follow this procedure if you have connected PhenoTyper 2 to EthoVision XT through the USB-IO box. With this setup you can control the white light.

1. In the Experiment Settings, next to **Trial Control Hardware**, select **Use of Trial Control Hardware** and click **Settings**.
2. In the window that pops up, select **Noldus USB-IO Box** and click **OK**.
3. In the **Device Configuration** window, locate the **TTL Port** of the USB-IO box that is connected to the PhenoTyper Control Unit (**TTL 1**).

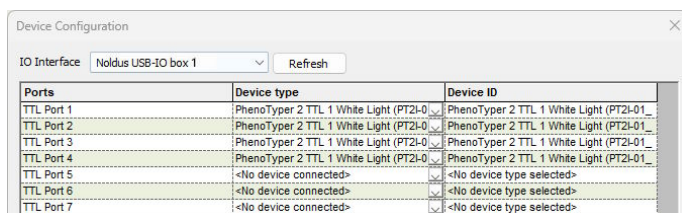
**NOTE** The SDI ports do not work with PhenoTyper 2.

4. Under **Device Type** select select **PhenoTyper 2 TTL 1 White Light** (PT2I-01\_TTL) to control the white light.

To control another module, select one of the other options (**PhenoTyper 2 TTL 2 Module** or **PhenoTyper 2 TTL 3 Module**). Note that modules other than the White Light are not standard; you have to purchase them separately.

5. Repeat the steps 3-4 for each PhenoTyper, then click **OK**.

Example with four PhenoTyper:



Ports	Device type	Device ID
TTL Port 1	PhenoTyper 2 TTL 1 White Light (PT2I-01_TTL)	PhenoTyper 2 TTL 1 White Light (PT2I-01_TTL)
TTL Port 2	PhenoTyper 2 TTL 1 White Light (PT2I-01_TTL)	PhenoTyper 2 TTL 1 White Light (PT2I-01_TTL)
TTL Port 3	PhenoTyper 2 TTL 1 White Light (PT2I-01_TTL)	PhenoTyper 2 TTL 1 White Light (PT2I-01_TTL)
TTL Port 4	PhenoTyper 2 TTL 1 White Light (PT2I-01_TTL)	PhenoTyper 2 TTL 1 White Light (PT2I-01_TTL)
TTL Port 5	<No device connected>	<No device type selected>
TTL Port 6	<No device connected>	<No device type selected>
TTL Port 7	<No device connected>	<No device type selected>

### ***Configure the devices of PhenoTyper 2 (Nortio Core + Nortio TTL Hub)***

Follow this procedure if you have connected PhenoTyper 2 to EthoVision XT through the Nortio Core + Nortio TTL Hub. With this setup you can control the white light.

1. In the Experiment Settings, next to **Trial Control Hardware**, select **Use of Trial Control Hardware** and click **Settings**.
2. In the window that pops up, select **Nortio Core** and click **OK**.

3. In the **Nortio Configuration** window, under **Nortio Hub**, expand the list and locate the TTL **Port** number connected to the PhenoTyper Control Unit.
4. Under Device select **Custom Hardware** for the white light.

Repeat the step for each PhenoTyper. In the following example, the first four TTL ports of Nortio Hub are connected to TTL ports of four PhenoTyper 2. In order to control the white light, you must connect Nortio Hub to the **TTL 1** ports of the PhenoTyper Control Unit, for all four PhenoTyper!

Nortio Hub ( Connected to backbone 1 )			
Name	Port	Device	State
01 NOHU-T12T (06008)			✓
	1	Custom Hardware	
	2	Custom Hardware	
	3	Custom Hardware	
	4	Custom Hardware	
	5	<No Device Selected>	

**NOTE** The items PhenoTyper Light and PhenoTyper Sound are for using PhenoTyper 1. See also **The Nortio system** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

### Analysis Options

- **Activity analysis.** Under **Analysis Options** select **Activity analysis** to detect freezing behavior.
- **Behavior recognition.** Under **Analysis Options** select **Behavior recognition**. If you work with rodents, you can let EthoVision XT automatically detect behaviors like grooming, drinking, eating and rearing. For this option, you need the Rat/Mouse Behavior Recognition Module.

## ARENA SETTINGS

For drawing arenas and zones, see page 63.

## ASSIGN THE PHENOTYPER DEVICES TO THE ARENAS

For this procedure you must have the Trial and Hardware Control Module.

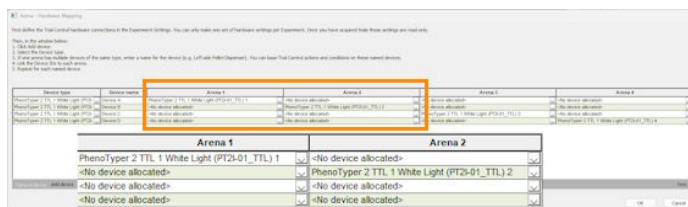
Open the Arena Settings and choose the section that applies depending on the interface device (either USB-IO box or Nortio Core + Nortio TTL Hub).

### *Assign the devices of PhenoTyper 2 (USB-IO box)*

Prerequisite: you have followed the previous step on page 89.

1. In the **Arena - Hardware Mapping** window, you should see as many rows as device added in the Experiment Settings. If not, click **Add device** for each device you want to add. For example, for four PhenoTypers, click **Add device** four times.
2. Under **Device Type**, select **Top Unit (Standard)** if it is not selected already. For four PhenoTypers, you should see four rows, each with **Top Unit Standard** selected.
3. Under **Device Name**, enter a name (e.g. PhenoTyper Light 1, etc.) or accept the default name (A, B, C...).
4. For each Arena, select the corresponding **PhenoTyper 2 TTL 1 White Light #**. Scroll the table to the right when needed.

For multiple PhenoTypers: Make sure that a each device is assigned to only one Arena (PhenoTyper cage), and that each Arena hosts only one of such devices.

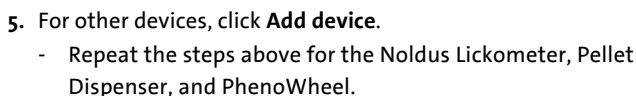


5. For other devices, click **Add device**.
  - Repeat the steps above for the Noldus Lickometer, Pellet Dispenser, and PhenoWheel.

- For more information about this step, see the *EthoVision XT 18 - Trial and Hardware Control - Reference Manual*.

Prerequisite: you have followed the previous step on page 89.

- In the example below, we have selected the Top Unit Light for each PhenoTyper. Make sure that each **Custom Hardware #** device is assigned to only one Arena, and that each Arena hosts only one of such devices.



- For a third-party TTL hardware device, under **Device Type**, select **Custom Hardware**. Enter a name under **Device name** and select one of the physical devices from the list for each Arena.

For more information about this step, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

## **TRIAL CONTROL SETTINGS**

For more information on Trial Control, see **Trial Control Settings** in the EthoVision XT Help, and the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

See also the chapter **Common tests with PhenoTyper**.

## **DETECTION SETTINGS**

For more information on Detection Settings, see **Configure Detection Settings** in the EthoVision XT Help.

## 7 Common tests with PhenoTyper

Short-term experiments can be carried out while the subjects are monitored in PhenoTyper. This can be, for example, learning tasks, anxiety tests, or tests on the effects of drugs.

For information on how to set up an experiment in EthoVision XT, see **Set Up an Experiment** in the EthoVision XT Help.

- **GENERAL BEHAVIOR MONITORING** - see below.
- **CREATE ROUTINES** - page 97.
- **FOOD RECEIVING TASK** - page 98.
- **ILLUMINATED SHELTER TASK** - page 101.
- **COGNITIONWALL™ TASK** - page 104.
- **ANXIETY TESTS** - page 105.

### GENERAL BEHAVIOR MONITORING

#### *Maximum trial duration (tested)*

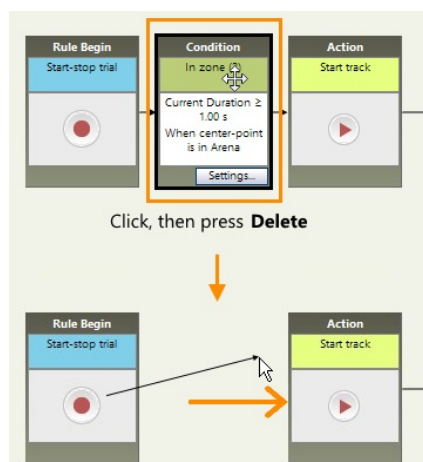
With or without simultaneous video recording: 72 hours.

For more information on tested configurations, see the EthoVision XT Help. To open the Help, in EthoVision XT choose **Help > Help Topics**.

#### *Prolonged video recordings*

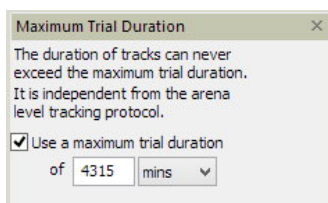
If you need to record video of the same subject for more than 72 hours, we recommend that you make a series of trials.

1. In the Trial Control Settings, remove the condition immediately before the **Start track** action box.

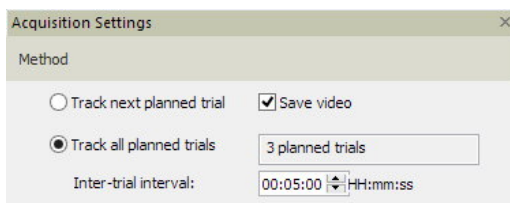


2. In the Trial Control Settings, set the Maximum Trial Duration.

**TIP** To make a trial of about 72 hours, set this time to 72 hour minus x minutes. The remaining x minutes are used as inter-trial interval (see below) to give the software enough time to save the video and the data. In this example 72 h minus 5 minutes = 4315 minutes.



3. In the Acquisition screen, select **Track all planned trials** with an inter-trial interval equal to the time x chosen above. If you want to select a shorter time, test it beforehand.



4. After data acquisition, and before running analysis, open a Data profile and group the trials based on the subject.

### ***Selected references***

Bass, J. S., Tuo, A. H., Ton, L. T., Jankovic, M. J., Kapadia, P. K., Schirmer, C., et al. (2020). On the digital psychopharmacology of valproic acid in mice. *Frontiers in Neuroscience* 14: 594612. doi: 10.3389/fnins.2020.594612

de Visser, L., van den Bos, R., Stoker, A. K., Kas, M. J. H., and Spruijt, B. M. (2007). Effects of genetic background and environmental novelty on wheel running as a rewarding behaviour in mice. *Behavioral and Brain Research*, 177: 290–297. doi: 10.1016/j.bbr.2006.11.019

Deiana, S., Platt, B., and Riedel, G. (2008). Scopolamine-induced changes in activity measured in a home cage observation system. *Proceedings of Measuring Behavior 2008* (Maastricht, The Netherlands, August 26-29, 2008): 312-313.

de Mooij-van Malsen, A. J. G., van Lith, H. A., Oppelaar, H., Hendriks, J., de Wit, M., Kostrzewa, E., et al. (2009). Interspecies trait genetics reveals association of *Adcy8* with mouse avoidance behavior and a human mood disorder. *Biological Psychiatry*, 66: 1123–1130. doi: 10.1016/j.biopsych.2009.06.016

Jankovic, M. J., Kapadia, P. P., and Krishnan, V. (2019). Home-cage monitoring ascertains signatures of ictal and interictal behavior in mouse models of generalized seizures. *PLoS One*, 14: e0224856. doi: 10.1371/journal.pone.0224856

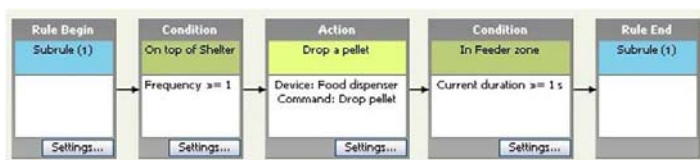
Namdar, I., Feldman, R., Glazer, S., Meninger, I., Shlobin, N. A., Rubovitch, V., et al. (2020). Motor effects of minimal traumatic brain injury in mice. *Journal of Molecular Neuroscience*, 70: 365–377. doi: 10.1007/s12031-019-01422-9



## CREATE ROUTINES

In a conditioning test, drop a food pellet when the animal has been detected on the shelter. Repeat this procedure as soon as the animal eats the food item. The trial must be stopped when the procedure is repeated 25 times. Between two subsequent repeats, an interval of 10 s is defined.

The corresponding sequence is defined as a Sub-rule in the Trial Control Settings.



With the following boxes:

- **Rule Begin** – Start of the sub-rule.
- **Condition** – Specifies that the animal must be in the zone *Shelter*.
- **Action** – Specifies the command *Drop a pellet* to the pellet dispenser. You must define this and all other devices in the **Port Device Mapping** and in the **Arena - Hardware Mapping** in order to be able to define actions on those devices.
- **Condition** – Specifies that the animal must be in the zone *Feeder*.
- **Rule End** – Stop of the sub-rule.

Define a Reference for this sub-rule and specify that the sub-rule must be repeated 25 times and the Stop to Start interval for subsequent repeats is 10 seconds.

Place the Reference box in the Start-Stop trial rule, immediately before the **Stop track** box.

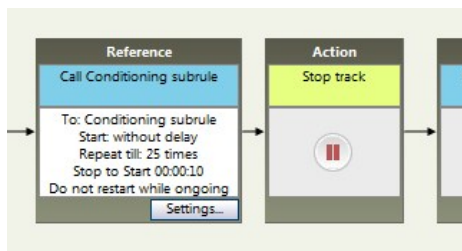
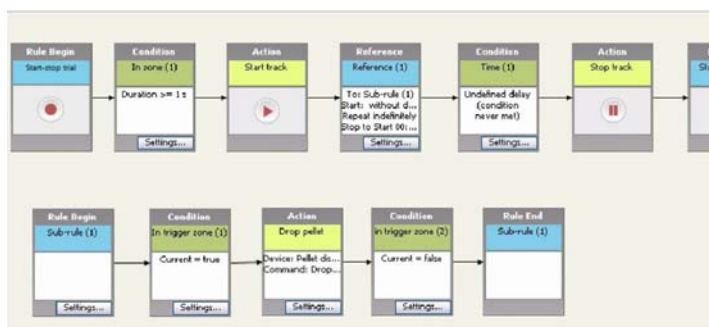


Figure 8 shows a rule with which a pellet is dropped every time the animal enters a trigger zone. In a similar way you can create a rule to switch on the light in a illuminated shelter when a particular entrance is chosen. See page 94 for those procedures.



**Figure 8** An example of a Trial Control rule to control the Pellet Dispenser.

For more details about creating routines, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

## FOOD RECEIVING TASK

In a commonly used learning test, the animal receives a food pellet from the pellet dispenser when it enters a certain zone, for example when it jumps on top of the shelter. The test generally lasts 15 minutes,

### Arena Settings

1. In the Arena Settings, create a zone for the top of the shelter. Note that this is not the same as the hidden zone used for detecting the animal when it is inside the shelter.
2. Define a zone for the area where the pellet is dropped. Check that the pellets always dropped in this zone. Especially when no bedding material is used, the pellets may roll far from where they are dropped.

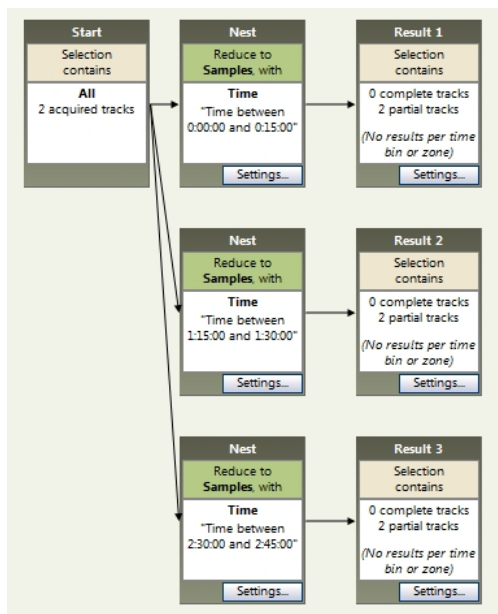
In the Trial Control Settings, create a Trial Control Rule for the Pellet Dispenser (for an example see page 97). Include conditions for the clock times the tests start and the action “PhenoTyper yellow light on”.

[illegible]

## 7 - Common tests with PhenoTyper

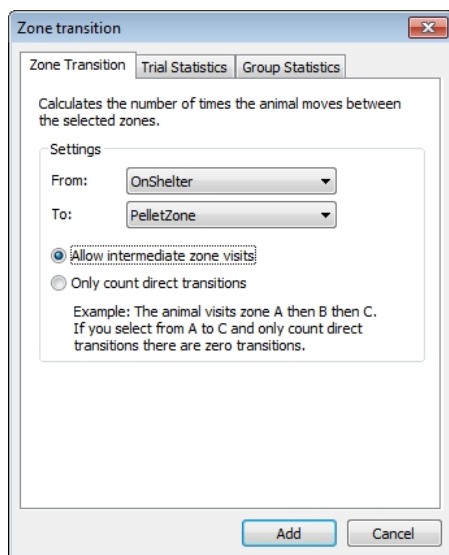
### Data profile

Create a Data Profile with multiple time conditions to filter out the 15 minutes periods. See below for an example.



### Analysis profile

Create an analysis profile and select the dependent variable **Zone transition**. Under **Settings** select from the zone *On top of Shelter* to the Pellet Dispenser zone. Select **Allow intermediate zone visits**. Click the **Trial Statistics** tab and select **Frequency**. If you run multiple trials, click the **Group statistics** tab and select the **Group mean** and **Standard Error** of the **Frequency**.



### ***Calculate the statistics***

Make sure the data profile that filters the 15 minutes task periods is active. In the Experiment Explorer click **Analysis output** under **Results**. Click **Calculate**. The frequency of transitions from the top of the shelter to the pellet dispenser zone is now given for each 15 minute task period. If the animal learns, this number increases over time.

## **ILLUMINATED SHELTER TASK**

The illuminated shelter has been developed to automate the Discriminative Avoidance Task in mice in the PhenoTyper 3000. The aversive stimulus is generated by two light boxes, each housing a bright white LED and mounted on two cage walls, shining inwards through the Perspex wall into the shelter. The light is switched on briefly or continuously, depending on the presence of the mouse near or inside the shelter. Mice can learn to avoid the “preferred entrance” of the shelter. In a study with 43 different mouse mutants, Maroteaux et

al. (2012) showed that mutants differ in their ability to associate a specific shelter entrance with the aversive stimulus

A typical protocol is the following:

- Day 1-4. First it is assessed over the first four days which of the entrances is preferred by the animal.
- Day 5-6. For this trial, a Trial Control Rule is defined in which a light in the shelter is switched on when the animal uses the preferred entrance.
- Day 7. In the probe trial, during a 24 hr period it is checked if the animal learns to use the other entrance of the shelter.

In the EthoVision XT experiment, define the following:

### ***Arena Settings***

In the **Arena Settings** create zones for both shelter entrances. These zones are predefined in the PhenoTyper template.

### ***Trial Control Settings***

Make a Trial Control subrule comparable with the example in Figure 8 on page 98. Create a condition for when the animal enters the zone “preferred shelter entrance” and an action to switch on the light in the illuminated shelter. Also create a condition and action to switch off the light in the shelter when the animal leaves it.

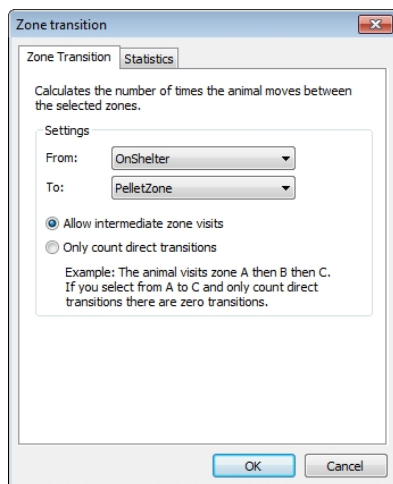
Create other necessary settings, like detection settings. Next, acquire the data.

### ***Data profile***

In the Data profile, select the testing trials (days 5-6) or if the testing phase is part of an uninterrupted trial, create a Time filter for the 48 hr testing phase. Click the **Settings** button on the Result box and select Results per time bin. Use time bins of, for example, 1 hour. Time bins help find trends in cognitive and aversive responses; for instance, an increase in the use of the shelter entry that did not trigger the light.

### Analysis profile

1. To calculate statistics of each entrance, create an Analysis profile and select the dependent variable **In Zone**. Select both shelter entrances. Click the **Trial Statistics** tab and select **Frequency**. If you run multiple trials, click the **Group statistics** tab and select the **Group mean** and **Standard Error of the Frequency**.



2. Make sure the data profile that filters the 48-hr testing phase is active. In the Experiment Explorer click **Analysis output** under **Results**. Click **Calculate**. The frequency of visits to both shelter entrances is now shown for every time bin.

### References

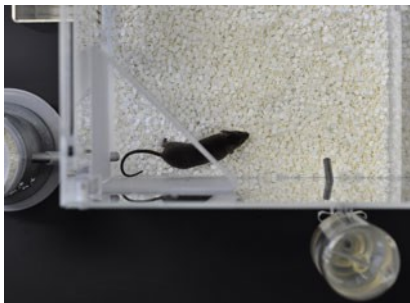
- Heer, R.C. de; Schenke, M.; Kuurman, W.W.; Spruijt, B.M. (2008). Learning (in) the PhenoTyper: an integrative approach to conducting cognitive behavioural challenges in a home cage environment. *Proceedings of Measuring Behavior 2008*, 6th International Conference on Methods and Techniques in Behavioral Research (Maastricht, The Netherlands, 26-29 August 2008), 57.
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## COGNITIONWALL™ TASK

The CognitionWall was designed for the development of operant cognitive tests in PhenoTyper home-cages. The CognitionWall is a wall with three entrances, which is placed in front of the Pellet dispenser. Mice are rewarded with a food pellet when they choose to pass through one of the three entrances. The rate at which a mouse gains a relative preference for the rewarded entrance is used as a measure of discrimination learning.

After an initial discrimination learning phase, the rewarded entrance is switched to another entrance (reversal). The rate at which a mouse gains a relative preference for the newly rewarded entrance is used as a measure of reversal learning.



## References

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Rommelink, E., Chau, U., Smit, A. B., Verhage, M., and Loos, M. (2017). A one-week 5-choice serial reaction time task to measure impulsivity and attention in adult and adolescent mice. *Scientific Reports*, **7**:42519. doi: 10.1038/srep42519

## ANXIETY TESTS

In anxiety tests, the exploratory behavior of the animals is tested. The animals are placed under mild stress by projecting a white light beam on the food area during the first three hours of the dark period, in which the animals are active. The animal must choose between feeding and avoiding the bright light. The time it takes before the animal starts feeding could reflect its level of anxiety.

### *Arena Settings*

Define a zone for the feeding area.

### *Trial Control Settings*

Create a Trial Control rule with an **Action White light on**, followed by a **Time** condition for the first three hours of the dark phase (i.e., “wait three hours”), followed by a second **Action White light off** which switches off the light after three hours.

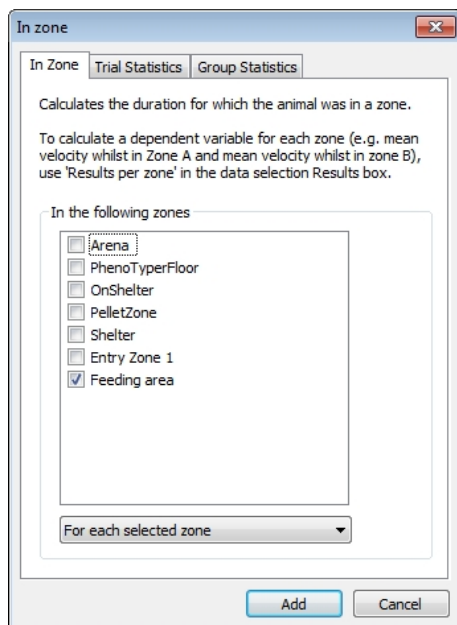
Create other necessary settings, like detection settings. Next, acquire the data.

### *Data profile*

- Create a data profile with a filter for the experiment time.
- Create a data profile with a filter for some time after the light on the feeding area has been switched off. For example, one hour.

### Analysis profile

Create an analysis profile and select the dependent variable **In zone**. Select the zone *Feeding area*. Click the **Trial Statistics** tab and select **Mean** (gives mean duration per visit), **Frequency** (gives the number of visits), and **Cumulative duration** (gives the total duration over all visits). If you run multiple trials, click the **Group statistics** tab and select the **Group mean** and **Standard Error** for each statistic.



Create an analysis profile and select the dependent variable **In zone**. Select the zone **Feeding area**. Click the **Trial Statistics** tab and select **Latency to first**. Next, select the correct combination of data profile and analysis profile. In the Experiment Explorer click **Analysis output** under **Results**. Click **Calculate**.

### Selected references

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Maroteaux, G., Loos, M., van der Sluis, S., Koopmans, B., Aarts, E., van Gassen, K., Geurts, A., Largaespada, D. A., Spruijt, B. M., Stiedl, O., Smit, A. B. and Verhage, M. (2012). High-throughput phenotyping of avoidance learning in mice discriminates different genotypes and identifies a novel gene. *Genes, Brain and Behavior*, **11**: 772–784. doi: 10.1111/j.1601-183X.2012.00820.x

Pham, J., Cabrera, S. M., Sanchis-Segura, C., Wood, M. A. (2009). Automated scoring of fear-related behavior using EthoVision software. *Journal of Neuroscience Methods*, **178**(2): 323–326.

## 8 Maintenance

Your PhenoTyper cage will last longer if you follow these few recommendations. Please always keep the cages clean and dry. Make sure that no liquid penetrates the equipment on top of the cage.

### CLEAN THE CAGE

#### *General information*

The cage walls, the bottom plate and the shelter of PhenoTyper are made of Acrylic, also known as Polymethylmethacrylate or PMMA. To clean the cage, please use water and soap only, or wipes for alcohol-sensitive materials (see below). Do not wash the cage with water at more than 80° C (176° F).

#### **IMPORTANT - READ THIS FIRST**

- Do not use chemicals to wash the cage except those listed in the next section.
- Whatever substance you use to clean PhenoTyper, wipe the surface completely dry. Make sure that droplets do not remain on any component of the cage.
- Do not use autoclaves to sterilize the cage or any of its components! It may damage it.

#### *Clean the cage walls provided with the pellet receptacle*

Beginning from items with serial number 132, the cage walls with a pellet receptacle can be cleaned without removing the pellet receptacle first. For those items, moisture is expected not to damage the circuits. You can also clean the inside of the receptacle by loosening the screws. However, if you want to clean the pellet receptacle thoroughly, follow this procedure:

1. Disconnect all cables and remove the wall with the pellet receptacle from the cage.

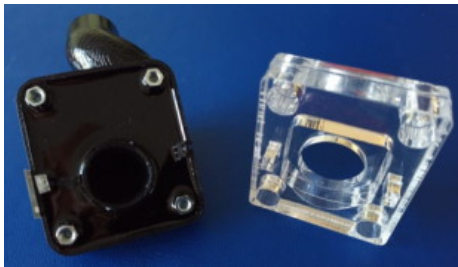
2. Unscrew the receptacle unit from the wall.



3. Remove the label with the serial number, or cut it through on the line between the black enclosure and the transparent support frame.



4. Gently pull out the two parts from the wall. Each part must be cleaned separately.



Clean those parts just like you do for the regular cage walls.

5. Reassemble the components and attach them back to the cage wall.

## ACCEPTABLE CLEANING TECHNIQUES AND SUBSTANCES

**IMPORTANT** Noldus takes no responsibility for any damage resulting from improper use of cleaning substances. Always test a substance when in doubt. Always remove remaining liquid after cleaning.

Use the following substances and techniques to clean the cage walls, the shelter and the bottom plate. For the drinking bottle and the Top Unit, see page 113.

- Electronic beam.
- Gamma radiation.
- Ethylene oxide gas.
- Hydrogen peroxide (HP), liquid (up to 3% concentration). Only use HP to clean the cage's acrylate/PMMA components.

**IMPORTANT** Do not use HP with any metal or electronic components, including the Top Unit! Metals can be severely damaged by HP.

If you use (vaporized) hydrogen peroxide, we recommend to clean the surfaces afterwards using water and mild soap, or a perspex/acrylic cleaner. Always dry all the surfaces.

- Vaporized hydrogen peroxide (VHP, up to 30% concentration). This technique requires specific apparatuses and procedures. See

<https://www.sciencedirect.com/topics/immunology-and-microbiology/vaporized-hydrogen-peroxide>

**IMPORTANT** Make sure that the VHP procedure includes the **dehumidification** process. Dehumidification reduces relative humidity (ideally to less than 30%). If dehumidification is not done, condensation of the gas occurs, resulting in a large number of droplets on the surface of the objects. This means that a much higher concentration of hydrogen peroxide than in the gas phase will be in contact with the objects. This is likely to damage plastic on the long term. When in doubt, always check with the manufacturer of the VHP cleaning equipment about the

compatibility of VHP with Acrylate (PMMA) and electronic components.

- The risk of damage increases with the number of times that an object is subject to VHP cycles during its lifetime.
- Only use VHP to bring material to the safe environment, not to clean the cages on a daily basis.
- We recommend to clean the surfaces afterwards using water and mild soap, or a perspex/acrylic cleaner. Always dry all the surfaces.
- Peracetic acid (also known as Peroxyacetic acid, or PAA; up to 2% concentration). This is a good alternative to autoclaving. A trade name for Peracetic acid is Nu-Cidex.
- UV radiation.
- High intensity visible light.
- Alkaline substances (albeit in low concentrations).
- Perspex/acrylic cleaner.
- Disinfection wipes that are compatible with acrylate. A good one is wipes with Meliseptol® by Carl Roth:  
<https://www.carlroth.com/nl/en/surface-disinfectants/disinfection-wipes-meliseptol-wipes-sensitive/p/xp57.1>
- Low-temperature hydrogen peroxide gas plasma.
- Diluted solutions of isopropyl alcohol can be used to wipe down acrylic without adverse reaction.

**IMPORTANT** Take care on the cleaning conditions below:

- Cleaning should take place in a well ventilated room.
- Take the cage apart and clean each component (bottom, walls, etc.) separately. Make sure that there are no parts together.
- Use spray or damp cloth to apply the isopropanol.
- After wipe down the surfaces, **make sure all isopropanol is evaporated**; droplets should not remain on the surfaces.

## SUBSTANCES THAT CAN DAMAGE PHENOTYPER



**IMPORTANT** Make sure that the following chemicals do not come in contact with PhenoTyper (cage and Top Unit). Noldus takes no responsibility for any damage resulting from improper use of cleaning substances. Always test a substance when in doubt.

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Acetaldehyde	Acetone	Allyl alcohol
Aniline	Acetic acid	Benzene
Benzyl alcohol	Butyl acetate	Butyl alcohol
Carbon disulfide	Carbon tetrachloride	Chloroform
Chlorine gas	Chlorobenzene	Dichloroethane
Dioxane	Ethanol	Ethoxyethane
Ethyl acetate	Ethylene oxide (wet)	Hydrocarbons
Hydrogen Fluoride	Hydrogen peroxide liquid (>3%)	Methanol
Methyl acetate	Methylated spirit	Methylene chloride
Methylethylketone	Nitric acid	Steam
Any strong acid		

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## CLEAN THE DRINKING BOTTLE

The bottle itself is made out of polycarbonate and can be autoclaved at 125 °C (257°F).

The stopper is made of neoprene and can be cleaned at temperatures up to 90 °C (194°F).



## CLEAN THE TOP UNIT

To clean the Top Unit, please use a damp cloth. Please do not use chemicals or soap to wash the cage.

- Make sure that no liquid penetrates the equipment inside the Top Unit or its connectors. Pay particular attention when cleaning the Top Unit of PhenoTyper 2.
- Always remove liquid after cleaning, whatever method you use.
- Do not use Vaporized Hydrogen Peroxide (VHP) or any of the substances listed on page 112 to clean devices or boxes that contain electronics.

## RECOMMENDED MATERIALS

For your animals in the PhenoTyper, you may want to use bedding and nesting material. Several suppliers offer different materials. We know that the products described below work well for tracking with EthoVision XT.

### *Bedding material*

- For black animals, tracking goes well if you use wood chips, like for example 5x5x1 mm Wood chips from ABEDD® - LAB & VET Service GmbH ([www.abedd.com](http://www.abedd.com)). Use this bedding material together with a white PhenoTyper floor.
- To track white animals you need a black background. In this case, use a black PhenoTyper floor. We know that tracking white animals goes well when using Paperchip pellets ([www.lbs-biotech.com](http://www.lbs-biotech.com)) as bedding material. This material appears black under infrared light.

**NOTE** When you try out different types of bedding materials, be aware that many materials that we see as black appear as white under infrared light.

When you track hooded animals, it depends on the proportion of black and white whether you can best track with a white or a black background.

### ***Nesting material***

A simple white tissue is often used as nesting material and placed inside the shelter. This goes well for tracking black animals. To track white animals, nesting material that appears as black under infra-red light should be most suitable. However, we are not aware that such material exists. Most black tissue-like materials unfortunately appear as white under infra-red light. Therefore, also for these animals a white tissue is often used. However, when you do so, check that the animals do not drag the tissue out of their shelter. If the tissue is present on the PhenoTyper floor, this tissue will be detected as the animal by EthoVision XT.

### ***Pellets for the Pellet dispenser***

For the Pellet dispenser you can use 20 mg or 45 mg pellets. For more information, see **The Pellet Dispenser and the Pellet Receptacle** in the PhenoTyper - EthoVision XT 18 - Service Manual.

# A Technical Support

## HELP DESK

If you run into problems, please contact us at [my.noldus.com](https://my.noldus.com).

Please check this manual carefully and the EthoVision XT Help before contacting the Help desk. To send experiment data, use the Backup procedure (see the EthoVision XT Help).

## REPLACEMENT OF COMPONENTS

If any component breaks or wears out, please contact Noldus to have it sent back to us. Please note that in any case you must send it to our international headquarters (Wageningen, The Netherlands) no matter where you are. Broken components will be fixed or replaced with new ones. See the next page for a list of the parts available.

To have components replaced, enter a support ticket at [my.noldus.com](https://my.noldus.com).

**IMPORTANT** Plastic components (such as the cage walls) will only be replaced or repaired free of charge if they are broken due to a defect of workmanship and the damage is reported within one month of shipment. Other components are covered by a one year guarantee; to claim under guarantee you must report the damage within one year of shipment. It is possible that you may have a service contract entitling you to free replacement of parts for a longer period, please contact the support department if you are not sure. In any case we can still fix or replace the parts, but it may be necessary to levy a charge. The guarantee is only valid if you treat the PhenoTyper as described in this manual. In any case, shipping broken parts is at your expenses, while sending repaired or replacing parts back to you will be at our expenses.

**IMPORTANT** If you connect PhenoTyper to any third-party products, such as video tracking system other than EthoVision XT, Noldus is not responsible for maintenance of that product, or for damage done to the PhenoTyper by that product.

To order spare parts, contact your Noldus representative.

## PARTS LIST

### *Top Units, Control Unit and cables*

Item Code	Description	Notes
<i>PhenoTyper 1</i>		
PT-T10.5/P	Top Unit V1.5 PAL/CCIR camera version	Includes camera, circuitry, lights, buzzer
PT-T10.5/N	Top Unit V1.5 NTSC/EIA camera version	Includes camera, circuitry, lights, buzzer
PT-CU-10	Control Unit	Includes cable to connect to PhenoTyper
PT-PS1	Power supply 120V AC-240V AC / 24V DC	
PTTO-SD01	Remote On Screen Device (OSD)	Available on request.
PT-PSC	Power supply feed-through cable 1.8 m	
PT-BNC30	Coaxial cable with BNC connectors, 75Ω, 3 m	
PT-BNC100	Coaxial cable with BNC connectors, 75Ω, 10 m	
<i>PhenoTyper 2</i>		
PT2TP-0020	Top Unit Top Plate	
PT2I-01_TTL	Top Unit TTL Control Unit	
MAPS-0014	Power supply 120V AC-240V AC / 24V DC	
PT-PSC	Power supply feed-through cable 1.8 m	
***	Network cable with RJ-45 connectors, *** m	

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The following cables are no longer available as a standard part (only on request):

- PT-LPT PC TTL cable 1.8 m.
- PT-LPT18 TTL extension cable 1.8 (Extension of PT-LPT).
- PT-LPT100 TTL extension cable 10 m (Extension of PT-LPT).
- PT-TTL18 TTL feed-through cable 1.8 m.

### ***Cage model 3000 (for mice)***

Below the standard cage components are listed. For custom solutions, please contact Noldus.

Item Code	Description	Notes
PTC3-W000	Wall plain, model 3000	Material: polymethylmethacrilate
PTC3-W001	Wall with air-gap, model 3000	Material: polymethylmethacrilate
PTC3-S001	Airgap shielding, model 3000	Material: polymethylmethacrilate
PTC3-W002	Wall prepared for feeder and bottle, model 3000	Material: polymethylmethacrilate
PTC3-W005	Wall prepared for shelter, model 3000	Material: polymethylmethacrilate
PTC3-S010	Cover plate, model 3000	Material: polymethylmethacrilate
PTC3-BT01	Cage bottom, model 3000	Material: polymethylmethacrilate, dual satin opaque. Side 31.5 cm Includes L piece (5x3x1.5 cm) to keep cage walls firm

**PT-C-S-MNT1**

Set of assembling  
material and tools for  
the cage, model 3000

Includes:

1x hexagon key 3 mm



1x hexagon key 2.5 mm



4x silicone tube, 1 cm



8x hexagon socket screw, cap  
head, stainless steel, M5 x 12



2x bolts M4x12



6x black nylon spacer 5 mm



6x white nylon screw  
CS M4 x 16



6x white nylon nut/washer



### ***Cage model 4500 (for rats)***

Below the standard cage components are listed. For custom solutions, please contact Noldus.

Item Code	Description	Notes
PTC4-W000	Wall plain, model 4500	Material: polymethylmethacrilate
PTC4-W001	Wall with airgap, model 4500	Material: polymethylmethacrilate
PTC4-S000	Airgap shielding, model 4500	Material: polymethylmethacrilate
PTC4-S001	Airgap shielding for mice, model 4500	Material: polymethylmethacrilate
PTC4-W002	Wall prepared for feeder and bottle, model 4500	Material: polymethylmethacrilate
PTC4-W005	Wall prepared for shelter, model 4500	Material: polymethylmethacrilate
PTC4-F001	Cover plate with air holes, model 4500	Material: polymethylmethacrilate
PTC4-F002	Cover plate with air holes for mice, model 4500	Material: polymethylmethacrilate
PTC4-BB01 (black) PTC4-BW01 (white)	Cage bottom	Material: polymethylmethacrilate, matted

**PT-C-L-MNT1**

Set of assembling  
material and tools for  
the cage, model 4500

Includes:

1x hexagon key 3 mm



1x hexagon key 2.5 mm



8x silicone tube, 1 cm



8x hexagon socket screw,  
cap head, stainless steel, M5  
x 12



4x hexagon socket bolts  
M4x12



10x black nylon spacer 5 mm



10x white nylon screw  
CS M4 x 16



10x white nylon nut/washer





**PT-C-L-MNT1**

Set of assembling material and tools for the cage, model 4500

(continued from previous page)

4x stainless steel screw, M3x12



4x hexagonal nuts



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### ***Bottles, feeder and shelters***

Item Code	Description	Notes
PT-B-00	Standard drinking bottle	Model 3000 can have 1 bottle. Model 4500 can have one or two
PTBM-0001	Drinking bottle mount	Comes with silicone tubes (see assembling tools, page 117 and 119)
PT-F-00	Feeder, model 3000	Made of stainless steel
PT-F-01	Feeder, model 4500	Made of stainless steel
PT-SH-BL-0	Shelter, small black	Size 100 x 100 x 50 mm, holes $\varnothing$ 32 mm
PT-SH-WH-0	Shelter, small white	Size 100 x 100 x 50 mm, holes $\varnothing$ 32 mm
PT-SH-BL-1	Shelter, large black	Size 150 x 150 x 120 mm, holes $\varnothing$ 75 mm
PT-SH-WH-1	Shelter, large white	Size 150 x 150 x 120 mm, holes $\varnothing$ 75 mm

# B Technical Specifications

## PHENOTYPER 1

<b>Power supply</b>	24V DC - 15 W
<b>Video output</b>	CCIR black/white 1 Vpp - 75 W Resolution 720x576 (PAL) or 640x480 (NTSC)
<b>Infrared lighting</b>	3 integrated IR arrays through diffuser. Wavelength 940 nm
<b>Audio stimulus (TTL on/off controllable)</b>	Frequency $\pm 2300$ Hz Amplitude $\pm 80$ dB
<b>Yellow light stimulus (TTL on/off controllable)</b>	Wavelength 590 nm
<b>White light gradient (TTL on/off controllable)</b>	Color temperature 5500°K Intensity 0 - approx. 1000 lux
<b>Connections</b>	Supply 24V DC in Supply 24V DC out (feed-through) TTL control in/out Sub-D 9 TTL control in/out Sub-D9 (feed-through) Video out (BNC) Control unit Sub-D15
<b>Remote On Screen Device (OSD)</b>	For Top Units with serial number 001051 to 1412. For 1471 and higher, OSD on request only.
<b>Cage components</b>	Clear or matte Acrylic (PMMA).
<b>Operating temperature</b>	0 to +40 °C (+32 to +104 °F)
<b>Storage temperature</b>	-20 to +70 °C (-4 to +158 °F)
<b>Operating humidity</b>	Up to 80%, no condensation
<b>Dimensions</b>	Model 3000: 300 x 300 x 435 mm / 11.8 x 11.8 x 17.1 in, Floor area 841 cm <sup>2</sup> (130.4 in <sup>2</sup> ) Model 4500: 450 x 450 x 660 mm / 17.7 x 17.7 x 26.0 in, Floor area 1936 cm <sup>2</sup> (300.1 in <sup>2</sup> ) Model 9000: 900 x 900 x 1000 mm / 35.4 x 35.4 x 39.4 in, Floor area 7744 cm <sup>2</sup> (1200.3 in <sup>2</sup> )

### *Top unit versions*

<b>PT-T10.0</b>	Introduced 09-2004; started from serial nr. 100 Features: CMOS type camera
<b>PT-T10.1</b>	Introduced 09-2005; started from serial nr. 196 Features: CMOS camera replaced by a CCD-type
<b>PT-T10.2</b>	Introduced 03-2011; started from serial nr. 801 Features: Camera mount with alignment method
<b>PT-T10.3</b>	Introduced 03-2012; started from serial nr. 1001 Features: new LED type for white stimulus/cue light
<b>PT-T10.4</b>	Introduced 10-2013; from serial nr. 1053 to 1412. Feature: On Screen Display for camera settings added
<b>PT-T10.5</b>	Introduced 4-2018; started from serial nr. 1471 OSD control on request only

### *Top Unit interface*

<b>Noldus type number</b>	PTTI-001x
<b>Power supply</b>	12V DC supplied by the USB IO-box
<b>Current consumption</b>	40 mA
<b>Connectors</b>	1x RJ45 8-pin modular to USB IO-box 4x RJ45 8-pin modular to PhenoTyper Top Units
<b>Dimensions</b>	115 x 65 x 32 mm / 4.5 x 2.6 x 1.3 in (L x W x H)
<b>Weight</b>	100 g
<b>Cable to USB IO-box</b>	1x 1m modular RJ45 (green)
<b>Cable to PhenoTyper Top Unit</b>	4x 2m modular RJ45 (green) with SubD-9 adapter

## PHENOTYPER 2

<b>Mains adapter</b>	Input 100-240 V AC Output 24 V DC 3 A maximum
<b>Power requirements</b>	24 V DC 0.4 A
<b>Standard camera</b>	Basler acA1300-60gm Interface: Gigabit Ethernet (RJ45 connector, CAT5e or higher) Sensor: 1/1.8" Progressive Scan CMOS, monochrome Max. resolution: 1280 x 1024 Max. frame rate: 60 fps
<b>Lens</b>	Evetar F1.8 4mm includes IR pass filter
<b>Infrared lighting</b>	Wavelength 940 nm Intensity adjustable manually, 0.1 to 0.35 mW/cm <sup>2</sup> (*); light permanent on
<b>White light</b>	Color temperature 4000°K Intensity adjustable manually, 0.5 to 2700 lux (*). Default 500 lux
<b>Connections</b>	24 V DC input/output feed-through 4x RJ-45 TTL control port (**)
<b>Cage components</b>	Clear Acrylic (PMMA)
<b>Operating temperature</b>	0 to +40 °C (+32 to +104 °F)
<b>Operating humidity</b>	20 to 80 %, relative, non-condensing
<b>Storage temperature</b>	-20 to +70 °C (-4 to +158 °F)
<b>Dimensions cage</b> L x W x H Top Unit excluded	Model 3000: 300 x 300 x 435 mm / 11.8 x 11.8 x 17.1 in, Floor area 841 cm <sup>2</sup> (130.4 in <sup>2</sup> ) Model 4500: 450 x 450 x 660 mm / 17.7 x 17.7 x 26.0 in, Floor area 1936 cm <sup>2</sup> (300.1 in <sup>2</sup> ) Model 9000: 900 x 900 x 1000 mm / 35.4 x 35.4 x 39.4 in, Floor area 7744 cm <sup>2</sup> (1200.3 in <sup>2</sup> )
<b>Dimensions Top Plate</b>	338 x 305 x 22 mm / 13.3 x 12.0 x 0.9 in
<b>Dimensions Control unit</b>	305 x 88 x 34 mm / 12.0 x 3.5 x 1.3 in
<b>Weight Top Plate</b>	2.1 kg (4.6 lbs) (camera module included)
<b>Weight Control Unit</b>	0.4 kg (0.9 lbs)

(\*) Measured at the bottom of a PT3000 cage. (\*\*) Ports 3 and 4 for future use

### ***Top Unit elements***

<b>PT2TP-0020</b>	PhenoTyper 2 Top Plate Introduced 01-2024; started from serial nr. 100
<b>PT2I-01_TTL</b>	PhenoTyper 2 Top Plate Control Unit Introduced 01-2024; started from serial nr. 100
<b>PT2MC-BGM-1300</b>	Digital camera module with Basler acA1300- 60gm

# C Declaration of Conformity

## **PHENOTYPER 1**

### *PhenoTyper 1*

We

Noldus Information Technology b.v.

Nieuwe Kanaal 5

NL-6700 AG Wageningen

The Netherlands

Declare that our product:

**"PhenoTyper"**

to which this declaration relates is in compliance with the requirements of the EMC Directive (89/336/EEC) because of the following reasons:

- It complies with the requirements of NEN-EN50082-1 (Generic immunity standard — Commercial and light industry).
- It complies with the requirements of NEN-EN50081-1 (Generic emission standard — Commercial and light industry).

The CE marking is affixed on the product according to article 10 of the EC Directive 89/336/EEC.

Wageningen, 15 October 2004



## EC declaration of conformity

Manufacturer:  
**Noldus Information Technology**  
Nieuwe Kanaal 5  
6709PA Wageningen  
The Netherlands

Declares that the following line of products:

### **PhenoTyper PT-T10 series**

Fulfills all relevant provisions of the EC EMC directive 89/336/EEC.

According the harmonized standards:

**NEN-EN 50081-1 January 1994**

Electromagnetic Compatibility - Generic Emission Standard Part 1:  
Residential, Commercial and Light Industry

**NEN-EN 50082-1 September 1993**

Electromagnetic compatibility - Generic immunity standard - Part 1:  
Residential, commercial and light industry

Fulfills all relevant provisions of the EC RoHS directive 2011/65/EU.

According the harmonized standards:

**EN IEC 63000 : 2018**

Technical documentation for the assessment of electrical and  
electronic products with respect to the restriction of hazardous  
substances

Fulfills all relevant provisions of the EC WEEE Directive 2012/19/EU.

Waste Electrical and Electronic Systems (WEEE)

The signatory on behalf of the manufacturer:

Date: September 30 2020

Name : Jeroen Kemerink  
Vice President Research & Development

## PHENOTYPER 2



### EC declaration of conformity

Manufacturer:  
**Noldus Information Technology**  
Nieuwe Kanaal 5  
6709PA Wageningen  
The Netherlands

Declares that the following line of products:

**PT2.0 TopPlate TTL Interface PT2I-xx\_TTL**

Fulfills all relevant provisions of the **EN55011-A** for :

- Industrial, scientific and medical equipment
- Radio-frequency disturbance characteristics

Fulfills all relevant provisions of the EC RoHS directive 2011/65/EU.  
According the harmonized standards:

**EN IEC 63000 : 2018**

Technical documentation for the assessment of electrical and electronic products with respect to the restriction of hazardous substances

Fulfills all relevant provisions of the EC WEEE Directive 2012/19/EU.  
Waste Electrical and Electronic Systems (WEEE)

The signatory on behalf of the manufacturer:

Date: September 30 2020

A handwritten signature in black ink, appearing to read 'Jeroen Kemerink', is written over a horizontal line.

Name : Jeroen Kemerink  
Vice President Research & Development





## PhenoTyper 2 digital camera - FCC Supplier's Declaration of Conformity

### FCC Supplier's Declaration of Conformity (No BD000560)



**Product Type:** Digital Camera  
**Series:** Basler ace GigE  
**Manufacturer:** Basler AG  
An der Strusbek 60-62  
D-22926 Ahrensburg / Germany [www.baslerweb.com](http://www.baslerweb.com)

**Authorised US Representative:** Basler, Inc., Exton  
855 Springdale Drive, Suite 203  
Exton, PA 19341  
USA

This declaration of conformity is issued under the sole responsibility of the manufacturer | authorised representative.

#### Object of declaration:

**Emission Class A Types:** acA640-90g\*, acA640-100g\*, acA640-120g\*, acA640-140g\*, acA645-100g\*, acA750-30g\*, acA780-75g\*, acA1280-60g\*, acA1300-22g\*, acA1300-30g\*, acA1300-60g\*, acA1600-20g\*, acA1600-60g\*, acA1920-25g\*, acA2000-50g\*, acA2040-25g\*, acA3800-10g\*, acA4600-7g\*

**Emission Class B Types:** acA640-121g\*, acA640-300g\*, acA720-250g\*, acA800-200g\*, acA1300-75g\*, acA1440-75g\*, acA1920-48g\*, acA1920-40g\*, acA1920-50g\*, acA2500-20g\*, acA2040-35g\*, acA2440-20g\*, acA2500-14g\*, acA3066-16g\*, acA4024-8g\*, acA4096-11g\*, acA4112-8g\*, acA5472-5g\*

\* represents placeholder combination zzzzzvvvvv, where:

zzzzz: placeholder up to 5 characters, indicates sensor subtype such as color ("m"= monochrome or "c"=color) or other spectral response  
vvvvv: optional placeholder up to 5 characters or symbols for product line or customized camera versions

The object of the declaration described above is in conformity with

FCC (2006) Part 15, Subpart B, Limit for digital devices, Class A and Class B

Signed for and on behalf of Basler Inc.

John Jenkins, Managing Director Americas  
Exton, January 10, 2023

Phone: +49 4102 463 0 · Fax: +49 4102 463 109 · [www.baslerweb.com](http://www.baslerweb.com)  
Management Board: Dr. Ing. Dietmar Ley (CEO) · Alexander Temme · Arndt Bake · Hardy Mehl  
Chairman Supervisory Board: Norbert Basler  
Companies' register of Luebeck local court (Amtsgericht Luebeck) · Commercial Registry Number 4090  
VAT ID: DE 135 096121 · WEEE-Reg.-No.: DE63888045

Template: Basler DNS A0001137 04

# Index

## A

- Acetic acid — 112
- Acetone — 112
- Acoustical stimuli — 14
- Acrylic — 108
- Action — 11
- Actions based on PhenoTyper — 11
- Airgap shield — 22, 117
- Alkaline substances — 111
- Analyzing data — 11
- Assembling PhenoTyper — 21
- Autoclave — 108
- Automatic gain control — 59

## B

- Backup — 46
- Bedding material — 113
- Behavior Recognition — 42
- Benzene — 112
- Benzyl alcohol — 112
- Bottle — 28, 112, 121
- Bottom of cage — 22
- Buzzer — 10

## C

- Cable connections
  - PhenoTyper 1 — 36
- Camera — 9

- PhenoTyper 1 — 58
- PhenoTyper 2 — 85

### Charts

- exporting — 131

- Cleaning the cage — 108

- CognitionWall — 104

- Condition — 11

- Conditioning test — 97

### Connect

- PhenoTyper 1 — 37, 40

- PhenoTyper 2 — 71, 74

- Control Unit — 116

- PhenoTyper 1 — 52, 53

- PhenoTyper 2 — 72

- Cover plate — 34

## D

- Declaration of Conformity — 126

- Documentation — 7

- Drinking bottle — 28, 112, 121

## E

- Ethanol — 112

- EthoVision XT

- general information — 6

- Ethylene oxide — 110, 112

- Experiments (multiple) — 49

- Extension cables — 116

## F

Feeder — 18, 31  
Focus (lens)  
    PhenoTyper 1 — 58  
    PhenoTyper 2 — 85  
Food receiving task — 98  
Frame grabber — 11

## G

Gamma radiation — 110

## H

Handling of subjects — 15  
Help — 115  
Hexagon key — 118, 120  
Home cage — 8, 18  
Hydrogen peroxide — 110, 112

## I

Illuminated shelter task — 101  
Import trials — 49  
Infrared light — 9, 51  
    copying settings — 55  
    PhenoTyper 1 — 51, 53  
    PhenoTyper 2 — 83, 84  
Infrared lighting — 122, 124  
Isopropyl alcohol — 111

## J

Jumper settings — 44

## L

LED arrays — 9  
Lens focus  
    PhenoTyper 1 — 58  
    PhenoTyper 2 — 85  
Lickometer — 50  
Light conditions — 6  
Light intensity  
    PhenoTyper 1 — 53, 54  
    PhenoTyper 2 — 84  
Light meter — 55, 85  
Light stimuli — 14  
Light-dark cycle — 10  
L-shape element — 27

## M

Manuals — 7  
Maximum trial duration — 94  
MediaRecorder — 43  
Methanol — 112  
Mixed video images — 41  
Model — 18  
Multiple experiments — 49

## N

Neoprene — 112  
Nesting material — 114  
Nortio  
    with PhenoTyper 1 — 38, 45, 61, 67  
    with PhenoTyper 2 — 72, 76, 89, 92

## P

- Pellet dispenser — 19, 50, 114
- Pellet receptacle — 19
  - cleaning — 108
- Pellets — 114
- Peracetic acid — 111
- Perspex/acrylic cleaner — 111
- PhenoTyper
  - assembling — 21
  - benefits — 14
  - communication with EthoVision — 10
  - configurations — 18
  - experiment — 108
  - general information — 6
  - maintenance — 108
  - setups — 11
- PhenoTyper 1
  - assign devices to arenas — 65, 67
  - cable connections — 35, 36
  - set up experiment — 60
- PhenoTyper 2
  - assign devices to arenas — 91, 92
  - cable connections — 69
  - set up experiment — 88
- PhenoWheel — 19
- Piccolo Alert encoder board — 37, 40, 43
- PMMA — 108
- Polycarbonate — 112
- Polymethylmethacrylate — 108
- Power cord — 36, 70
- Power supply — 116
- Protecting sheet — 22

## Q

- Quad unit — 41

## R

- Resolution
  - PhenoTyper 1 — 40, 42
  - PhenoTyper 2 — 74
- Restore backup — 46
- Rodent — 6

## S

- SDI Control port — 44
- SDI control port — 38, 44
- SDI devices — 50
- Separate video images — 43
- Shelter — 18, 30, 121
- Shutter speed — 59
- Sound intensity — 55
- Sound stimuli — 10
- Specifications — 122
- Stimuli — 10

## T

- Technical support — 115
- Testing
  - PhenoTyper 1 — 51
  - PhenoTyper 2 — 83
- Tools — 118
- Top Unit — 8, 116
  - cleaning — 113
- Top Unit Interface — 38, 44
- Trial Control — 11, 68, 93
- Trial Control Settings — 94, 97, 99, 102, 105
- TTL devices — 50
- TTL In — 38, 44
- Tube lamps — 51

## U

USB-IO box — 38, 44, 50, 72, 76  
    with PhenoTyper 1 — 38, 44, 61, 65  
    with PhenoTyper 2 — 71, 75, 89, 91  
USB-IO box jumpers — 44  
UV radiation — 111

## Y

Yellow light spot — 10, 53, 54

## V

Vaporized hydrogen peroxide — 110  
Ventilation holes — 18  
VHP — 110  
Video cable  
    PhenoTyper 1 — 36  
    PhenoTyper 2 — 70  
Video camera — 9  
Video images (mixing) — 41  
Video processor — 41  
Video resolution  
    PhenoTyper 1 — 42  
    PhenoTyper 2 — 42, 74  
Video tracking — 11  
Visual stimuli — 14

## W

Walls — 18, 117  
    protecting sheets — 22  
Water bottle — 121  
Wavelength — 122, 124  
White light (PhenoTyper 2) — 73, 84  
White light spot (PhenoTyper 1) — 10, 53, 54