Reference Manual

DanioVision™

Version DVOC-0041

Noldus

Information Technology

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For EthoVision XT version 16

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Contents

1 What is DanioVision?

DANIOVISION

DanioVision™ is a system for high throughput tracking of zebrafish larvae in multi-well plates. It consists of:

The DanioVision Observation Chamber (DVOC-0041)

See Figure 1. This chamber contains a transparent holder with backlight that fits an ANSI SBS compatible multi-well microtiter plate, infrared (IR) and white light sources (underneath the microtiter plate), and a Gigabit Ethernet (GigE Vision) video camera. The optical design provides a stable and undistorted top view of each well and a controlled and constant environment within the observation chamber.

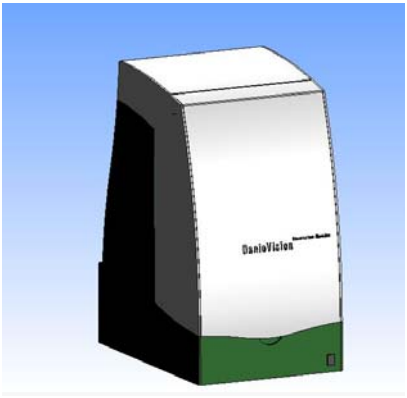


Figure 1 *The DanioVision Observation Chamber* **DVOC-0041**.

For technical specifications and for cleaning instructions, see **System specifications**.

TIP You find your DanioVision' version number on the label at the back of the chamber.

EthoVision® XT

Video-tracking software with the Multiple Arenas Module and Trial & Hardware Control Module. Using Trial & Hardware Control, you can adjust the white light stepwise based on time or behavior of your fish. You have the option to connect external devices, such as sound or shock generators.

NOTE DanioVision DVOC-0041 works with EthoVision XT 10.1 or newer.

DANIOVISION ADD-ONS

DanioVision Temperature Control Unit

With the DanioVision Temperature Control Unit you can set up a water flow at constant temperature underneath the well-plate. For details, see the Reference Manual - DanioVision Temperature Control Unit DV-TCU.pdf, which you can download from the Noldus website (see page 10).

DanioVision Tapping Device

With the DanioVision Tapping Device you can trigger a startle response in zebrafish. For details, see **The DanioVision Tapping Device** in this manual.

DanioVision Toplight Unit

With this add-on you can generate top-down light stimuli in your experiments. Four types of light are available: white, red, blue and green. For details, see **The DanioVision Toplight Unit** in this manual.

DanioVision with Optogenetics

DanioVision can be provided with one to three LEDs that illuminate the well plates based on your Trial Control protocol. The Optogenetics add-on allows to accurately control and time the application of optogenetic stimulation to up to 96 individuals simultaneously (one for each well in a 96-well plate). For details, see **The DanioVision Optogenetics add-on** on page 88.

Custom solutions

As research evolves, so do research needs. If you are interested in a custom solution, don't hesitate to contact us!

A few recent examples of custom solutions:

- **Light-Dark Grid**. Commonly used to investigate anxiety behavior in zebrafish. The grid is placed underneath the well plate (available for 6, 12, 24, 48 or 96 wells) and creates a dark and light half in each well. The grid is IR light translucent, leaving the tracking of the zebrafish unaffected.
- **High-speed camera**. DanioVision is equipped with a camera capable of 1000 frames per second. The aim is to measure the startle response of larvae to a stimulus during a Pre-Pulse Inhibition test (Burgess and Granato 2007).
- **Split basin**. The DanioVision basin has been modified so that it receives water at two different temperatures. The aim of this solution is to measure temperature preference in zebrafish larvae.
- **Ultraviolet Toplight Unit**. This is a modification of the Toplight unit (see the previous page), which shines UV light. Light intensity is controlled by EthoVision XT.
- **Turbo-cooling TCU**. This is a modified Temperature Control Unit which keeps water at a constant temperature of 12° in a room where temperature is about 25°.

FOR MORE INFORMATION

Manuals

For more information, see

- The EthoVision XT Help (press **F1** in the EthoVision XT).
- The Reference Manual - Trial and Hardware Control in EthoVision XT. You can find this manual under **Noldus** > **EthoVision XT 16 Other Documentation**.

- The Reference Manual - DanioVision Temperature Control Unit DV-TCU. You can find this manual on the Noldus website (my.noldus.com). Note that you have to log in to access the downloads page.

You can also find other manuals on the EthoVision XT installation USB stick.

Sample experiments

EthoVision XT download page you can find sample experiments that make use of DanioVision. Browse to my.noldus.com, there under **Downloads** choose **Sample projects**, then **EthoVision XT**.

Experiment templates

EthoVision XT contains templates that help you setting up an experiment with arenas, analysis profiles and other elements already defined. See **CREATE A DANIOVISION EXPERIMENT** on page 41.

References

See our web page of selected publications:

www.noldus.com/daniovision/resources

Support center

If after reading this manual you still have questions, please contact our Support center at my.noldus.com.

2 What's new in DanioVision

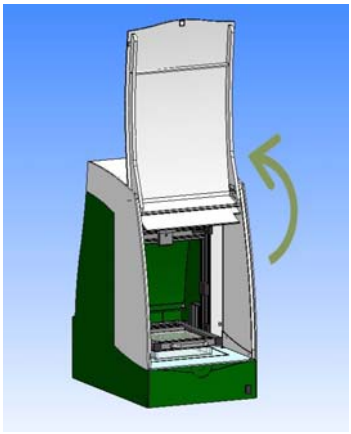
DANIOVISION OBSERVATION CHAMBER

For users of DanioVision DVOC-0040

The white light LED array has been improved. The White light can be set to higher intensity.

For users of DanioVision DVOC-0030

- The housing has been completely redesigned. You open the observation chamber by tilting the lid along the top side. This way you have easier access to the camera and opening/closing the lid produces less vibration.



NOTE The DanioVision Observation Chamber is designed to block light from the outside. However, it cannot be guaranteed that it is completely dark inside the Chamber. For experiments that require absolute darkness (like experiments with day-night rhythms), place the DVOC in a dark room.

- The housing is now larger to create more space for accessing the video camera or adding devices. For this purpose, the housing can

be disassembled more easily than before. For the instructions see page 116.

- The basin has been redesigned. It has now a more flexible retainer that can hold a wider range of well plates. DanioVision can now work with ANSI, SBS compatible micro plates (L =127.76± 0.5 mm, W=85.48 ± 0.5 mm) with a maximum height of 27 mm, and with Petri dishes with a diameter up to 90 mm. To place small well plates and petri dishes, slide the bridge to a new position in such a way that the well plate/petri dish is in the middle of the camera view (left). For details see **PLACE WELL PLATES AND PETRI DISHES** on page 37.

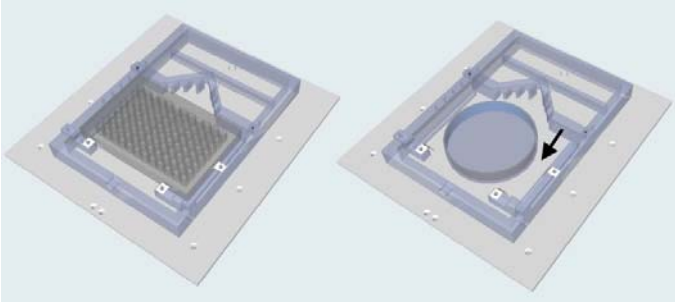


Figure 2 *Positioning a well plate (left) and a petri dish (right).*

- The mechanism for moving the Fresnel lens located above the well plate has been improved. You can now move the lens all the way out of the camera view when you need to place containers higher than 27 mm.
- Communication between DanioVision and EthoVision XT has improved via a new version of the hardware interface software. See **INSTALL ETHOVISION XT** on page 22.
- All four TTL lines **TTL 1** to **TTL 4** are available for communication with external devices. For connecting custom hardware, additional expansion connectors **Expansion 1** and **2** are available. See Figure 3 on page 15.

- The **White Light** is now dimmable at very low intensity, without the need to operate the Low light switch located behind the camera.
- The **DanioVision Tapping Device** has been designed to provide DanioVision with a reliable device which evokes a startle response in zebrafish larvae. See **The DanioVision Tapping Device** on page 72.

  NOTE The DanioVision Tapping Device is a separate add-on to the DanioVision system.

For users of DanioVision DVOC-002x

- The DanioVision camera is now a Gigabit Ethernet (GigE) digital camera connected to the EthoVision XT computer through a 1-Gb Ethernet card. With a GigE camera, tracking can be done with a higher sample rate and higher resolution.
- The intensity of the infrared LED back-lighting has been increased. This results in a more even distribution of back-lighting over the well-plate and a higher contrast between animals and the background. Therefore, it is easier to configure the detection settings and detection is better.
- It is possible to use very low light levels for the DanioVision White Light in addition to the default, standard White Light levels. The *standard* White light levels range from ±20 to ±10000 lux. The *low* White light levels range from 0 to ±20 lux.
- The anti-condensation mechanism can now be easily switched on and off, or set to automatic. The anti-condensation mechanism prevents the lens from condensing during an experiment.

ETHOVISION XT

Here the main additions relevant to DanioVision are listed.

For a complete list of the new features, see the EthoVision XT Help "What's new" section.

For users of EthoVision XT 15

- *Improved Subject Contour filter*. In the Detection Settings, a second Erosion filter is added to the **Subject Contour** settings. This filter is added after the pre-existing Erosion and Dilation, and improves detection in some difficult situations. For more information, see **Advanced detection settings: Subject contour** in the EthoVision XT Help.
- *Trial Control variable in the analysis*. In the Analysis profile you can now select the Trial Control variables used in the Trial Control protocols. This way you can analyze or visualize its values just like the other dependent variables. You can also visualize a Trial Control variable in the Integrated Visualization for testing purposes. For example, to check that a variable gets the expected value at a specific time in the trial.
- **NOTE** The Deep learning functionality in EthoVision XT 16 is not designed specifically to track fish larvae. In DanioVision experiments, always choose Contour-based as the body point detection technique.

For users of EthoVision XT 14

- Heatmaps are plotted together with a time scale. This helps you quantify the time that the subjects spend in specific locations.

3 Set up DanioVision

CONNECTIONS

The back panel of the DanioVision Observation Chamber contains the following connections (see Figure 3 below):

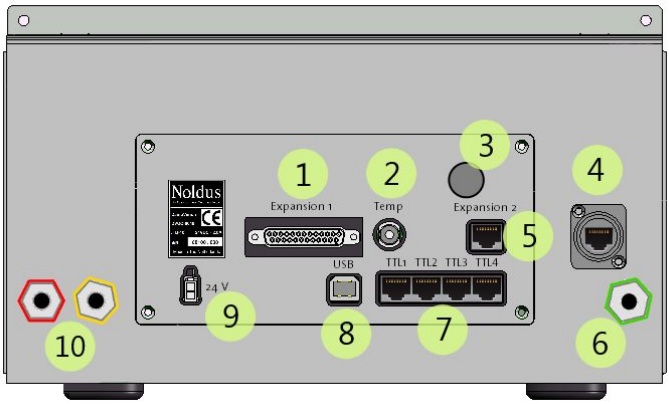


Figure 3 *The back panel of the DanioVision Observation Chamber. See text for explanation. The label with the CE marking contains the product version.*

1. **Expansion 1**. You can optionally use this connector to set up to 4 TTL out lines, to control external devices. For details, see page 127.
2. **Temp**. Connector for the temperature sensor. Use this in combination with the DanioVision Temperature Control Unit (TCU).
3. Cable grommet. If you install additional hardware inside the observation chamber, puncture the rubber membrane to let the cables pass through. For example, to connect the sensor of a digital thermometer placed in the water basin to a digital display outside the DanioVision Observation Chamber.
4. Gigabit Ethernet (GigE) camera connector. Use this output to connect the DanioVision camera to the EthoVision XT computer.

5. **Expansion 2**. This is a RJ45 type, 8-pin feed through connection. You can use it to connect future internal add-on hardware to get external signals in the DanioVision Observation Chamber.
6. Connector for water flow (see page 18 for details).
7. **TTL1** to **TTL4**. You can optionally use these TTL input/output connections to control external devices such as a sound or shock generator, or the DanioVision Temperature Control Unit.
8. **USB** port (type Standard-B) to connect the DanioVision Observation Chamber to the EthoVision XT computer.
9. Connection for the 24 V power supply.
10. Connectors for water flow (see page 18 for details).

CONNECT DANIOVISION

Make sure the DanioVision Observation Chamber is placed on a level surface!

Power

Connect the DanioVision Observation Chamber's **24 V** socket (**9** in Figure 3) to the power supply using the power cable and adaptor.

Video

Use the Ethernet cable with the large round connector to connect the digital video output of the DanioVision Observation Chamber (**4** in Figure 3) to the Ethernet port labeled **DanioVision camera** on the EthoVision XT computer.

The DanioVision camera must be connected to a 1-Gb Ethernet card. See page 23 for how to install this card on your computer. If you have purchased a PC from Noldus together with DanioVision, this card is already installed. Contact Noldus if you need a new Ethernet card for the DanioVision camera.

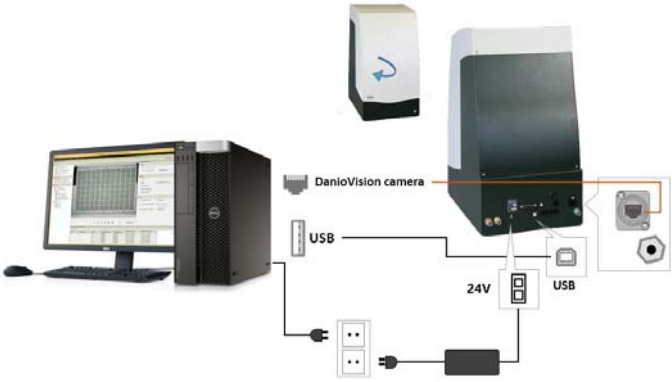


Figure 4 *Basic connections between the EthoVision XT computer and the DanioVision Observation Chamber. For controlling other devices like the Toplight Unit and the Temperature Control Unit, see the corresponding sections.*

White Light, Tapping Device, and other hardware

Use the USB cable that comes with DanioVision to connect the **USB** port of the DanioVision Observation Chamber (**8** in Figure 3) to a USB port on the EthoVision XT computer. Do this only if:

- You want to control the White Light or the Tapping Device in the DanioVision Observation Chamber during data acquisition in EthoVision XT.
- You have the DanioVision Temperature Control Unit and you want EthoVision XT to take action if the water temperature gets too low or too high.
- You want to control any custom hardware connected to the DanioVision Mini USB-IO box.

SET UP A WATER-FLOW SYSTEM

If you require a temperature-controlled water flow underneath the well plate, the Noldus DVTCU Temperature Control Unit is the preferred method.

Figure 5 *The DanioVision DVTCU Temperature Control Unit.*

The back panel of the DanioVision Observation Chamber contains three connections for controlling the water flow (see Figure 6): a water inlet (marked in green), a water outlet (marked in yellow) and a water overflow (marked in red).

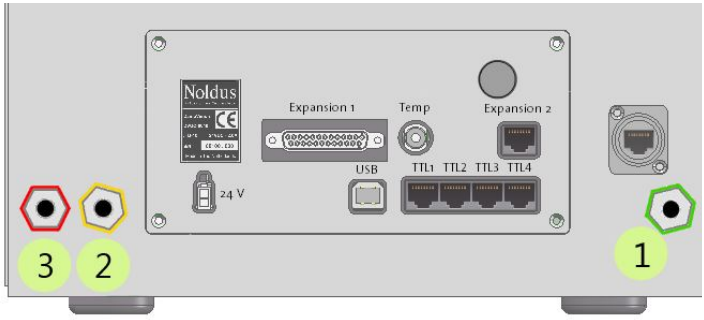


Figure 6 *The back panel of the DanioVision Observation Chamber, with the numbers indicating the connections of the water-flow system:*
1 *- water inlet;* **2** *- water outlet;* **3** *- water overflow.*

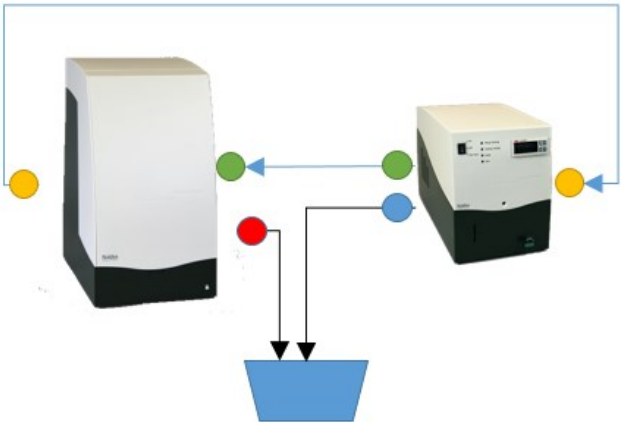


Figure 7 *Tube connections between the DanioVision Observation Chamber and the DanioVision Temperature Control Unit. Color dots represents colors on the tubes. Arrows indicate the water circulation.*

Connect the DanioVision Observation Chamber to the DVTCU using the tubes that match the color of the connectors. Please see the Reference Manual - DanioVision Temperature Control Unit DV-TCU for the complete instructions.

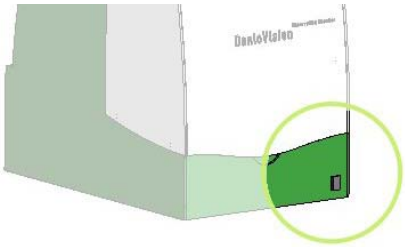
Suggested temperature

For *Danio* larvae, the temperature of the water flowing through the system is usually 28 degrees Celsius. You can set a different temperature when needed.

LIGHT AND SWITCHES

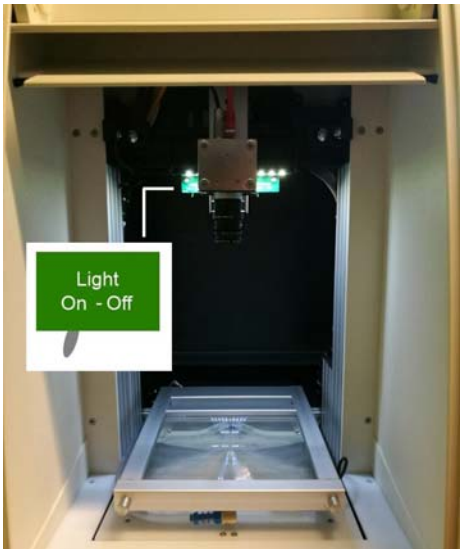
Main switch

The main switch is located on the front panel. After you switch on DanioVision, wait at least 15 minutes for it to 'start up' and to avoid condensation in the system.



Internal LED light

The DanioVision Observation Chamber is equipped with a LED light to illuminate the inside of the chamber while you set up your experiment.



Operate the **Light** switch at the left of the camera to turn the light on and off.

Anti-Moisture switch

This switch is located at the right of the camera. Operate this switch to adjust the anti-condensation mechanism (page 35).

Low light switch

This switch is located on the Mini USB-IO box, behind the camera at the back of the observation chamber.

- When the switch is set to **H** (default), the standard light levels are activated, from ±20 to ±10000 lux.
- Set this switch to **L** to allow very low light intensities, from 0 to ±20 lux. For details, see page 36.

INSTALL ETHOVISION XT

- If you have purchased the DanioVision Observation Chamber including a computer from Noldus Information Technology, all software has been installed and tested. You can skip this section.

See **Set up EthoVision XT** on page 41

- If you use your own computer (or a computer you bought from Noldus IT in the past; in that case, make sure it meets the EthoVision XT system requirements), see below.

EthoVision XT 16 is supported with Windows 10 Pro, 64 bit. For more information about system requirements, press **F1** in EthoVision XT.

Mini USB-IO box drivers

The Mini USB-IO box is the embedded electronics unit that communicates with EthoVision XT. The Mini USB-IO box makes it possible for EthoVision XT to control for example the white light stimulus.

Before installing EthoVision XT, remove older versions of the drivers for the Mini USB-IO box. To do so:

1. In the Windows Control Panel select **Programs and features**.
2. Click each of the items whose name begins with **Noldus_Hardware Interface..** and click **Uninstall**.
3. Insert the EthoVision XT Installation USB stick into your computer and follow the instructions to install EthoVision XT.

When you install EthoVision XT, the drivers of the Noldus Mini USB-IO Box are automatically installed.

IMPORTANT When you open an experiment from EthoVision XT 12 or lower, a warning appears, informing you that you cannot open upgraded experiments in the old version of the software anymore. Make a backup of your DanioVision experiment in the older version of EthoVision XT before opening it in EthoVision XT. To make a backup, choose **File** > **Make Backup**.

IMPORTANT If you uninstalled the older version of EthoVision XT, you cannot use the Backup function. Instead, make a copy of your

experiment using Windows Explorer before you open it in the newer EthoVision XT version. Always copy the complete experiment folder and not only the *.evxt file otherwise your experiment does not open.

For more information

See **Installation** in the EthoVision XT Help for instructions on how to install EthoVision XT.

For more information on the Mini USB-IO box, see the Reference Manual - Trial and Hardware Control in EthoVision XT, which you can find on your EthoVision XT computer, under **Noldus** > **EthoVision XT 16 Other Documentation**.

INSTALL THE NETWORK CARD FOR THE DANIOVISION CAMERA

DanioVision works with a Basler Gigabit Ethernet (GigE) video camera, connected via an Intel PRO/1000 CT 1-Gb Ethernet card installed on your computer.

If you ordered a computer from Noldus Information Technology when you purchased DanioVision, it came with that Ethernet card. The card has already been installed and tested. In that case, skip the sections below and continue with Chapter 4 on page 32. If you bought your computer somewhere else, you will have to install the Ethernet card yourself. This involves a few steps (see below).

Figure 8 *The Intel PRO/1000 CT Ethernet card.*

Step 1. Insert the Ethernet card in the DanioVision computer

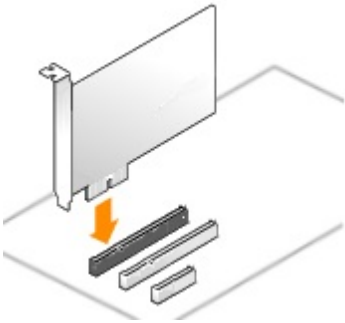
1. Turn off your computer and all connected peripherals, such as the monitor and printer. Make sure that the computer is unplugged.
2. Remove the PC's casing according to the instructions provided in the PC's user manual.
3. Select a free PCIe expansion slot, and remove the corresponding extension cover.

   When possible, choose the slot that gives maximum performance. To estimate performance, take note of the slot version (2, 3, etc.) and compare it with the following values:

   - PCIe v1.x: 250 MB/s
   - PCIe v2.x: 500 MB/s
   - PCIe v3.0: ~1 GB/s
   - PCIe v4.0: ~2GB/s
   - PCIe v5.0: ~ 4GB/s

   Note that values are given per lane; they should not be multiplied by the number of lanes in a slot (e.g. x4) since the card has a 1x connector). For example, SLOT1-PCIe3x4 means version 3.0, with four lanes.

   IMPORTANT Do not insert the PCIe board in the blue or white slots.
4. Unpack the Ethernet card, place it into the slot, and press it carefully into position. If the card does not fit into place easily, remove it and repeat the operation.



When touching the board, its electronic components can be damaged by static electricity. To avoid any such risk, make sure that you are grounded. You can ground yourself by putting on an earthing wristlet, and attaching its clip to the metal frame of the computer. If an earthing wristlet is not available, you can hold the metal frame with one hand while holding the Ethernet card in your other hand. Ensure also that your clothing does not touch any components while handling the card.

5. Fix the card to the chassis and re-fit the computer's cover.

   For more information about installing the camera and the Ethernet board, see **Camera Installation** in the EthoVision XT Help.

Step 2. Install the camera drivers

1. Insert the EthoVision XT installation USB stick into your computer.
2. Double-click EthoVision XT Setup.exe. If you have already installed EthoVision XT, select Modify.
3. Under **Drivers and tools**, select **Basler GigE camera driver**.
4. Continue with installation. Under **Profile**, select **Camera User**, then click **Next**.
5. Under Interfaces, select **GigE**, then click **Next**.
6. Connect the DanioVision camera to the port of the Ethernet card using a network cable (see **D** in Figure 3 on page 15).

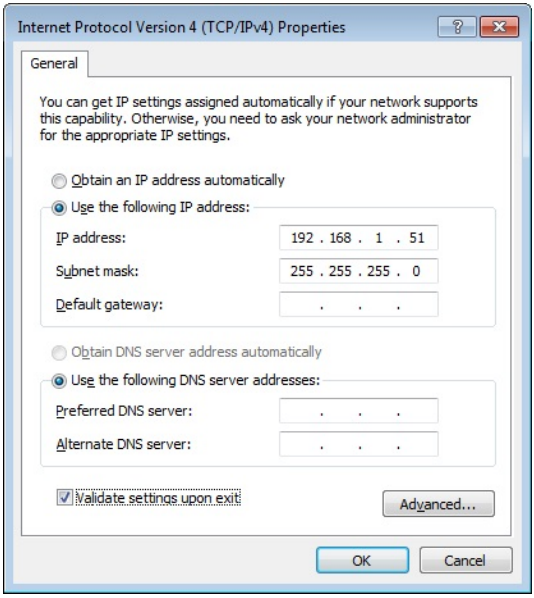
Step 3. Assign the IP address to the Ethernet card

TIP Watch the video tutorial **Set Up the Camera**. In EthoVision XT, choose **Help** > **Video Tutorial**.

1. In Windows (here version 10), search for **Network Connections**.
2. Right-click the **Local Area Connection** and click **Properties**. If your computer has more than one Local Area Connection, choose **Basler GigE Vision Adapter**. Write down the number of this connection.

3. Select **Internet Protocol Version 4 (TCP/IPv4)** and click **Properties**.

4. Select the options: **Use the following IP address** and **Use the following DNS server addresses** and fill in the details as shown in the next figure.



Also select the checkbox **Validate settings upon exit**. When done, click **OK** and then **Close**.

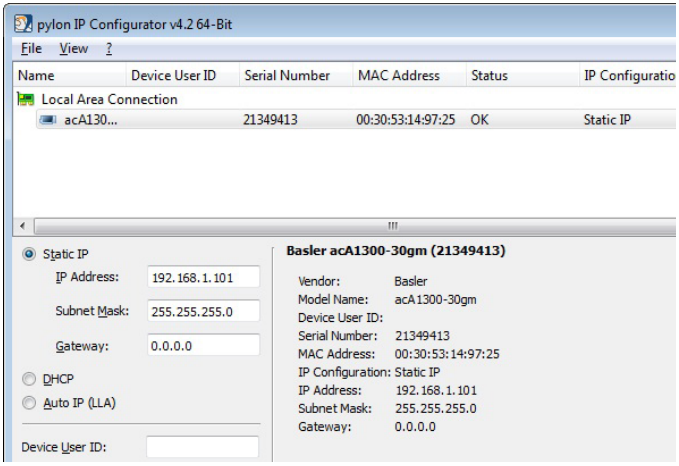
Step 4. Set the IP address of the DanioVision camera

TIP Watch the video tutorial **Set Up the Camera**. In EthoVision XT, choose **Help** > **Video Tutorial**.

1. In Windows, search for **IP Configurator** and start this application.

2. Select the camera.

3. Fill in the details in the **IP Address** and **Subnet Mask** fields under **Static IP Address** as shown in the figure below.



4. Click **Save** and then **Close**.

5. Follow the instructions below to configure the DanioVision camera.

CONFIGURE THE DANIOVISION CAMERA

Camera settings

The DanioVision camera that you received is already configured according to your setup. Noldus created three main settings profiles, called *User Sets*, stored in the **Pylon Viewer** software which controls the camera: **User Set 1**, **User Set 2** and **User Set 3**. Only one User Set can be active at a time.

For each video resolution, you must use different camera settings and in some cases a different camera lens. For example, the video resolution determines the maximum frame rate. The higher the required resolution (in number of pixels), the lower the maximum frame rate (in frames per second). The three User Sets are specific for a combination of video resolution and frame rate (Table 1).

Which User Set is currently selected?

Make sure that EthoVision XT is closed. Start the **Basler Pylon Viewer** software and in the **Features** panel (for details, see the procedure in the next pages), under **Configuration Sets**, look for the item **Default Startup Set**. This is the User Set that contains the current settings.

Always check that the settings in Pylon Viewer for a particular User Set correspond to those in the table!

Note

EthoVision XT reads the settings from the camera driver software. For a specific EthoVision XT experiment you can override the values of video resolution and frame rate values that are stored in the driver, not other settings; see page 45.

The maximum frame rates have been measured with a 96 well plate setup. Other values may be optimal in other setups.

Table 1 *Overview of predefined User Sets in the camera driver software and associated video frame rate, video size and required camera lens.*

	Resolution and maximum frame rate	1280 x 960, 30 fps	640 x 480, 60 fps	800 x 600, 60 fps
	Camera lens	12 mm	12 mm	8 mm + 0.5 mm spacer ring
Settings in Pylon Viewer				
A	**User Set**[1]	User Set 1	User Set 2	User Set 3
B	**Pixel Format**	Mono 8	Mono 8	Mono 8
C	**Analog Gain**	**1**	**0**	**1**
D	**Center X** and **Center Y**	✖	✖	✖
E	**Horizontal binning**, **Vertical binning**[2]	1, 1	2, 2	1, 1
F	**Exposure TIme (Abs)** in microseconds [3]	4000	2000	4000
Settings in EthoVision XT				
G	**Resolution**	1280 x 960	640 x 480	800 x 600
H	**Frame rate** (fps)	30	60	60
I	**Color space**	Y800	Y800	Y800

[1] User Set 1 is the default User Set in most applications. User Set 2 is pre-selected if you purchased a 12-mm lens. User Set 3 is pre-selected if you purchased a 8-m lens.

[2] For User Set 2, Binning mode must be **Summing**. For other User Sets either option has no effect with binning equal to 1.

[3] For all User Sets: **Enable Acquisition Frame Rate** must be selected. 

Adjust the camera settings in EthoVision XT

In EthoVision XT, choose **Setup** > **Experiment Settings** and click the camera button. Adjust the settings according to the table on the previous page.

For details, see **Adjust camera Settings in EthoVision XT** in the EthoVision XT Help.

Adjust the camera settings in Pylon Viewer

You can also adjust the camera settings directly in the camera software Pylon Viewer. Adjust the settings according to the configuration you want to use in **Table 1**. First, choose a video resolution and frame rate combination, then mount the recommended camera lens, and next make the appropriate settings in Pylon Viewer. Finally, adjust the appropriate settings in EthoVision XT.

For more information about Pylon Viewer, see the **Configure the digital camera** in the EthoVision XT Help.

- Pixel format (column **B** inTable 1).
- Analog gain (column **C** in Table 1).
- Center X and Center Y options (column **D** in Table 1).
- Horizontal and Vertical binning (column **E** in Table 1).
- Acquisition frame rate and Exposure time (column **F** in Table 1).

TIP To preview the camera image, click the **Continuous Shot** button on the toolbar. 

TIP You can increase the **Analog gain** to **2** if you need a brighter camera image. However, the image noise will also increase, which may influence tracking.

To save the settings, click the **Stop** button and open the **Configuration Sets** item. From the **Configuration Set Selector** list, select the User Set you want to save the settings to. Next to **User Set Save**, click **Execute**. Select the User Set you want to use at a daily 

basis. From the **Default Startup Set** list, select the User Set of your choice.

Camera view

Check that the camera view is correct by placing a wellplate in the DanioVision Observation Chamber.

Make sure that:

- The well plate is in the middle of the field of view, not rotated or skewed. If not, see page 125.
- The well plate is in focus. If not, operate the focus ring of the camera lens.
- Fixation screws of all rings on the lens are tightened.
- The aperture is chosen such that the image appears slightly overexposed. With water in the well plate, the image is fine.

When you create an EthoVision XT experiment, check that the video Resolution, Video frame rate and Color space are as in Table 1 (page 29) for the User Set you want to use.

See the next chapter and also **Fine camera adjustments** on page 122.

4 Check the DanioVision system

Before you start setting up EthoVision XT, you need to check the following:

Camera

- The camera lens should have the correct aperture and must be in focus.

See page 33

- The camera is correctly configured.

See page 28

Anti-condensation mechanism

- You need to do this depending on the temperature inside the DanioVision chamber and the desired water temperature around the well-plate.

See page 35

White Light stimulus

- You need to do this when you want to use very low intensities for the DanioVision White Light.

See page 36

Well plates and Petri dishes

- The well plate or Petri dish must be inserted correctly.

See page 37

- Always control evaporation in the basin and in the well plate, especially when you carry out long trials (> 1 day).

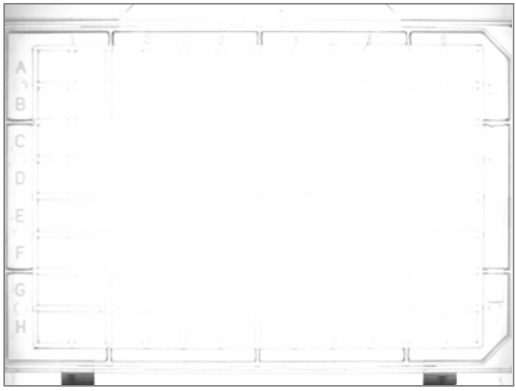
See page 40

CHECK THE CAMERA LENS

Although the camera and lens are set up before shipment, the settings of the lens can shift during transport. Before you start to work with the DanioVision system, please check that the lens is set up correctly by doing the following:

1. The lens you use depends on the video resolution and frame rate you want to use. See the table on page 29.
2. Insert a well plate (o petri dish) in the basin.
3. Create a new EthoVision XT experiment (see **CREATE A DANIOVISION EXPERIMENT** on page 41) and open the Arena Settings to see the camera image.

   When water is not present in the basin, the image of the well plate should look overexposed, like the following:



4. Check the focus (see Figure 9). If the camera image is not in focus, loosen the screw, adjust the focus and tighten the screw.

   The camera should be focused on the animals. The distance between animals and camera does not change much between types of well-plate and water level in the wells, however it is always a good idea to check the image before an experiment and adjust the focus until it is optimized.

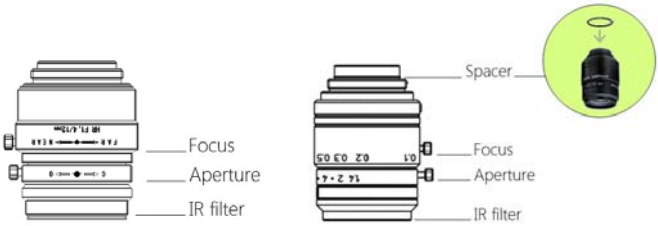


Figure 9 *The 12-mm standard camera lens (left) and the 8-mm camera lens (right). The 8-mm lens has a spacer ring (already installed if you purchased this lens from Noldus). See also Table 1 on page 29 for which camera settings apply to which lens.*

5. Check the aperture (see Figure 9).

   The aperture has been adjusted in such a way that when the basin is filled with water, the video image is optimal for tracking (see **EXAMPLE OF A GOOD VIDEO IMAGE** on page 126). However, in the Detection Settings (page 64) check that detection of the larvae is good. If necessary, adjust the aperture to get a better contrast between larvae and background.

6. Make sure the camera is correctly configured (see page 28).

Notes

The more the aperture is closed, the larger the depth of field, which means that an object is 'sharp' at different distances from the lens. This is especially relevant when you work with a zoom lens and zoom in to focus on a smaller region of a well plate. On the other hand, closing the aperture decreases the contrast between animals and the background. Adjust the aperture until the image is optimized.

CONFIGURE THE ANTI-CONDENSATION MECHANISM

Anti-condensation switch

The DanioVision Observation Chamber is equipped with an anti-condensation mechanism to prevent water droplets forming on the lens during an experiment. A temperature sensor inside the DanioVision Observation Chamber checks whether the anti-condensation mechanism should be switched on or off when it is set to **Auto** mode. Besides the **Auto** mode, you can also permanently switch the anti-condensation mechanism **On** or **Off**. The switch to set the anti-condensation mode is located behind the camera, at the right side.

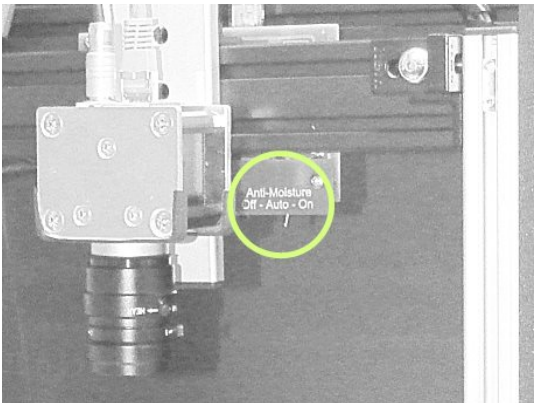


Figure 10 *Position of the anti-condensation switch (labeled: Anti-Moisture) inside the DanioVision Observation Chamber.*

Auto

Default and recommended when the DanioVision TCU is **not** used. The anti-condensation mechanism is automatically switched on when the air temperature inside the chamber drops below 28 °C (82.4 °F) to

prevent condensation on the lens. When the air temperature inside the chamber exceeds 28 °C, the anti-condensation mechanism is automatically turned off to prevent heating of the water in the wells.

The value of 28 °C is a default setting of the embedded controller.

NOTE If you use the DanioVision TCU, follow the instructions on the Reference Manual - DanioVision Temperature Control Unit DV-TCU, and set the switch to either **On** or **Off**.

Off

The anti-condensation mechanism is permanently turned off. Use this mode when the air temperature inside the chamber is equal to or higher than the actual water temperature of the basin/well plate water (in most cases 28 °C). You can use this option in combination with the DanioVision TCU.

On

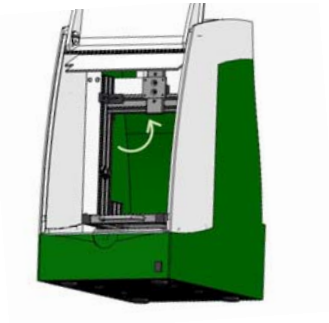
The anti-condensation mechanism is permanently turned on. Use this mode when the air temperature inside the chamber is lower than the water temperature in the wells. You can use this option in combination with the DanioVision TCU.

CONFIGURE THE DANIOVISION WHITE LIGHT

In the DanioVision Observation Chamber it is possible to use very *low light* levels for the DanioVision White Light in addition to the default, *standard* white light levels. The *standard* light levels range from ±20 lux to ±10000 lux. The *low light* levels range from 0 to ± 20 lux.

Both *standard* and *low light* can be controlled in the same way by EthoVision XT's Trial and Hardware Control.

1. Locate the switch, behind the camera, at the bottom-left of the black mini USB-IO Box.

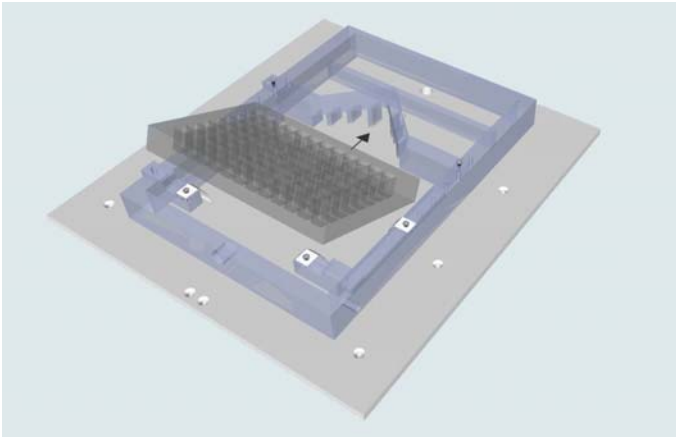


2. Set the intensity of the White light.
   - Right position **H**, standard high light level.
   - Left position **L**, low light level.

PLACE WELL PLATES AND PETRI DISHES

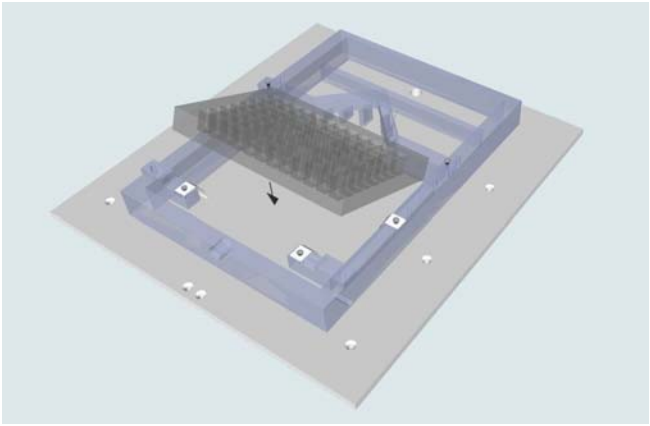
Well plates (ANSI standard)

1. Put the back of the well plate against the bridge and place it on the basin floor.



2. Tilt the front of the well plate down to the floor. This way the water in the basin pushes the air from underneath the well plate. Make sure the well plate is accommodated correctly between the metal springs at the front and at the right side.

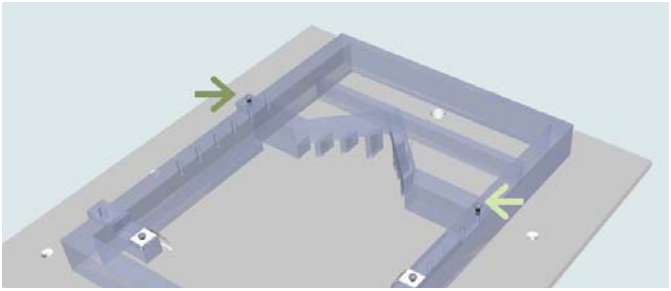
   TIP Drill a few holes on the sides of the well plate to allow air to escape when placing the well plate.



Well plates (non-ANSI standard)

For smaller or larger well plates, place the bridge in a different position. The default position (ANSI standard) corresponds to the *second* hole on the side walls of the basin, when counting from the inner side of the observation chamber.

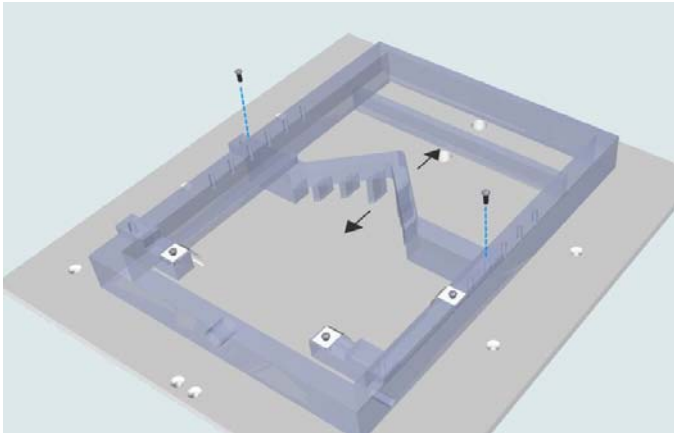
1. Remove the screws as indicated in the picture.



2. Slide the bridge by one or more positions.

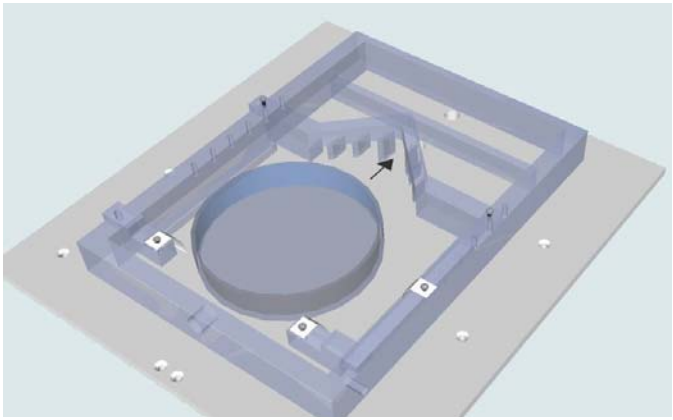
   Check the video image in EthoVision XT, and make sure that the well plate is centered in the camera view.

3. Fix the screws in the corresponding holes.



Petri dishes

1. Put the Petri dish against the 90° angle of the bridge.



2. If necessary, remove the screws of the bridge (see above) and slide the bridge of a few positions until the dish is centered in the camera view.

With DanioVision you can use Petri dish of diameter up to 90 mm.

We recommend not to let water flow in the basin when using Petri dishes. Water may flow underneath the bottom of the dish, making it shift from its original position during a trial.

CONTROL EVAPORATION

Wells on the sides of the well plate tend to show a higher rate of evaporation than wells in the center. Also, wells at the front have a higher evaporation rate than those at the back.

Our recommendations

In long experiments (> 24 h), evaporation may occur for both the wells in the plate and the basin.

- Wells: Check the wells every 24 hours and if necessary, refill them.
- Basin: Refill the basin every 2-3 days using the Basin Refill tool. For details, see the Reference Manual - Temperature Control Unit DV-TCU.

The environmental conditions (temperature, relative humidity and air flow) and experimental conditions (lens heater on/off, White light intensity) influence the rate of evaporation (and condensation) of water from the basin and well plate. If you plan to carry out long experiments, always test evaporation at your environmental and experimental conditions, and plan refills to make sure that water drop never reaches a critical value.

TIP You can use a special syringe to refill the basin without having to open the DanioVision Observation Chamber. Contact Noldus for more information.

5 Set up EthoVision XT

PREREQUISITES

Before you can start working with the DanioVision system, you need to connect the DanioVision Observation Chamber to the EthoVision XT computer (see page 15) and, optionally, set up the water-flow system (see page 18). After you have turned on the DanioVision Observation Chamber with the switch on the front panel, wait at least 15 minutes for the DanioVision chamber to 'start up'.

Also make sure to remove the lens cap from the camera.

Assign treatments to wells

To prevent systematic errors as a result of potential variation in temperature, light intensity or other unknown variable across a well-plate, it is recommended to either assign larvae from different treatments randomly to the wells or make sure that wells containing different treatments (that is, different solutions or different concentrations of the same solution) are evenly distributed across the well-plate (so, for example, one type of solution is not only put in the center wells).

CREATE A DANIOVISION EXPERIMENT

Preferred method

With this method, All arenas are created automatically, but need to be manually adjusted, using the **Arrange Arenas** option. Unnecessary functionality like manual scoring is removed from the interface and detection settings are optimized for DanioVision.

If you select a template which contains more arenas than needed, you can exclude the unused arenas with Data selection (see page 106).

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

1. Do one of the following:
   - In the **EthoVision XT** Startup window, under **New experiment**, click **New from template**.
   - Choose **File** > **New from Template**.
2. In the **Select a template option** window, click **Apply a pre-defined template**. Next, follow the instructions in the guided setup as described below.
3. **Which video source will you use?**

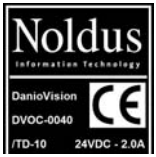
   Select the option **Live tracking (and saving video files)**, click the **Sources** button and select the Basler camera. Click **Next >**.
4. **Which subjects will you track?**

   Select **Fish** and then **Zebrafish larvae** from the list. Click **Next >**.
5. **How is the arena configured?**

   Select an Arena template from the list, then click **Next >**.
   - **DanioVision DVOC 004x, 24 round wells**.
   - **DanioVision DVOC 004x, 48 round wells**.
   - **DanioVision DVOC 004x, 48 square wells**.
   - **DanioVision DVOC 004x, 96 round wells**.
   - **DanioVision DVOC 004x, 96 round wells**.
   - **DanioVision DVOC 004x/T, 24 round wells**. *
   - **DanioVision DVOC 004x/T, 48 round wells**.
   - **DanioVision DVOC 004x/T, 48 square wells**.
   - **DanioVision DVOC 004x/T, 96 round wells**.
   - **DanioVision DVOC 004x/T, 96 round wells**.

     *) Select one of the options with **/T** when you have installed the DanioVision Tapping Device and you want to use it in this experiment. For details see page 72.

     TIP To know which DVOC version you have, see the **serial number** on the label located on the back panel.



   - **Open field, round**.
   - **Open field, square**.

- **Well plate, round wells** - Select this option if you have a different number of round wells.
- **Well plate, square wells** - Select this option if you have a different number of square wells.

NOTE Arena templates are also present for older DanioVision DVOC versions: **DanioVision DVOC 001x -003x**.

NOTE If you selected a template for an older DanioVision version, it is possible that the **DanioVision** detection settings method does not give optimal detection of the larvae with images from your camera. If that is the case, use another detection method in the **Advanced** section of the **Detection Settings** pane. Consult the DanioVision manual that came with your setup, or see **Configure Detection Settings** in the EthoVision XT Help.

6. **Initialize template experiment**. Check here that all settings are correct and, if so, click **Finish**.
7. Enter the name of the experiment and click **OK**.

   The **EthoVision XT** Overview window opens.
8. Follow all the next steps:
   - **Experiment Settings**. See page 45.
   - **Arena Settings**. See page 49.
   - **Trial Control Settings**. See page 57.
   - **Detection Settings**. See page 64.

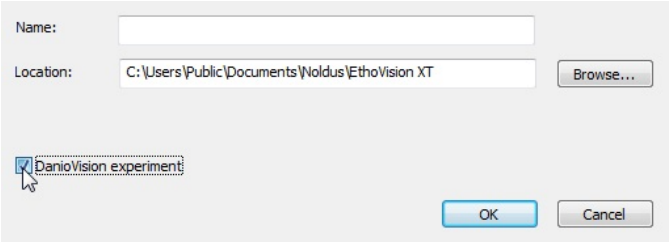
Other ways to create a DanioVision experiment

No matter which method you choose, when you create a DanioVision experiment unnecessary functionality like the Manual Scoring Settings is removed from the interface and detection settings are optimized for DanioVision.

- **File** > **New from Template** > **Apply a custom template**. Choose this method to create an experiment based on an existing one. For example when you want to replicate an experiment with the same number of arenas, the same trial control procedures, etc. For details,

see **Create a new experiment based on an existing experiment** in the EthoVision XT Help.

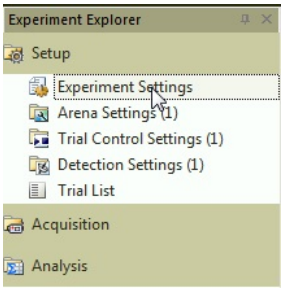
- **File** > **New** and in the window that appears select the option **DanioVision experiment**.



In the Experiment Settings, next to **Number of Arenas** enter the number of wells. With this option you have to draw the arenas manually. For more information on creating an experiment manually, see **Set Up an Experiment** in the EthoVision XT Help. Instead, we advise to create an experiment with a predefined template (first method), because all arenas are then defined automatically.

EXPERIMENT SETTINGS

Choose **Setup** > **Experiment Settings**.



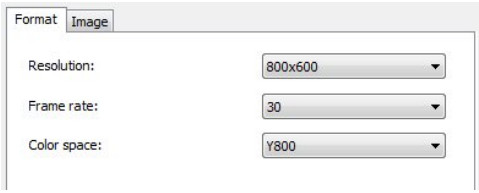
Video Source

1. Under **Video source**, click the **Video** icon on the right side of the row of your camera.

2. In the **Video Settings** window that appears, click the **Formats** tab and select the **Resolution** and **Frame rate**. Default resolution is **1280 x 960** at **30** frames/s.

   If you create an experiment based on a template with the Tapping Device, set the frame rate to 60 fps and reduce the resolution according to your setup (see the table on page 29).
   See also the guidelines in columns **G**, **H** and **I** in Table 1 on page 29.

   From the **Color space** list, choose **Y800**. This is the color space for a monochrome video image. The other options, suitable for color images, do not apply to DanioVision.



Tracked Features

Specify whether you want to track the center point of the fish (default) or the center point, the nose point and the tail base. Choose the latter option only if you track fish in a close-up image.

Body Point Detection Technique

The **Contour-based** technique is selected. The option **Deep learning** is not available for fish tracking.

Analysis Options

If you are interested in how long and how frequent your subjects have been active vs. inactive, under **Analysis Options** select **Activity analysis**. Make sure you also adjust the **Activity settings** in the Detection Settings (see page 66).

For more information about setting up an experiment, see **Set Up an Experiment** in the EthoVision XT Help.

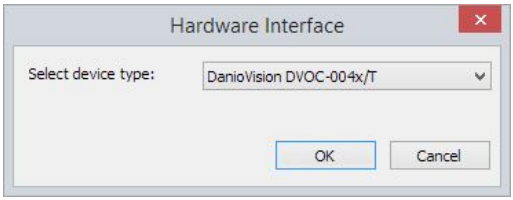
USE OF TRIAL CONTROL HARDWARE

Follow this section if you use stimulus devices like the white/color lights and the tapping stimulus device.

1. In the Experiment Settings, under **Trial Control Hardware**, make sure that **Use of Trial Control hardware** is selected and click the **Settings** button.

2. In the **Hardware Interface** window that opens, choose the type of system you have.

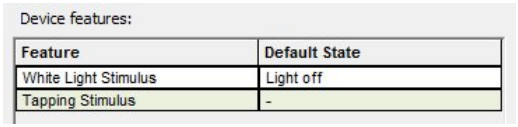
   If you have DanioVision DVOC-0041:
   - Choose **DanioVision DVOC-004x/T** if you use the Tapping Device in this experiment.
   - In all other cases choose **DanioVision DVOC-004x**.



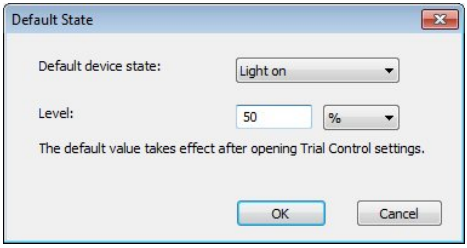
The other options **DanioVision DVOC-001x** - **003x** are meant for older versions of the DanioVision Observation Chamber.

NOTE The option **Mini USB-IO Box** only applies when you use a Mini USB-IO box instead of DanioVision DVOC.

3. In the **Device Configuration** window, under **Device features**, the hardware devices activated for this experiment are listed.



4. **OPTIONAL** The default status of the DanioVision White Light Stimulus if **Light off**. This means that, when you start a trial, the white light is switched off. To use the white light as a *stimulus*, program its activation with Trial Control. If, however, you want to use the white light as a *default lighting condition*, and use the darkness as a stimulus, do the following:
   - **a** Click the **White Light Default** button.
   - **b** Under **Default device state**, select **Light on**.
   - **c** Choose a percentage of intensity (0-100) and click **OK**.



Then in the Trial Control procedure you can program when to switch the light off. See **Trial Control Settings** on page 57.

5. **OPTIONAL** If you connect additional hardware, under **Plug-in devices**, select the devices in the corresponding rows for **TTL Port 1-4**, or **Expansion 1 / Port 1-4** depending on which port on the DanioVision back panel you use.
6. Click **OK**.
7. **OPTIONAL** If you set the default state of the White Light Stimulus to **Light on**, open the Trial Control Settings. This will confirm your selection.

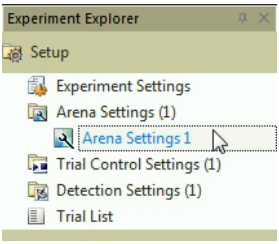
Notes

- The **Tapping Stimulus** is listed under **Device features** when you select **DanioVision DVOC-004x/T** in step **2**.
- If you upgraded from an older version of EthoVision XT, and you use an experiment created in the older version as a template for a new one, the **DanioVision** detection method is not available. Therefore, we recommend to create a new experiment with the newest version, and use this as a template (**File** > **New From Template** > **Use a custom template**).
- If you upgraded an experiment from an older EthoVision XT version, do not change the Trial Control Hardware Settings in the Experiment Settings. Leave **Noldus Mini USB-IO Box** selected in the **Hardware Interface** window. Otherwise the Trial Control rules, for example to control the white light, may not work.
- When you change the default state of the White Light Stimulus to **Light on**, note that the white light is only switched on in one of the following cases:
  - When you open the **Trial Control** screen.
  - When you open the **Arena - Hardware Mapping** window.
  - When you start a trial.
- For more information on Trial and Hardware Control, see the Reference Manual - Trial and Hardware Control in EthoVision XT.

6 Arena Settings

DEFINE THE ARENAS

Choose **Setup** > **Arena Settings** > **Arena Settings 1**.

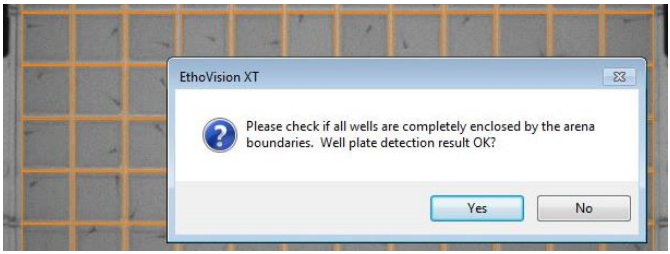


To define the arenas automatically

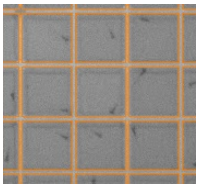
Follow these instructions if your experiment was created for well plates with 6, 12, 24, 48 or 96 round wells, or 96 square wells, and this is the first new Arena Settings in your experiment. In other cases, go to **To define the arenas manually** on page 52.

TIP Use well plates with no notches between wells. These result in better well detection. Also make sure that the borders of all wells are within the camera image.

1. In the **Grab Background Image** window, click the **Grab** button to grab a background image of an empty well-plate from the camera image.
2. If EthoVision XT can detect the wells, the outline of the arenas are displayed over the background image. A message appears asking whether the arenas overlap with the wells in the image.

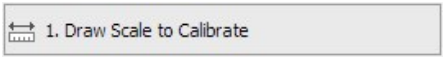


3. Do one of the following:
   - If there is good overlap like in the picture below, click **Yes**.

     If needed, you can always make small adjustments using the **Arrange Arenas** function (see page 53).
   - If the arenas clearly do not overlap with the wells, or arenas overlap with each other, click **No**. The **Arrange Arenas** window appears automatically. See ***arrange the arenas*** on page 53 for details.

4. Click the **1. Draw Scale to Calibrate** button.

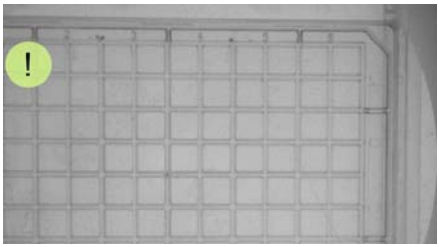
   

   Draw a line over the entire well-plate. Next, enter its size. Do this horizontally as well as vertically.

5. Click **Validate Setup** to validate the Arena Settings. Common errors are overlapping arenas, or zones falling outside an arena. If the setup is valid, continue with the instructions below.

Notes

- Arenas are marked with labels **A1**, **A2**, ..., **B1**, **B2**, ... according to your well plate (letters indicate rows; numbers indicate columns)
- Automatic detection of the wells in the video image has been tested with the DVOC-0040 and DVOC-0041. Success of automatic detection much depends on image contrast, focus, and distortion, and therefore we cannot guarantee it will work in all situations and with video made with previous DanioVision versions. Automatic detection also does not work if water is not present in the well plate.
- If automatic detection of the wells does not work, it could be due to the well plate being not centered in the video image.



See **Fine camera adjustments** on page 122.

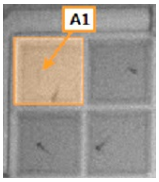
- When the well plate image is optimal and centered, and still EthoVision XT shows the message **Well plate detection failed**, click **OK** and proceed with ***To define the arenas manually*** on page 52.
- To start automatic well plate detection, click the **Well plate detection** button  at the bottom of the **Arenas and Zones** section of the **Arena Settings** pane.

To define the arenas manually

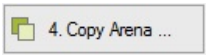
The following instructions apply when the automatic well plate detection does not work properly. In that case you must define one arena and then make duplicates.

If you used a predefined template to set up your experiments, go to ***arrange the arenas*** on page 53.

1. Click the **1. Draw Scale to Calibrate** button, draw a line over the entire well-plate and enter its size. Do this horizontally as well as vertically.
2. If the current arenas are of shape different from that of the wells, select all arenas and press **Delete**.
3. Click **2. Select Shape and Draw Arena** and use one of the shapes to create the first, top-left, arena. Make sure the label of the first arena (for example **A1**) points to the inside of the drawn arena.



4. Click the outline of the arena or its label and then click the **4. Copy Arena** button.

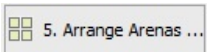


   In the window that appears select **All other arenas**. Select the number of rows and click **OK**.

5. Make sure the predefined arenas cover the wells. If you need to adjust the arena size and position, see ***arrange the arenas*** on page 53.

ARRANGE THE ARENAS

1. in the **Arena Settings** window, click the **5. Arrange Arenas** button.



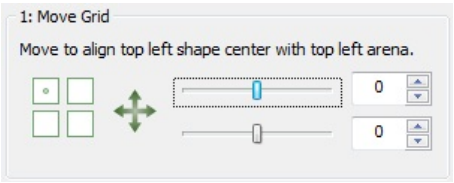
In the **Arrange Arenas** window, do the following:

2. *To move all arenas*: Under **1. Move Grid**

To move up/down: Click the first slider. Move it the right (to move the arenas down) or left (to the arenas move up).

To move to the right/left: Click the second slider. Move it the right (to move arenas to the right) or left (to move them to the left).

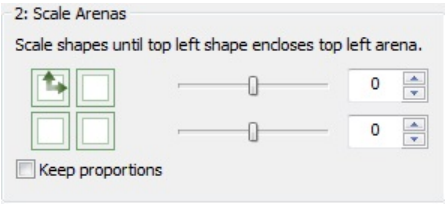
You can also click the up/down arrow buttons of the corresponding box or enter a number in the box.



3. *To scale all arenas:* Under **2. Scale Arenas**

Resize all shapes by using the sliders, by pressing the up/down arrows of the corresponding box or by entering a number in a box.

To increase the height of all arenas, use the first slider. To increase the width of all arenas, use the second slider

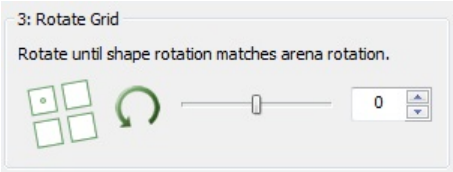


By default the option **Keep proportions** is not selected. Then you can resize the height and width of all shapes separately. If you

select **Keep proportions**, you can change either the height, or the width while keeping the aspect ratio.

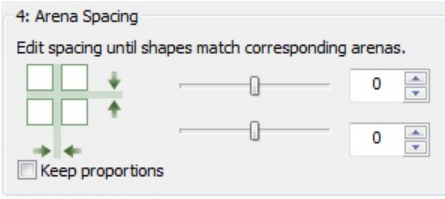
4. *To rotate all arenas:* Under **3. Rotate Grid**

   Optionally, rotate all shapes. This is only necessary if the well-plate is not positioned straight. The shapes rotate around the center of gravity of Arena 1.



5. *To change the space between arenas:* Under **4. Arena Spacing**

   Change the space between the shapes.



   By default, the option **Keep proportions** is not selected. Then you can change the space between the shapes separately in the vertical and horizontal direction (relative to the first row and first column, respectively). If you select **Keep proportions**, change the spacing in either the horizontal or vertical direction while keeping the aspect ratio.

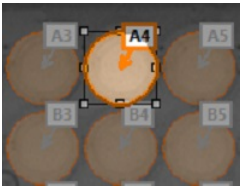
   TIP First position the upper left arena in the desired position and make desired size (use scale). Next adjust arena spacing so all arenas are correctly positioned. See **Using the Multiple Arena Setup** in the EthoVision XT Help for information on setting up multiple arenas.

6. Click **Validate Setup** to validate the Arena Settings. Common errors are overlapping arenas, or zones falling outside an arena. If the setup is valid, continue with the instructions below.

To edit a single arena

Click the row in the Arena Settings pane for that arena.

The corresponding arena is highlighted.



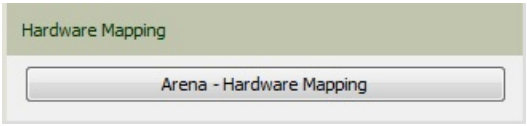
For details on editing shapes, in the EthoVision XT Help see **Arena Settings** > **Work with shapes**.

ASSIGN THE STIMULI TO THE ARENAS

Follow this procedure if you want to apply stimuli during a trial, for example a light pulse or a tapping stimulus.

IMPORTANT If you created an experiment with a pre-defined template and selected the correct DanioVision arena template, the White Light, or Tapping Stimulus has been assigned automatically to the arenas. The steps below are then not necessary.

1. At the bottom of the **Arena Settings** pane, click the **Arena - Hardware Mapping** button.

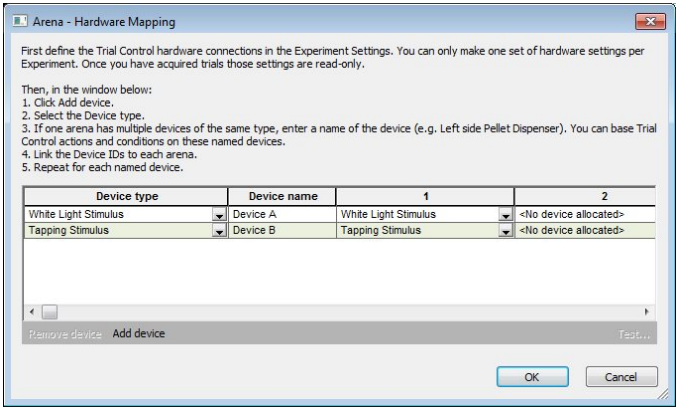


2. In the **Arena - Hardware Mapping** window, do one or both depending on what you want to use:

   a Click **Add device**. Under **Arena 1**, select **White Light Stimulus**.

   a Click **Add device**. Under **Arena 1**, select **Tapping Stimulus**.

   The devices **White Light Stimulus** and **Tapping Stimulus** only need to be assigned to Arena 1. They are in fact applied to all arenas.



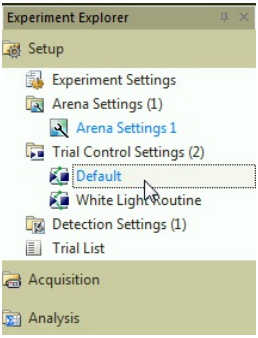
3. Click **OK**.

For more information

- See also **Test the hardware devices** in the Reference Manual - Trial and Hardware Control in EthoVision XT.
- To set up the Tapping Device, see also **The DanioVision Tapping Device** on page 72.

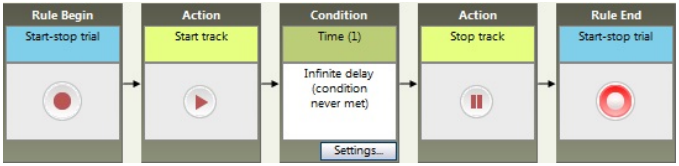
7 Trial Control Settings

Choose **Setup** > **Trial Control Settings** > **Default** or **White Light Routine**.

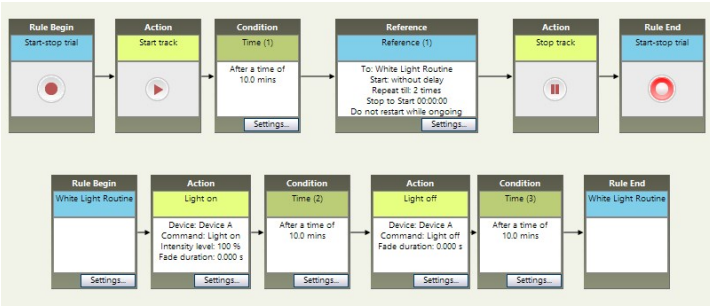


The template experiment contains two examples of trial control settings:

- **Default** - The default Trial Control Start-Stop rule. Use these settings to start and stop tracking manually.



- **White Light routine**. Use these settings when you want to provide a light stimulus during the trial. Ten minutes after the start of the trial, the DanioVision white light immediately turns on to 100% intensity for 10 minutes, then the white light immediately turns off for 10 minutes. This sequence of actions is repeated twice, using a sub-rule, after which the trial stops.



- For using the White Light, see below.
- For using the Tapping Device, see page 72.

Maximum trial duration (recommended)

- If you record video: 60 minutes.
- Without recording video: 72 hours.

Set the duration of your trials in the Maximum Trial Duration pane or in the Start-Stop trial rule, by placing a Time condition immediately before the **Stop track** action box.

CREATE YOUR OWN TRIAL CONTROL PROCEDURE

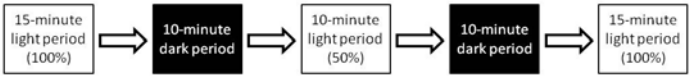
You can adjust these trial control settings to your own needs. The procedure below describes how to define Trial Control Settings from scratch.

For more information on Trial Control Settings:

- **Trial Control** in the EthoVision XT Help.
- The Reference Manual - Trial and Hardware Control in EthoVision XT, which you can find under **Noldus** > **EthoVision XT 16 Other Documentation**.

With the Trial Control Settings you can control the DanioVision White Light.

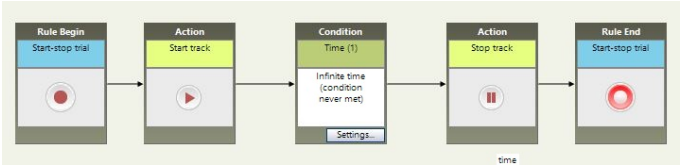
Example: After a 24-hour dark period in the DanioVision Observation Chamber, you want to expose your animals to alternating periods with the white light on and off, in which the light-on periods also vary in intensity. This schedule is shown in the figure below.



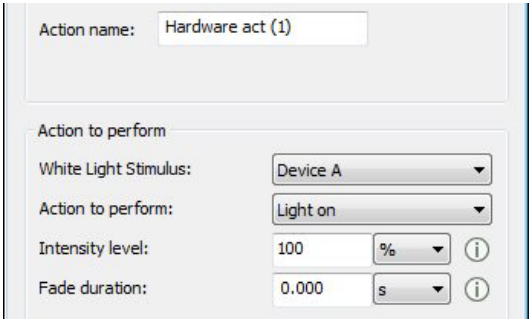
To program this schedule:

1. To open the Trial Control, in the Experiment Explorer, right-click **Trial Control Settings**, select **New**, enter a name and click **OK**.

   The Trial Control screen opens with the default Start-Stop trial control rule.



2. Click the **Condition Time** box with "Infinite time" and press **Delete**.
3. In the **Components** pane, under **Actions** - **Hardware**, click the button in the **Add** column next to **White Light Stimulus**. Select **Create a new action**.
4. In the **Hardware Action** window, set the following:



- **Action name**. Enter a description of the action, for example "Light on 100%".
- **Action to perform**. Select **Light on** from the list.
- **Intensity level**. Select the maximum intensity level of the light, in percentage (**0** - **100%**) or in Steps (**0**-**4096**). See some reference values on page 131.
- **Fade duration**. For example, with Intensity level of 50% and a Fade duration of 2 seconds, the white light gradually turns on until 50% of its maximum intensity in 2 seconds time.

5. Click **OK** and insert the **Action** box in the sequence.

6. In the **Components** pane, under **Conditions**, click the button next to **Time**. Click **Create a new action** and click **OK**.

   In the **Time condition** window, you can set the following:
   - **Condition name**. For example, enter 'Wait 15 mins'.
   - **Condition is met**. For the option After a time of, enter '15 mins'.

7. Click **OK** and insert the **Condition** box in the sequence.

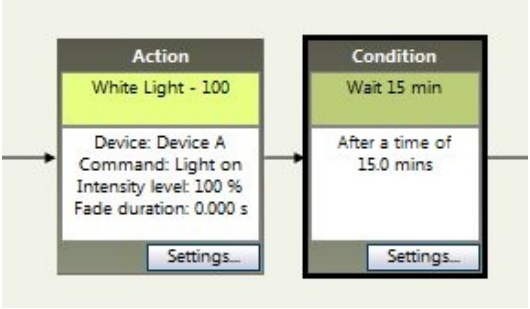


Figure 11 *This combination of Action and Condition box turns the White Light on at 100% intensity and keeps it on for 15 minutes.*

8. Repeat steps 3-6 to complete the Trial Control rule example described on page 59:
   - Add an **Hardware Action** box with **White Light - Off** and a **Condition Time** box with "After **10 mins**".

- Add an **Hardware Action** box with **White Light - On 50%** and a **Condition Time** box with "After **10 mins**".
- Add an **Hardware Action** box with **White Light - Off** and a **Condition Time** box with "After **10 mins**".
- Add an **Hardware Action** box with **White Light - On 100%** and a **Condition Time** box with "After **15 mins**".

TIP Combinations of Action and Condition boxes that are repeated in a sequence can be replaced with a *sub-rule*. This is especially handy when the entire sequence is repeated multiple times during a trial. For example: [White Light - On - 100%] > [After 10 min] > [White Light - Off] > [After 10 min]...

For more information on using sub-rules, see also the Reference Manual - Trial and Hardware Control in EthoVision XT.

9. Insert all the boxes in the correct order into the sequence and make sure you connect all boxes (see Figure 12 and Figure 13 on the next page for an example).

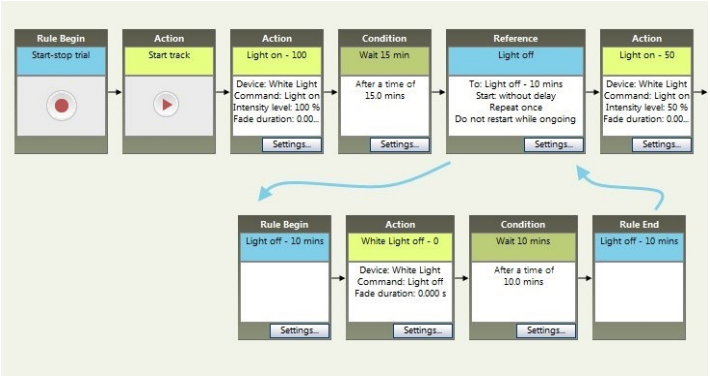


Figure 12 *Example of Trial Control Settings. This is the first half of the Start-Stop Trial rule. In this example of Trial Control Settings, the repeated combination of Action box 'Light off - 0' and Condition box 'Wait 10 mins' to turn the White Light off for 10 minutes, has been replaced by a Sub-rule (see also the Figure on the previous page).*

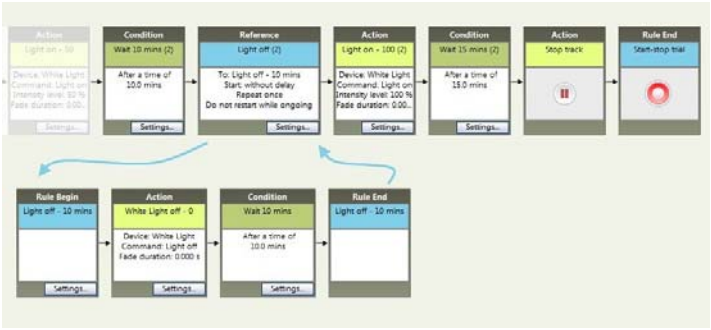


Figure 13 *Continued from Figure 12. This is the second half of the Start-Stop Trial rule. The Sub-rule is called a second time by the Reference box* **Light off (2)**.

Notes

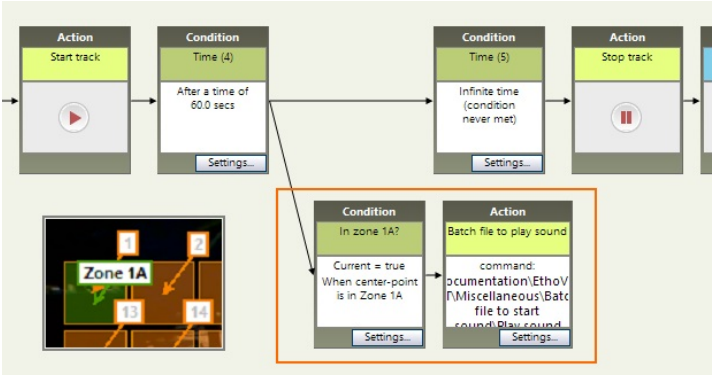
- Click **Test** in the **Hardware Action** window to test the hardware action.

- Click **Reset** to reset all **Hardware Action** windows in your Trial Control procedure to their default values (for example a White Light action is set to **off**; however if you set the default White light to **on** (page 47), then after clicking **Reset** the White light switches on.
- To analyze data in the light or dark period, create first a Data profile that specifies that period using *Trial Control State* (see page 107). Then, calculate the statistics.

Use an external command

If you want to use an external command, for instance to play a sound during the trial, the command is executed for each arena. For example, the command to play the sound is repeated 96 times for a 96 arena experiment. To prevent this from happening, put a condition before the External command box, which will only become true for one of the arenas, for example Arena 1. In the Arena Settings, create a zone in

Arena 1 and call it “Zone 1A”. Make the zone as large as the arena. In the Trial Control Settings, make a condition like “Is subject in Zone A?”, followed by the external command. Make sure that the sequence does not interfere with the rest of the Trial Control rule, like in the following example.



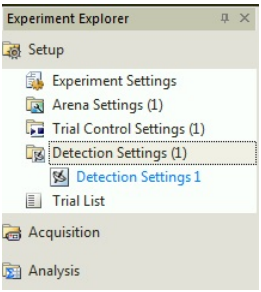
8 Detection Settings

What do you want to do?

- Track one subject per well/enclosure. See below.
- Track two or more subjects in the same enclosure. See page 67.
- Use Activity. See page 66.

ONE SUBJECT PER ENCLOSURE

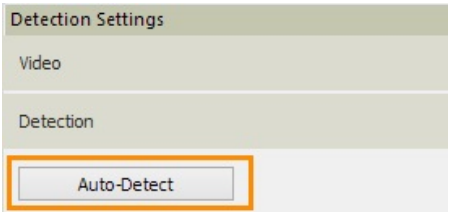
Choose **Setup** > **Detection Settings** > **Detection Settings 1**.



The template experiment contains a Detection Settings profile with **DanioVision** selected as method.

IMPORTANT If you have upgraded EthoVision XT to version 15 while you have a DanioVision system older than the DVOC-0040, it is possible that the **DanioVision** detection settings method does not give optimal detection of the larvae with images from your camera. If that is the case, use another detection method in the **Advanced** section of the **Detection Settings** pane. For details, see the EthoVision XT Help.

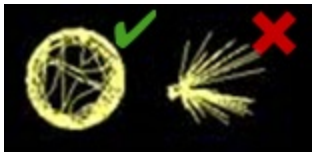
1. Click the **Auto-detect** button.



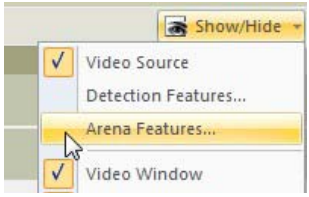
IMPORTANT Use the **Auto-detect** button every time you change a well-plate.

2. The **Auto-detect** window with a timer appears. Wait until it has disappeared. All larvae should now be detected well. If not, do one or more of the following:
   - Open the **Advanced** section and move the **Sensitivity** slider. Moving the slider to the left results in detecting more of the body of the larvae, but also more noise. Moving the slider to the right results in detecting less noise, but also gives less good detection of the larvae.

     If you see erratic tracks like that in the picture below (right), it means that small groups of pixels at the border of the well are sometimes detected as the larva. Move the Sensitivity slider to the right.

   - If some larvae are not detected while they are in the corner or border of a well, check that the arenas are drawn well. Click the **Show/Hide** button on the toolbar and select **Arena Features** and then select the check box in front of **Arenas**.

Check that the arenas cover the entire wells. In the picture below, the arena on the right does not cover the left margin of the well. Therefore, the larva is not detected when swimming in that region.

- Try to maximize the contrast by opening the aperture of the camera lens (See "check the camera lens" on page 33.) However, make sure the light intensity is equal for all wells (that is, the center and outer wells).
- Certain compounds dissolved in the water can affect the pigmentation in the larvae, thereby decreasing the contrast of the animal and making detection of the animal more difficult. Make sure that EthoVision XT can still detect all animals after treatment.

3. If detection remains unsatisfactory, adjust the Advanced Detection Settings. The **Dynamic subtraction** method may give better results. For an explanation of the options, see **Configure Detection Settings** in the EthoVision XT Help.

ACTIVITY ANALYSIS

Use Activity to quantify overall activity of your subjects. In the Experiment Settings (see page 46) select **Activity analysis.** In the **Detection Settings** pane, open the **Activity** section. Activity is shown by purple pixels in the video window. Set the **Activity threshold** in such a way that movements of the animals are detected and some noise is left.

For more information on activity, see **Activity settings** in the EthoVision XT Help. Also watch the video tutorial on Activity analysis.

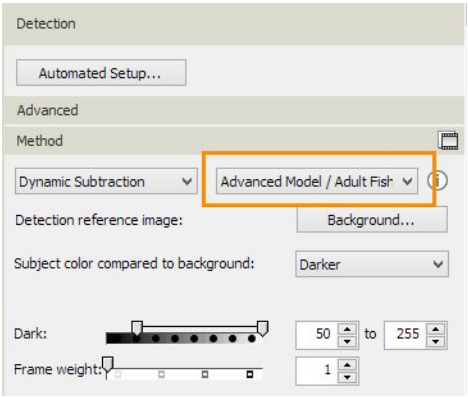
For more general information on Detection Settings, see **Configure Detection Settings** in the EthoVision XT Help.

MULTIPLE SUBJECTS IN THE SAME ENCLOSURE

Prerequisites: in the Experiment Settings, **Number of Subjects** is set to 2 or higher.

1. Choose **Setup** > **Detection Settings** > **Detection Settings 1**.
2. Click **Automated Setup** and draw a rectangle around each subject.
3. Under **Method**, select **Advanced Model / Adult Fish** or **Advanced Model / Other** if the first does not give the expected results when tracking fish larvae.

   NOTE The **Advanced Model /Other** method is not based on a modeled fish shape. With this method you cannot track the fish snout and tail. It is only available if the experiment is set to Center-point detection only.



9 Acquire data

There are three ways of acquiring data with your DanioVision system. For every option it is possible run single trials, but it is also possible to carry out batch acquisition.

- **ACQUIRE DATA LIVE** (page 69). The larvae are tracked as they move in their wells.
- **ACQUIRE DATA LIVE AND RECORD VIDEO SIMULTANEOUSLY** (page 70). The larvae are tracked as they move in their wells while at the same time a video is recorded. This gives you to opportunity to redo the tracking if necessary, either per trial or using batch acquisition.
- **RECORD VIDEO, THEN ACQUIRE DATA** (page 71). You record a video first and then track the subjects offline from the video. Please note that if you only record video with EthoVision XT, Trial Control is not applied. So, for example, the white light is not switched on and off, if you only record video.

Before you start acquiring data, make sure you have carried out all the necessary checks of the system (see page 32).

Notes

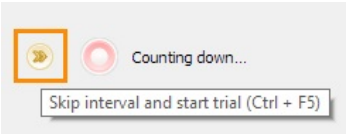
- The DanioVision Observation Chamber is designed to block light from the outside. However, it cannot be guaranteed that it is completely dark inside the chamber. For experiments that require absolute darkness, place the DVOC in a dark room.
- **IMPORTANT** In long experiments, evaporation may occur for both the wells in the plate and the basin. Check the wells every 24 hours and if necessary, refill them.

  Refill the basin every 2-3 days using the Basin Refill tool. For details, see the Reference Manual - DanioVision Temperature Control Unit DV-TCU.
- Please read the section in the next pages that applies to your setup. For more information, see **Acquire Data** in the EthoVision XT Help.

ACQUIRE DATA LIVE

1. Make sure the camera is connected to the EthoVision XT computer and that **Live tracking** is selected as **Video Source** in the Experiment Settings.
2. Optionally, plan your trials in the **Trial list** and select the **Arena settings**, **Detection settings** and **Trial control settings** for each trial.
3. Choose **Acquisition** > **Open Acquisition**.
4. If no trials are planned, click the **New trial** button in the **Playback Control** window or press **Ctrl+F3**.
5. In the **Acquisition Settings** pane, select whether you want to track only the next planned trial or whether you want to track all planned trials without having to start the next trial manually.

   If you want to track all planned trials, specify the inter-trial interval. Enter a time long enough to be able to change well plates. When you change the well plate between trials, you can start the next trial without waiting that the inter-trial interval is reached. To do this, click the **Skip remaining interval** button in the **Playback Control** window.



6. Select whether you automatically want to start the analysis after the tracks are acquired. If that is the case, first define your dependent variables in an Analysis Profile (see **Analyze data** on page 113). Optionally, first make a Track Smoothing profile and a Data profile to make a selection out of your data.
7. Select whether you want to use the Track smoothing profile, Data profile, and Analysis profile that are highlighted in blue in the Experiment Explorer for analysis, or that you want to run analyses for all possible profile combinations. In the latter case, first delete all profiles you do not need for analysis. If you want to carry out analyses for only a selection of profiles, deselect the check box **Auto-**

start analysis and carry out batch analysis after acquisition (see step 5 on page 115).

8. Put the well-plate into the DanioVision chamber.
9. Click the **Auto-detect** button. A **Re-initialize** window with a timer appears. Wait until it disappears.

   IMPORTANT Use the **Auto-detect** button every time you change a well-plate.

10. To start the trial, click the **Start trial** button in the **Playback Control** window (or press **Ctrl+F5**).
11. To stop the trials, wait until Trial Control automatically stops the trials. To stop acquisition manually click the **Stop trial** button in the **Playback Control** window (or press **Ctrl+F6**). If you stop the trials manually, automatic analysis does not start. Also, for batch analysis, the next trials are not started if you stop a trial manually.

NOTE The recommended maximum trial duration when acquiring live without recording video is 72 hours (3 days).

ACQUIRE DATA LIVE AND RECORD VIDEO SIMULTANEOUSLY

To save video when doing tracking, follow the procedure above, with the difference that in step 4 you select the check box **Save video** in the **Acquisition Settings** pane.

If you realize tracking was not optimal, you can re-do tracking from the acquired video.

NOTE The recommended maximum trial duration when acquiring live and recording video simultaneously is 60 minutes. Longer trials produce video files of excessive size, more difficult to manage. Furthermore, if one large video gets corrupted then you lose more data.

RECORD VIDEO, THEN ACQUIRE DATA

If you use the option **Save video only, track later**, Trial Control is not applied so you cannot control the white light of the DanioVision Chamber with EthoVision XT.

To save video when doing tracking, follow the procedure on page 69, with the difference that in step 4 you select **Save video only, track later** in the **Acquisition Settings** pane. Also make sure that the trial is time limited, either with a time condition in the Trial Control start-stop rule, or with a Maximum Trial duration setting (page 58).

Next, you can acquire data using batch acquisition. Plan your trials in the Trial list and specify the video and optionally Arena Settings, Trial Control Settings and Detection Settings for each trial.

NOTE The recommended maximum trial duration when recording video is 60 minutes. Longer video recordings produce files of excessive size, more difficult to manage. Furthermore, if one large video gets corrupted then you lose more data.

DRAIN AND CLEAN THE SYSTEM

At the end of the experiment, we recommend to remove all the water from the basin and the tubes.

If you use the Temperature Control Unit, DV-TCU, set it to **Fill/Drain** and follow the instructions in the manual Reference Manual - DanioVision Temperature Control Unit DV-TCU.pdf.

To prevent the growth of algae in the system:

- Always drain the system after use.
- Do not expose the system and the tubes to sunlight.

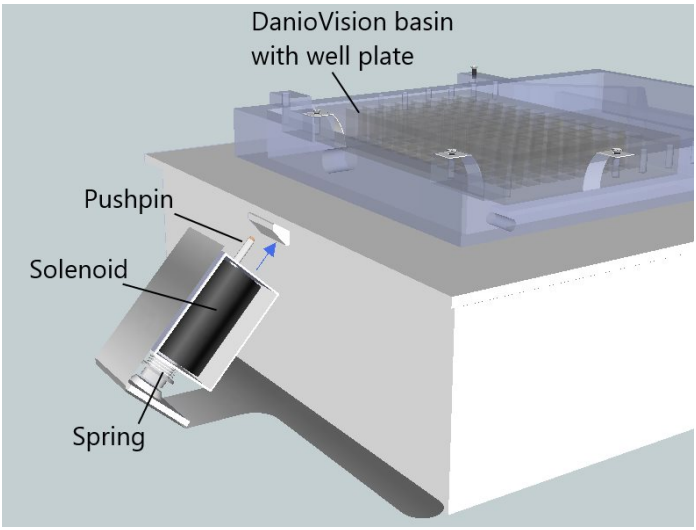
10 The DanioVision Tapping Device

INTRODUCTION

Larval stage zebrafish display a robust startle response, which is mediated by neural pathways similar to that in higher vertebrates. With the DanioVision Tapping Device you can evoke a startle response in zebrafish larvae.

How the Tapping Device works

The DanioVision Tapping Device is based on an electromagnetically inductive coil wound around a metal core. The core is made of two parts, a fixed one and a plunger connected to a push pin. When a current flows in the Tapping Device, magnetic force is created between the plunger and the core, causing the plunger and the push pin to quickly move forward (as indicated by the blue arrow in the picture). The pushpin hits a metal plate attached to the basin in which the well plate is kept. When deactivated, a spring makes the plunger resume its position.



How you control the Tapping Device

With the Trial and Hardware Control module of EthoVision XT you can plan when to activate the Tapping Device during a trial. For example, activate the Tapping Device 10 times at intervals of two minutes.

Noldus test results: a summary

- In both light and dark conditions, the Tapping Device is capable of inducing a startle response in animals of 3 dpf and older.
- It is in principle possible to design experiments where the animals are subject to stimuli of different intensity. You can set the tap intensity in EthoVision XT (page 76).
- There is no evidence that the startle response measured by EthoVision XT depends on light conditions and location of the subject in the well plate.

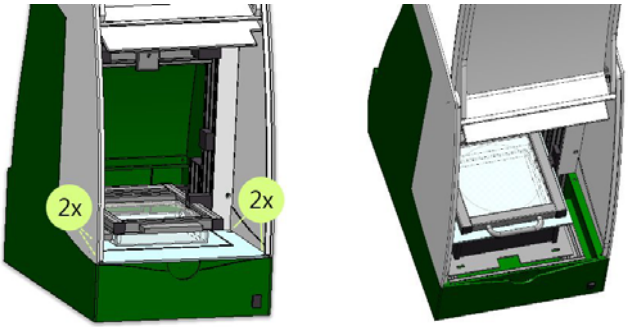
See also the white paper **Using the DanioVision Tapping Device**, which you can download from our web site.

IMPORTANT We advise you not to compare the results between two DanioVision Observation Chambers provided with the Tapping Device. Slight differences in the position of the Tapping Device may result in differences in the actual stimulus intensity, all else being equal!

INSTALL THE DANIOVISION TAPPING DEVICE

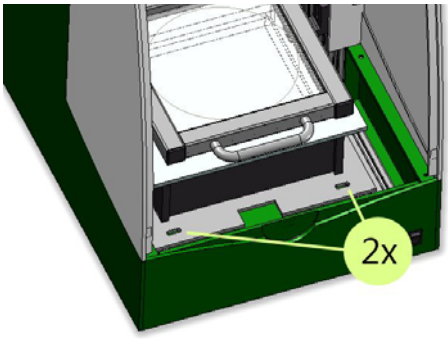
If you order the Tapping Device together with a DanioVision Observation Chamber, the Tapping Device is already installed. In all other cases:

1. Open the lid of the DanioVision Observation chamber, and remove the bench plate (see also page 116).

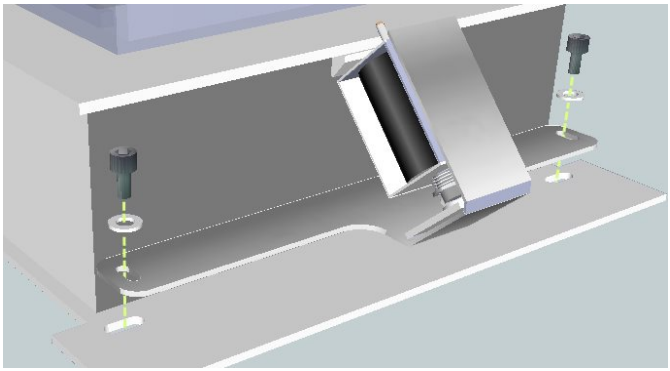


2. Remove the two bolts from the aluminium plate at the bottom of the chamber as indicated in the figure below. **Make sure that the backlight box does not move**.

   TIP Use a pencil to mark the exact position of the backlight box on the frame so that you can place it in that position if it accidentally moves.



3. Put the metal frame of the Tapping Device over the aluminium profiles, and fix the bolts with the supplied spring washers. Use both the already connected spring washers and the new ones.

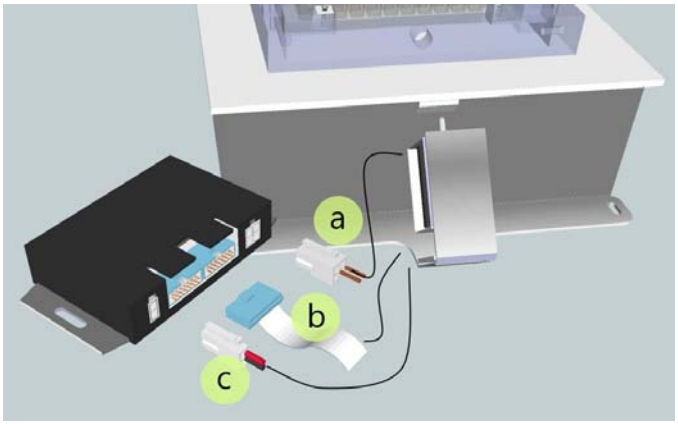


4. Connect cables as indicated here.

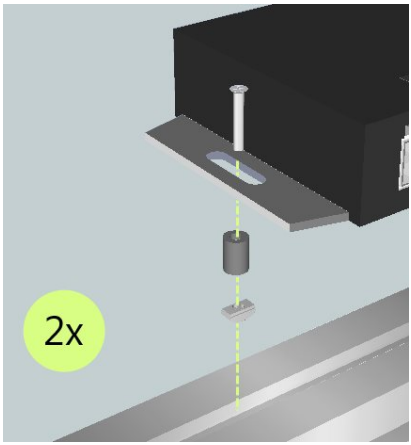
   a The cable with a 4-pin white connector comes from the Tapping Device.

   b The flat cable with a blue connector comes from the bottom of the DanioVision chamber. Raise the metal hooks, insert the connector, then lower the hooks to fix the connector.

   c The cable with 2-pin connector comes from the bottom of the DanioVision chamber.



5. Attach the control box to the aluminium profile at the front side of the observation chamber.
   a Insert the screw in one of the hole at the sides of the control box.
   b Attach the spacers and the sliding nuts to the screw.
   c Insert the sliding nuts into the profile, and turn the screw.



6. Put the bench plate back into place and fix the four screws.

SET UP THE TAPPING DEVICE IN ETHOVISION XT

Make sure that the DanioVision Observation Chamber is connected to your EthoVision XT computer.

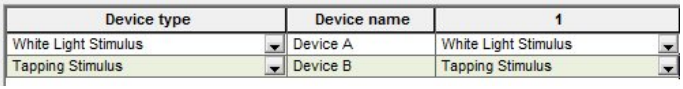
Experiment Settings

Under **Trial Control hardware**, click **Settings**. In the Hardware Interface window, select **DanioVision DVOC-004x/T**.

Arena settings

NOTE If you created an experiment with a pre-defined template and selected an arena template for DanioVision with the Tapping device (DanioVision DVOC 004x/T), the Tapping Stimulus has been assigned automatically to the arenas. The steps below are then not necessary.

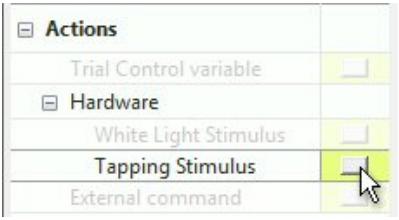
1. In the **Arena Settings** pane click **Arena - Hardware Mapping** and then click the **Arena - Hardware Mapping** button.
2. Click **Add device**. Under **Device type**, select **Tapping Stimulus**. Under **Arena 1** select **Tapping Stimulus**.



3. To test the Tapping Device, select the cell under **Arena 1** and click **Test**.

Trial and Hardware Control Settings

1. Open the Trial Control Settings containing the protocol in which you want to insert the Tapping Stimulus. Under **Actions - Hardware** click the button next to **Tapping Stimulus**.



2. In the **Hardware Action** window specify the **Intensity level** (1-8). Click Test to get an impression of the tap intensity.
3. Click **OK** and insert the **Action** box in your Trial Control protocol.
4. Test the protocol before carrying out the real trials.

IMPORTANT Allow at least 300 milliseconds between two consecutive tapping events. To do so, place a **Time** condition box that specifies this time between two Tapping Stimulus **Action** boxes.

ANALYZE THE RESPONSE TO THE TAPPING STIMULUS

You can analyze the startle response by looking at dependent variables like *Activity*, *Distance moved*, or *Acceleration (state)* immediately before and after the administration of the stimulus.

In the following example, we examine the *Total Distance moved* in the 1-second periods immediately before and after the tapping stimulus command was given. We expect that the total distance moved increased from "before" (assumed to be a baseline) to "after". The stimulus command was given at exactly 1 minute after the start of the trial.

1. In the Data profile define two **Nesting** time intervals, one from 00:59.00 to 01:00.00, the other from 01:00.00 to 1:01.00. Each of the resulting **Nest** box is connected to a separate **Results** box.
2. In the Analysis profile define **Distance moved**.

The following picture shows the Distance moved plotted in Integrated Visualization.

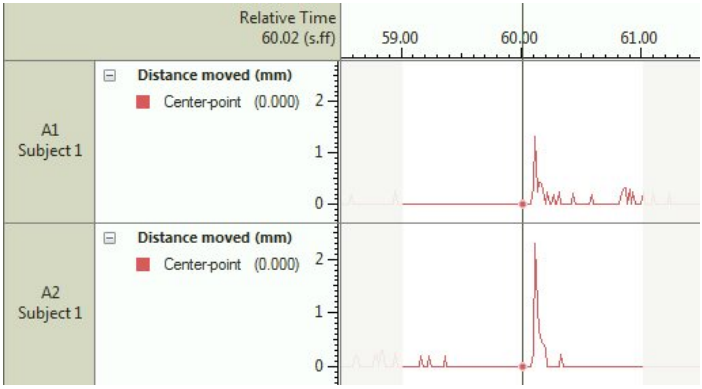


Figure 14 *An example of per-sample distance moved plotted against time for two subjects, and in the 1-s period before (59 s – 60 s) and after triggering the Tapping Device (60 s – 61 s). The hairline indicates the time that the stimulus was administered.*

11 The DanioVision Toplight Unit

This manual explains the setup and operation of the DanioVision Toplight (type DVTL-0020) for the DanioVision Observation Chamber DVOC-0041.

NOTE The DanioVision Toplight Unit is an add-on to DanioVision; it does not come with a standard DanioVision system. For the specifications, see **DANIOVISION TOPLIGHT UNIT** on page 135.

CONNECT THE TOPLIGHT UNIT

Components

- DanioVision Observation Chamber with built-in Toplight Unit.
- Desktop power supply, TTL-to-28V interface and cabling (see the figure below).

Operation

With this setup, two of the LED colors, or the white light can be switched on and off from EthoVision XT. The TTL-to-28V interfaces enable switching the power from the desktop power supply.

NOTE Whenever you would like to use all three colors (R-G-B) within one experiment you'll need a second TTL-to-28V interface.

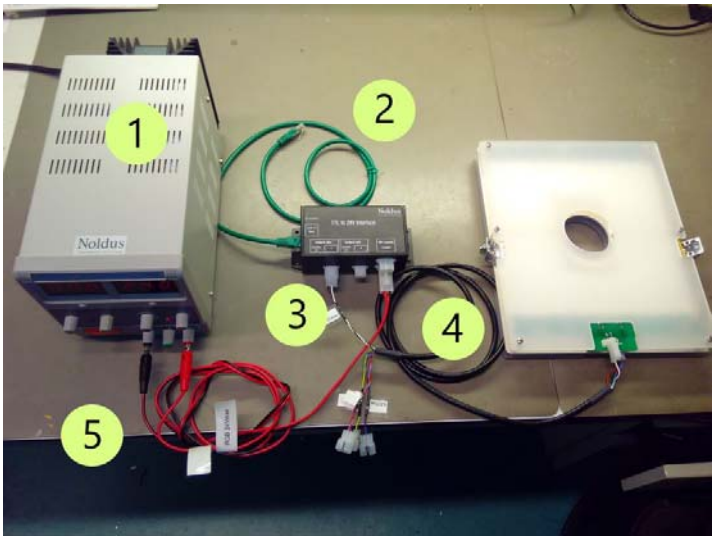
IMPORTANT The LEDs get hot during operation. Please let the ceiling light cool down before touching it

IMPORTANT Do not exceed the maximum specified voltage for each color as doing so may damage the LEDs

Setup for the white light

1. Desktop adjustable supply set to max 18 V for white light.
2. TTL cable to free **TTL** port on the DanioVision Observation Chamber's (DVOC) back panel.

3. White light cable plugged in.
4. Top light cable from the DVOC's back panel.
5. Main LED power cable.

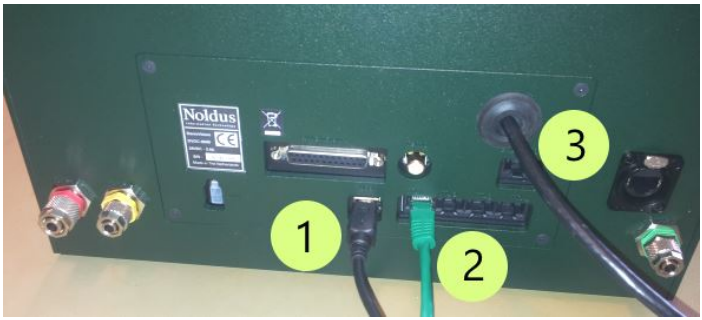


Connect the components

The LED power cable (coming from the back of the DVOC) has 4 connectors, one for each LED color. The connectors belonging to the appropriate colors should be connected to the TTL-to-28V interface on outputs 1 and 2.

The TTL network cable (green) should be connected between the TTL-to-28V interface and port 1 on the DVOC (see the next figure).

A main power cable connects the desktop power supply to the input of the TTL-28V interface.



1. USB cable to EthoVision XT computer.
2. TTL cable running to TTL-to-28V interface.
3. LED power cable.

OPERATE THE POWER SUPPLY

On the power supply the voltage can be set which determines the amount of light that the LEDs emit. The voltage may not exceed the maximum voltage for each LED color. This is 24V for the Red, Green and Blue LEDs and 18V for the white LEDs.

IMPORTANT Higher voltages may damage the LEDs. Before connecting to the mains outlet, check that the mains voltage matches the **voltage setting** of the power supply.

To power up, press the power button.

To adjust the voltage

Turn the Voltage knobs for coarse and fine adjustments. Or when in presence of one knob, press once for coarse adjustments, and press once more for fine adjustment. The actual output voltage can be read from the display.

Both “Current” knobs can be used to set a current limiting value (maximum current). This feature is not needed; just set to the maximum level (turn full clockwise).

If not set properly, the supply may come in Constant Current mode (CC) and limit the output voltage.

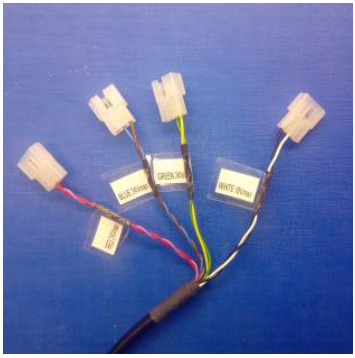
It is good practice to first set your desired voltage before actually connect the main LED power cable to the supply.

For more information, see the supply manual.

CHANGE THE LIGHTS

Two switch between red, green and blue

To change between colors, simply unplug the current connector of the LED power cable from the TTL-to-28V interface and plug in the correct one. Each connector is labeled.



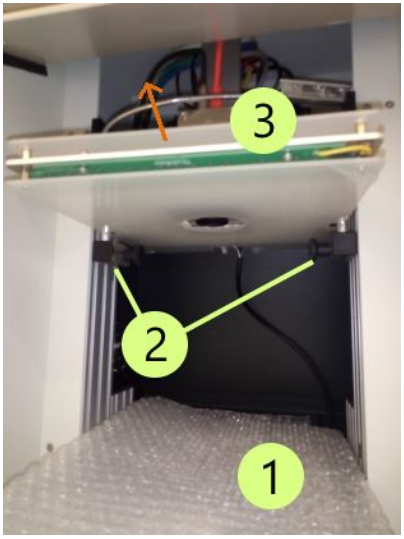
To switch between white and colors

If you want to switch from RGB to white light (or vice versa), turn the complete unit in order to have the right LEDs facing down towards the well plate.

The RGB LEDs and white LEDs are mounted on the opposite sides of the same plate and can be identified by the label **WHITE** and **RGB** attached.

1. Cover the lens bracket with some protective material.
2. Untighten both left and right round black knobs to enable the unit sliding down a few centimeter, just below the camera.
3. Pull the unit out and turn it upside down (facing the preferred LED's down).

   IMPORTANT Be careful not to twist the cable at the back. Unplug temporarily both connectors to prevent this from happening.



4. Insert the unit and slide it up again.

5. Tighten both round knobs.

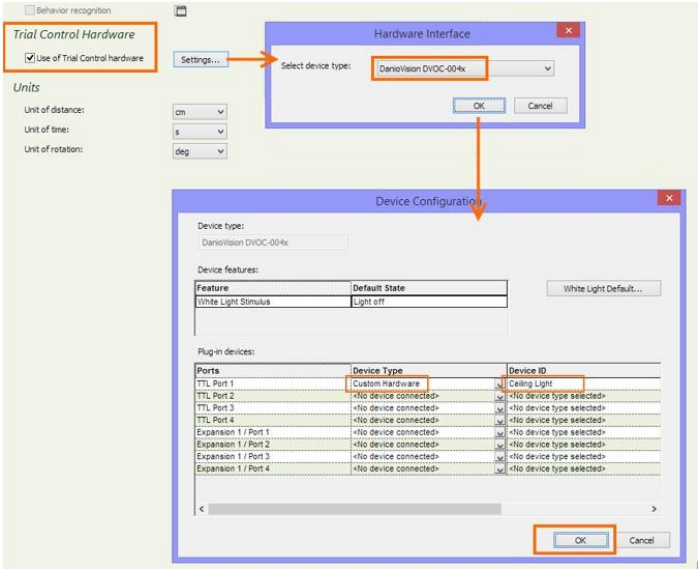
CONFIGURE ETHOVISION XT TO CONTROL THE TOP LIGHTS

1. Start EthoVision XT and create a new experiment using a template (**File** > **New from template**).

   During the guided setup, choose **Live Tracking**, **Zebrafish larvae**, **DanioVision** [with the type of well plate you use].

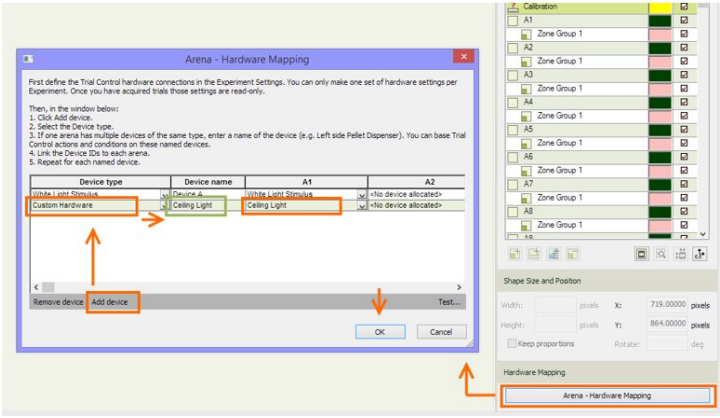
2. Choose **Setup** > **Experiment Settings**.

   Under **Trial Control Hardware**, select **Use of Trial Control Hardware** and choose the options as in the figure below. Choose the TTL port of the DVOC that you connected (see above). You can rename **Device ID** to something like “Ceiling Light”.

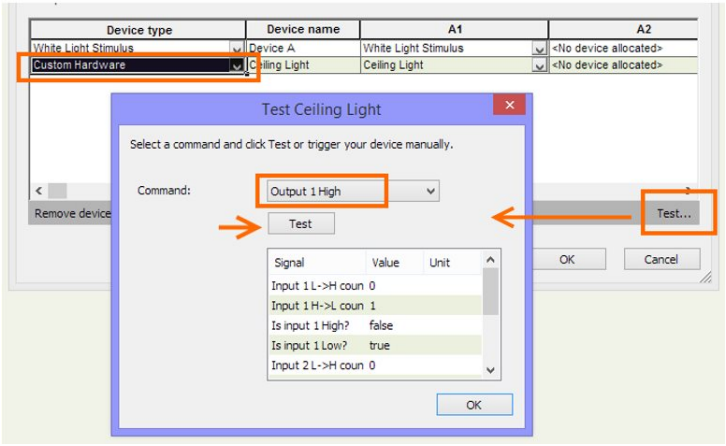


3. Choose **Setup** > **Arena Settings**.

   After calibrating and drawing the arenas, click **Arena Hardware Mapping**. Click **Add device** and follow the picture below. You can rename the text under **Device name** to something like “Ceiling Light”. Under **A1** select the device. Then Click **OK**.



4. To test the light, select **Output 1 High** or **Output 2 High** from the **Command** menu and click the **Test** button. To turn them off, select **Output 1 Low** or **Output 2 Low**.



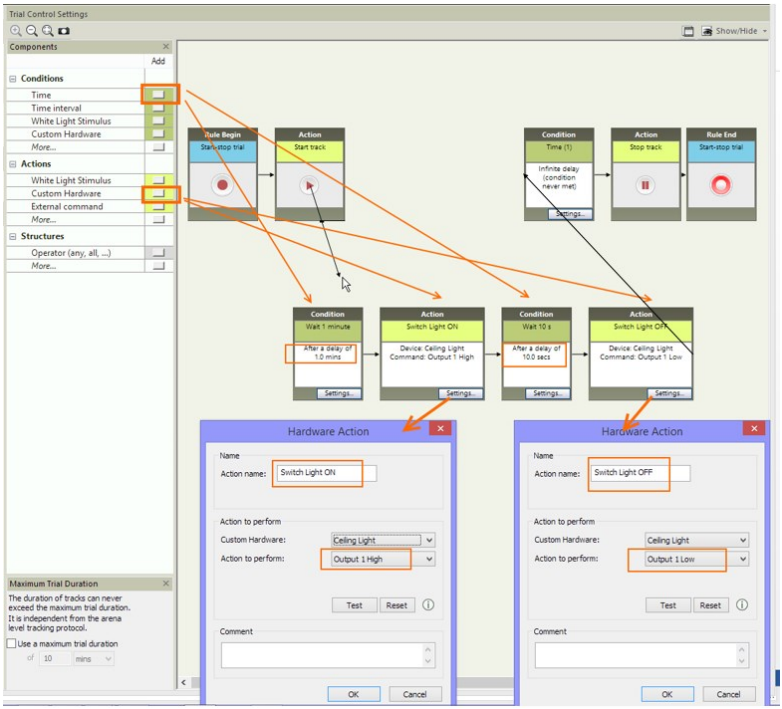
PROGRAM THE TOP LIGHTS IN ETHOVISION XT

Choose **Setup** > **Trial Control Settings**.

Example: After 1 minute during the trial, switch the light on, then after 10 seconds switch it off.

- Under **Conditions**, choose **Time** to create a time condition (e.g. Wait 1 minute, Wait 10 seconds).
- Under **Actions**, choose **Custom Hardware** to define commands (Switch on/off). This can be done for both output 1 and output 2.

Remember to connect all boxes with the start-stop procedure.



Create routines

With the **Subrule** function you can create routines, to switch on and off the lights in repeated sequences.

For more information, see the Reference Manual - Trial and Hardware Control in EthoVision XT.

Also adjust the Detection Settings (**Setup** > **Detection Settings**) before running the trials.

12 The DanioVision Optogenetics add-on

INTRODUCTION

Optogenetics allow scientists to control the activity of specific neurons and study their downstream influence on a variety of biological processes, including behavior. In rodent studies, this technique requires optical fiber implantation. When working with zebrafish larvae, mostly transparent, fiber optics are not needed Light simply needs to shine in the right direction, making it easy to assess the effect of stimulation of specific, light-sensitive neurons on the behavior of the fish.

By inserting light-sensitive receptor proteins into neurons in vivo, it is possible to make those neurons sensitive to activation by light of specific wavelengths. In zebrafish studies, the light-sensitive receptor protein halorhodopsin (NpHR) has successfully been used to inhibit swimming behavior in zebrafish larvae (Arrenberg *et al*. 2009). Other research showed that channelrhodopsin-2 (ChR2) activation induced backward swimming in a sparse transgene expression line (Zhu *et al*. 2009).

Optogenetics add-on

Application of optogenetic stimulation in zebrafish larvae requires the correct wavelength (i.e., color) of light. When using the DanioVision Observation Chamber, the Optogenetics Add-on provides a way to accurately control and precisely time the application of optogenetic stimulation to up to 96 individuals simultaneously (working with 96 well plates).

The Optogenetics add-on for DanioVision is an optogenetic LED light source based on the Prizmatix Modular LED system. It consists of one, two or three LED wavelengths. The Optogenetics add-on can be programmed and controlled using the EthoVision XT software included with your DanioVision system. You can set user-defined time conditions for the optogenetic stimulation. In comparison to manual

control, this offers far better temporal precision and adds efficiency to longitudinal studies.

References

- Arrenberg, A.B., Del Bene, F., Baier, H. 2009. Optical control of zebrafish behavior with halorhodopsin. *PNAS* **106**: 17968-17973.
- Zhu, P., Narita, Y., Bundschuh, S.T., Fajardo, O., Scharer, Y.P., Chattopadhyaya, B., Bouldoires, E.A., Stepien, A.E., Desseroth, K., Arber, S., Sprengel, R., Rijli, F.M., Friedrich, R.W. 2009. Optogenetic dissection of neuronal circuits in zebrafish using viral gene transfer and the Tet system. *Frontiers in Neural Circuits* **3**: 21.

Sample experiment

Your DanioVision system comes with an EthoVision XT sample experiment: **DVOC_Optogenetics_Demo_Recommended.evxt**. It contains examples of Trial Control rules for triggering pulse sequences in one up to three Pulsers.

Additional manuals

For connecting hardware

- Prizmatix UHP-T LED Illuminator User Manual.pdf

For programming the Pulser

- Prizmatix Pulser User Manual.pdf

For connecting the DanioVision Temperature Control Unit

- Reference Manual - DanioVision Temperature Control Unit DV-TCU.pdf

INSTALL THE DICHROIC MIRROR IN DANIOVISION

When you receive the DanioVision Observation Chamber DVOC-004x with the Optogenetics add-on, the LEDs are already mounted so you only have to connect the cables (see the next section). However, the dichroic mirror comes in a separate box to prevent damage during shipping. This section explains how to install it. Please follow the instructions below.

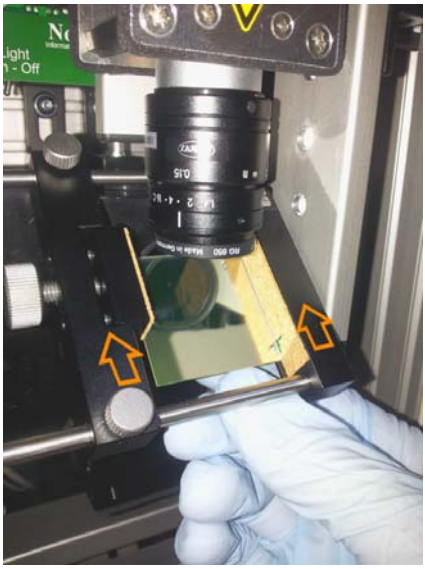
NOTE The function of the dichroic mirror is to direct the color light beams to the well plate, and at the same time allow light to reach the camera, so you can film and track the fish during the test.

1. Locate the box containing the mirror.

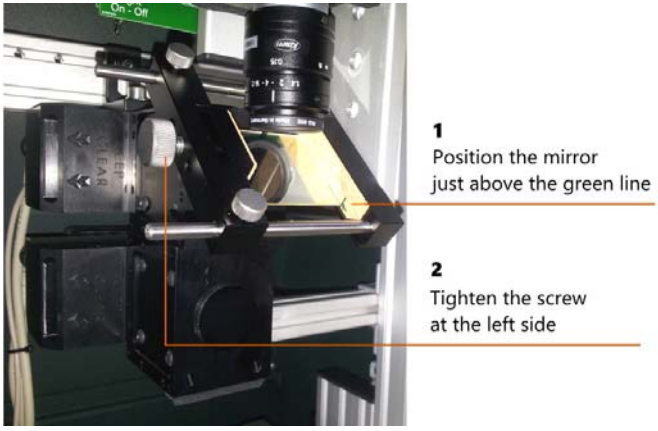
important Please wear the supplied gloves to avoid damaging or dirtying the mirror. Avoid touching the mirror surface and only hold its edges.

2. Locate the arrow on one side of the mirror. Once the mirror is mounted, this arrow should be at the top of the mirror and point towards the light source, that is, the backside of the Daniovision chamber.

3. Attach the mirror (see the next picture). Slide the mirror upwards along the brackets as indicated by the arrows.



4. Position the mirror just above the mark on the right-hand bracket (see the picture below, **1**). When the mirror is in that position, carefully tighten the screw on the left to fix the mirror (**2**). Do not apply any force as this may damage the mirror.



5. The mirror is now ready to use. See the next sections.

IMPORTANT The LED is extremely powerful and may cause eye injury when looking directly into the light source. For testing, we recommend to set the LED intensity to low.

THE PULSER SOFTWARE

Please skip this section if you do not use the Pulser/PulserPlus devices.

Follow the procedure below if you use one or more Pulser/PulserPlus devices. To control multiple devices, multiple instances of the Pulser software must be running on the EthoVision XT computer.

IMPORTANT Do not connect the Pulser/PulserPlus device to the computer until the software setup process is complete.

Install the Pulser software once

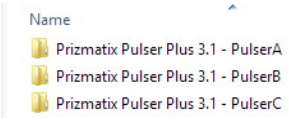
1. Copy the installation files and folders to your computer's hard drive.
2. Run the **setup.exe** file and follow the instructions as they appear on the screen. Next, restart the computer to complete the software installation.
3. Open the folder where the software has been installed (default: C:\Program Files (x86)\Prizmatix Pulser Plus 3.1) and make a shortcut to the EXE file. Give the shortcut a name that is easily recognized, like *Pulser A* or the name of the first LED.
4. To install or update the Pulser/PulserPlus drivers, see the Pulser/PulserPlus User Manual.

Install multiple instances of the Pulser software

To control two or three LEDs independently using the dedicated Pulser/PulserPlus devices, install one instance of the Pulser software for each Pulser/PulserPlus.

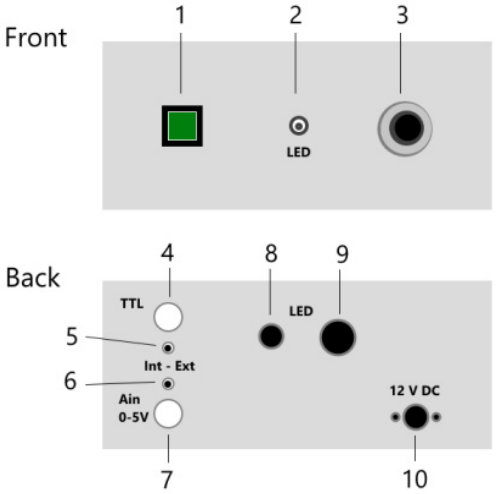
1. Install the first instance of the Pulser software (see above).
2. Browse to the folder C:\Program Files (x86) and copy the entire folder **Prizmatix Pulser Plus 3.1**.

3. Paste the folder in the same location C:\Program Files (x86). Rename the folder to ... **Prizmatix Pulser Plus 3.1 PulserB**.
4. Open the new folder and make a shortcut for the EXE file that you find there. Place the shortcut on your desktop. Name it like *Pulser B* or the name of the second LED.
5. If you have a third LED, repeat the steps above from 2 to 4 to create a new folder which will contain a third instance of the Pulser software. Make a shortcut to the new EXE file and rename it to e.g. *Pulser C*.
6. You should at this point have a folder structure like the following:



and three shortcuts on your desktop.

THE LED CONTROLLER



To control the LED manually:

1. Turn on the main power (see **1** in the figure above).
2. Set the switch near **TTL** (**5**) to **Int**.
3. Set the switch near **Ain** (**6**) to **Int**.
4. To turn the LED on, flip the switch (**2**) on.

To control the LED from EthoVision XT (through TTL)

1. Turn on the main power (see **1** in the figure above).
2. Set the switch near **TTL** (**5**) to **Ext**.
3. Set the switch near **Ain** (**6**) to **Int**.
4. To turn the LED on, flip the switch (**2**) on.
5. Connect the TTL cable with BNC connector from the pulser (or the USB-IO box, depending on the setup) to the connector TTL on the back panel of the LED controller (**4**).
6. In EthoVision XT you can now give the “high” command. See page 100 or 101 depending on the setup.

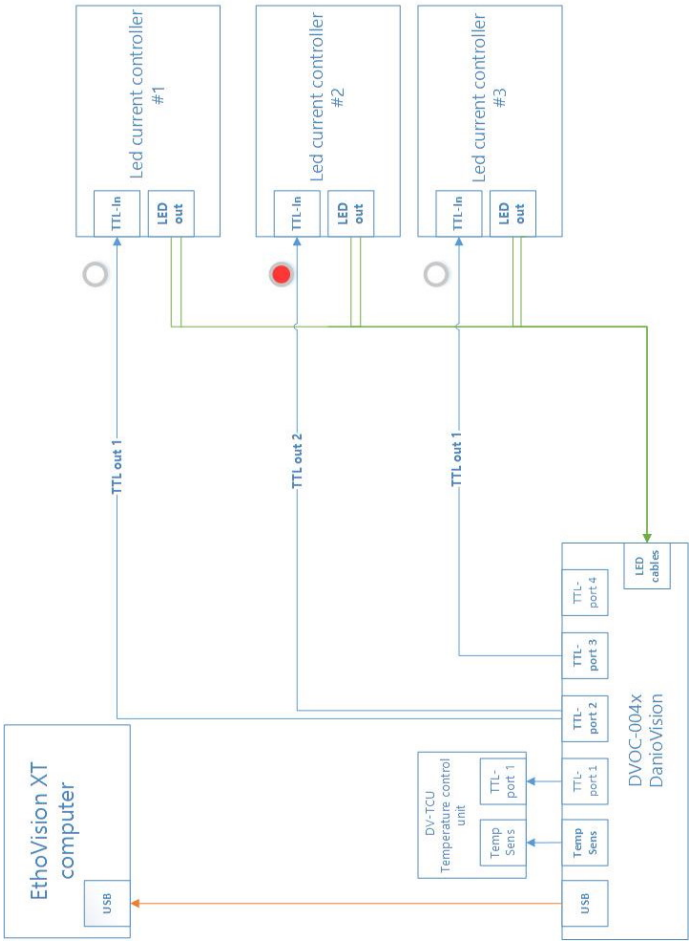
Other controls

- Power adjustment dial (**3**).
- Analog input (**Ain**) connector (**7**).
- LED control cable connector (**8**).
- LED current cable connector (**9**).
- DC power jack (**10**).

CONFIGURATION 1 - WITHOUT THE PULSER

Connection diagram

In the following example, EthoVision XT controls three LED controllers through the TTL ports in the DanioVision Observation Chamber. Note that each port contains two lines, with connectors white (Line 1) or red (Line 2). Each line controls one LED controller.



Define the pulse sequence

To define a pulse sequence, use actions and conditions in the EthoVision XT's Trial Control Settings. For example a 1-s pulse of light in the first LED can be defined as: Action Light On on port 1 > Time Condition (1 second) > Action Light Off on port 1.

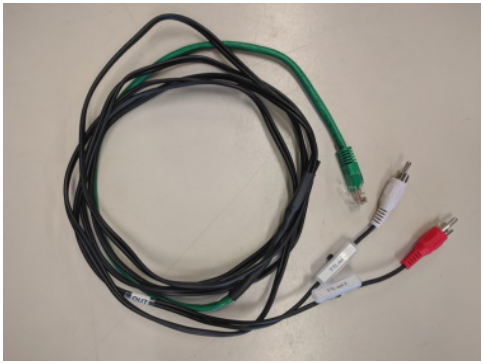
For information about creating pulse sequences in EthoVision XT, see the Chapter Optogenetics experiments in the Application Manual - EthoVision XT, which you can find on the EthoVision XT computer under **Noldus** > **EthoVision XT Other Documentation**.

USB ports

You need one USB port on the EthoVision XT computer, to control DanioVision.

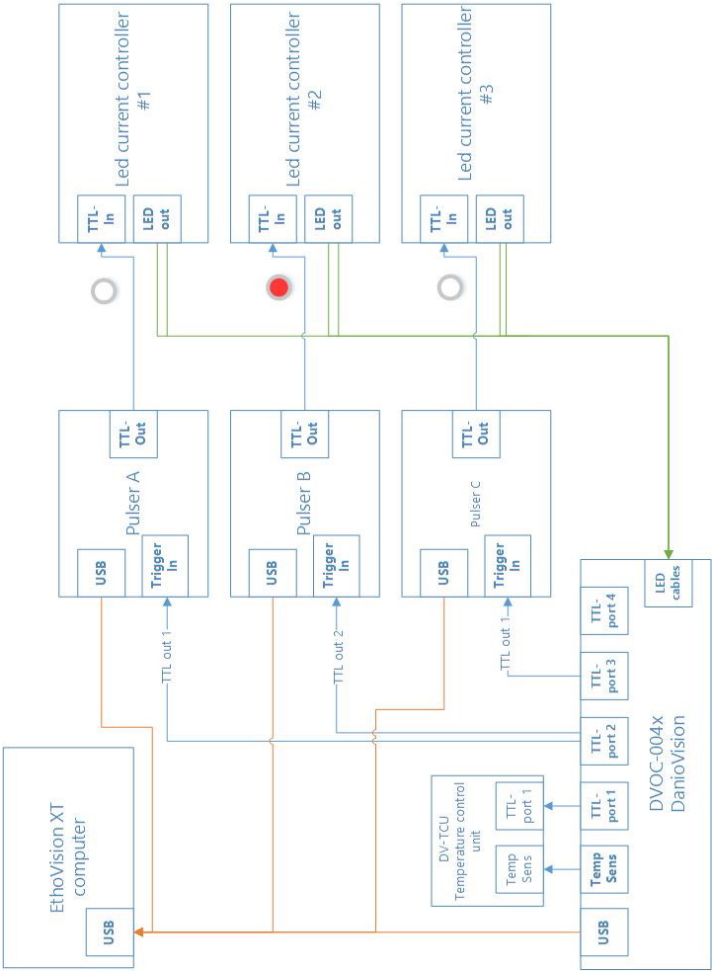
TTL ports

- When using one LED, you can use the TTL-port 2 of DanioVision. Use the white connector at the other end of the cable to connect the LED controller.
- When using two or three LEDs, you can control two LEDs with one TTL port. Use TTL-port 2 for LED 1 and 2, and TTL-port 3 for LED 3. Remember that the white connector at the other end of the cable means Line 1, and the red connector means Line 2.



CONFIGURATION 2 - WITH PULSERS

Connection diagram



USB ports

On the EthoVision XT computer, you need:

- One USB port, to connect DanioVision (see page 17)
- Additional USB ports, one for each Pulser (see page 97). Connect the USB port of each Pulser to a USB port of your computer.

TTL ports

- When using one Pulser, you can use the TTL-port 2 of DanioVision. Use the white connector at the other end of the cable to connect the Pulser.
- When using two or three Pulsers, you can control two Pulsers with one TTL port. Use the DanioVision TTL-port 2 for Pulser A and B, and TTL-port 3 for Pulser C. Connect the other end of the cable to the **Trigger In** port of each Pulser. Remember that the white connector at the other end of the cable means Line 1, and the red connector means Line 2.

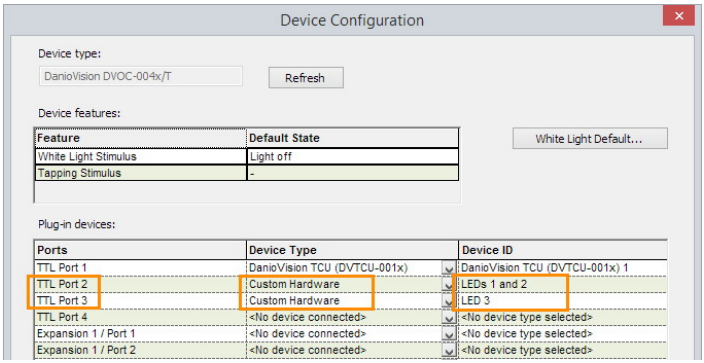
EXPERIMENT SETTINGS

Choose **Setup** > **Experiment Settings** > Click **Settings** next to **Use of Trial Control hardware**. Then follow the section below that applies to choose the port numbers in the Device Configuration window.

TIP Open the sample experiment DVOC_Optogenetics_Demo_Recommended.evxt. This is a live tracking experiment where basic settings in Experiment Settings and Trial Control are already selected for when you work with three pulsers.

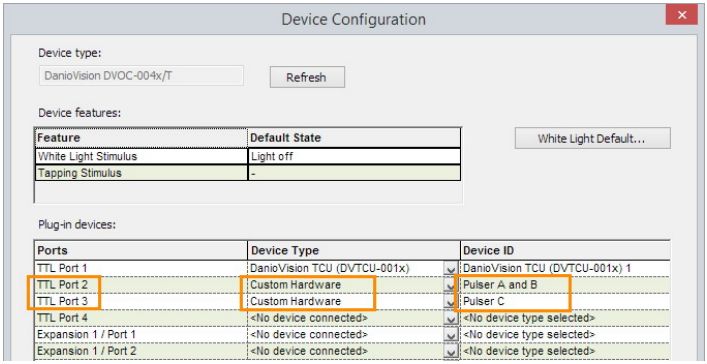
Configuration 1 - without Pulser

Each LED controller is connected directly to one TTL port of DanioVision. If for example you have three LEDs, choose Port 2 for the first two LEDs, and Port 3 for the third one. See the corresponding scheme on page 95.



Configuration 2 - with Pulser

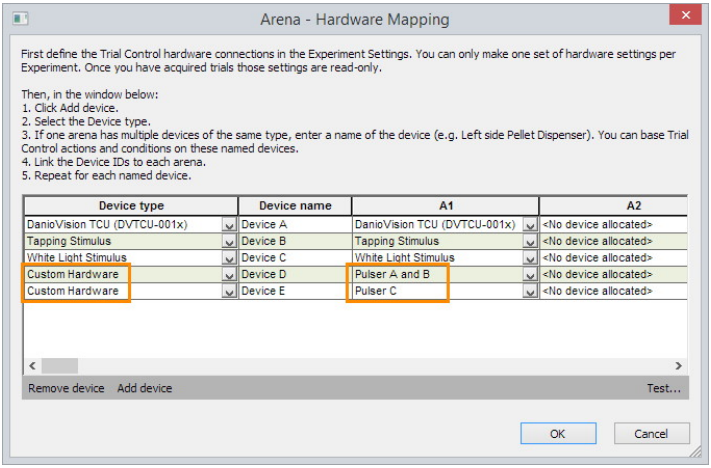
Each Pulser is connected to one TTL port of DanioVision. If for example you have three Pulsers, choose Port 2 for the first two Pulsers, and Port 3 for the third one. See the corresponding scheme on page 97. Under **Device ID**, give each port a name that can be easily recognized.



ARENA - HARDWARE MAPPING

Choose **Setup** > **Arena Settings**. Draw the arenas (see this manual). When the arenas are ready, click the **Arena - Hardware Mapping** button and make sure that the devices are selected under Arena 1 (**A1**).

The figure below refers to a configuration with three Pulsers.



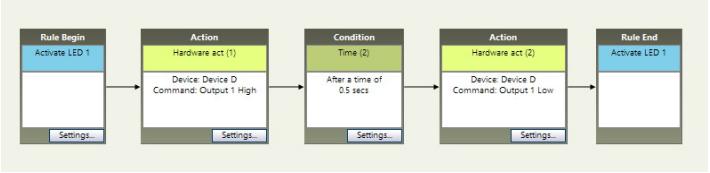
TRIAL CONTROL (NO PULSER)

IMPORTANT Before you follow this section, make sure that (1) the dichroic mirror is installed in the DanioVision chamber (page 90), and (2) all components are connected (page 95) and powered up, and (3) that the LED controller is set to **TTL** control (page 93).

When the LED is controlled directly from EthoVision XT, activation occurs in Trial Control, using the Action boxes. The duration of the light pulse is determined by a Time condition placed between the Action **Output 1 High** and **Output 1 Low**.

In the example below, LED 1 (Device D in the Arena - Hardware mapping window; see above) is activated with a TTL signal **Output 1 High** and after 0.5 it is deactivated with a TTL signal **Output 1 Low**. The LED Controller must be connected to DanioVision through TTL Port 1 - Line 1 (see page 95).

Use **Output 2 High**/**Low** commands to control the other LED controller connected to the same DanioVision TTL port.



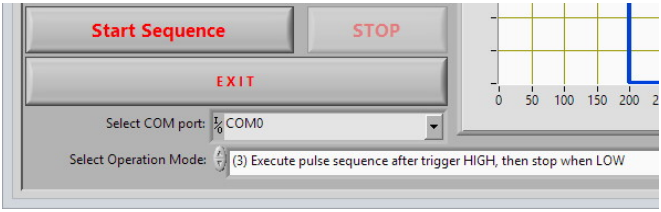
Make sure that the Int-Ext switch near TTL on the LED controller is set to **Ext**!

TRIAL CONTROL (WITH PULSERS)

IMPORTANT Before you follow this section, make sure that (1) the dichroic mirror is installed in the DanioVision chamber (page 90), (2) all components are connected (page 97) and powered up, and (3) that the LED controller is set to **TTL** control (page 93).

Procedure

1. Open all the instances of the Pulser software by clicking on the desktop shortcuts **Pulser A**, **Pulser B**, and **Pulser C**. Each instance controls one pulser.
2. For each instance of the software, next to **Select COM port**, choose the correct COM port for the corresponding Pulser. These can be found on the pulser or on the connection diagram.



3. Next to **Select Operation Mode**, choose one of the following:
   - When testing the Pulser, choose **(0) Execute pulse sequence after START**. By pressing the **Start Sequence** button, the LED flashes the configured sequence.

- When running the actual trials, choose **(3) Execute pulse sequence after trigger HIGH, then stop when LOW**. If you now click the **Start Sequence** button, the Pulser waits for a TTL trigger to start the sequence.

4. Enter the other settings in the dialog to control the pulse duration and the time between pulses. For details, see the Prizmatix Pulser/ PulserPlus User Manual.

5. Start EthoVision and open the example experiment: DVOC_Optogenetics_demo_recommended.evxt

   In the Trial Control Settings you find predefined routines (called *subrules*) to control three Pulsers. The main procedure (top row) has been configured to consecutively trigger the three Pulsers using the corresponding subrules. Make sure the pulsers all have the correct COM port, are all in mode **3** and the start sequence button has been pressed. See also **CONFIGURE THE COM PORT FOR EACH PULSER** on page 103.

   Here below you find the basic actions to control the triggering of the Pulsers. For more information, see also the Chapter Optogenetics experiments in the Application Manual - EthoVision XT, which you can find in **Start** > **Noldus** > **EthoVision XT Other Documentation**.

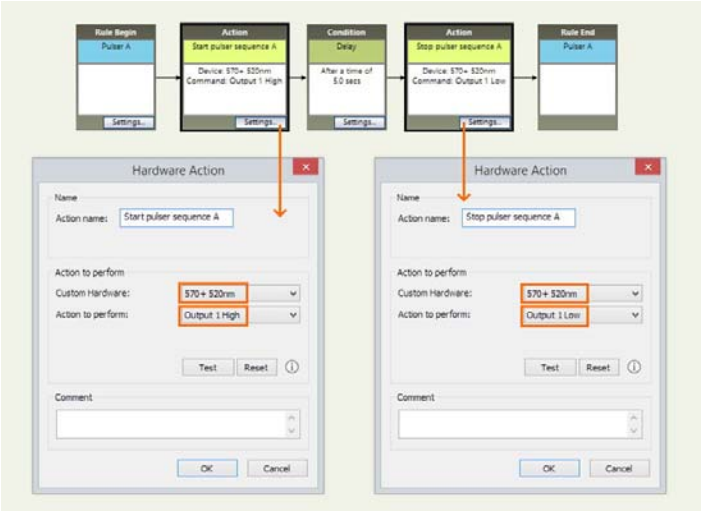
Basic subrule

A basic set of instructions in a subrule activates the Pulser a number of times, as specified in the Subrule Reference box.

The boxes within the subrule specify an action to trigger the pulse sequence (Send a signal **High** to a device through line 1 or 2), a Time (wait a number of seconds) and a second action which stops the pulse sequence (Send signal **Low** to a device through line 1 or 2).

In the following example, the subrule triggers the sequence in the Pulser A through Output Line 1, for 5 seconds. The name of the device selected next to **Custom Hardware** is **570 +520 nm**, because it control two Pulsers, one for a LED of 570 nm (through Output Line 1) and one for a LED of 520 nm (through Output Line 2). You find the name **570 +520 nm** in the Arena - Hardware Mapping window of the Arena Settings in the demo experiment.

In practice, the subrule would look like the following:



Make sure that the Int-Ext switch near TTL on the LED controller is set to **Ext**!

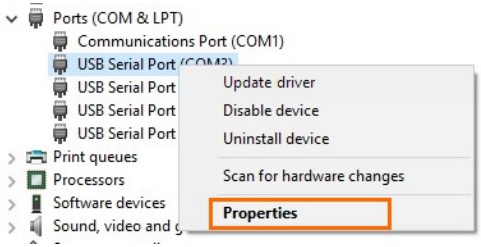
CONFIGURE THE COM PORT FOR EACH PULSER

Aim

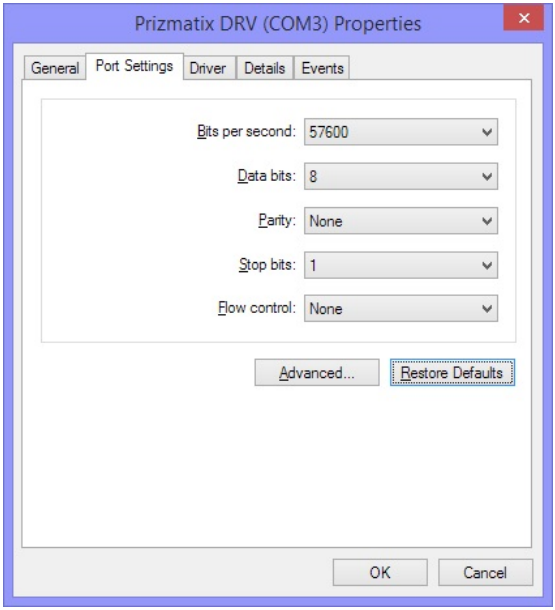
To configure the COM port in such a way the Pulser/PulserPlus accepts commands from EthoVision XT.

Procedure

1. Connect the Pulser/PulserPlus to the PC using the USB cable with type-A to type-B connectors.
2. In the **Control Panel**, open the **Device Manager**.
3. Under **Ports (COM & LPT)**, right-click **USB Serial Port** and select **Properties**.



4. Click the **Port Settings** tab.
5. Make sure that the parameters are as below, then click **OK**.



6. Take note of the port number (in the example above it is COM3).
7. Repeat the procedure for each Pulser/PulserPlus.

Notes

- The COM port number is retained in the Pulser, so if you disconnect the Pulsers and reconnect them, you do not have to assign the COM ports again.
- **TIP** To know which COM port is assigned to which Pulser/PulserPlus device, disconnect the USB cable from one Pulser/Pulser Plus. Then, re-connect it. The item that appears under **Ports (COM & LPT)** indicates the COM port.
- **TIP** To change the COM port for a specific Pulser, in the **Control Panel**, open the **Device Manager**. Under **Ports (COM & LPT)**, right-click the COM port, select **Properties**, **Port Settings**, then **Advanced**. From the **COM Port Number** list select the COM port among those available.

13 Data preparation

SMOOTH THE TRACKS

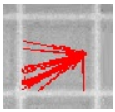
Track Smoothing profiles in the template experiment

- **No Smoothing**. Smoothing filters are not activated.
- **MDM 0.2 mm** - With this Minimal Distance Moved filter, you can reduce the effect of 'jitter' on the Distance moved variable. Applying this filter is recommended, however, it results in longer calculation times in the analysis.

Change these settings for your setup, if necessary.

More options

Use the Maximum Distance Moved filter to remove outliers in the track, due to erratic detection of objects within the arena. when the margin of the well is sometimes detected as the fish.



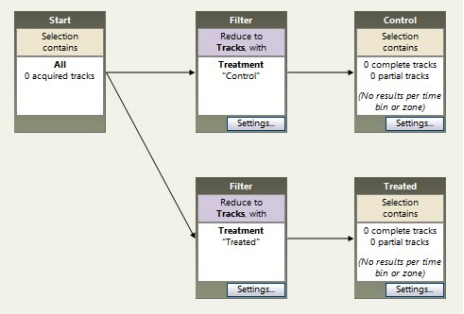
For more information on smoothing data, see **Smooth the Tracks** in the EthoVision XT Help.

SELECT DATA

Data profiles in the template experiment

- **All Data**. With this Data profile you analyze all tracks separately.

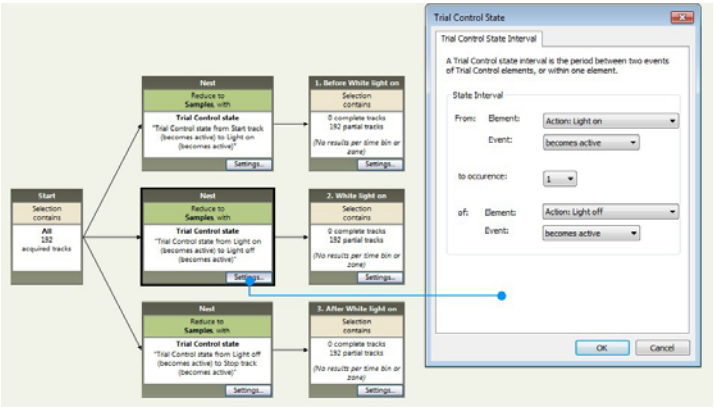
- **Treated vs. Control** - The data are split for analysis based on the two values of the user-defined variable Treatment: Treated and Control.



Analyze dark vs. light periods

In this example, the researcher wants to analyze the data in different light and dark periods, determined by the activation of the white light. In the Data profile, the function **Trial Control State** under **Nesting** is used to create intervals corresponding to the state of the white light.

1. Choose **Analysis** > **Data Profile** > **New**. Give the new Data profile a name and click **OK**.
2. In the **Components** pane, under **Nesting**, click the button next to **Trial Control State**.
3. Select the period you are interested in, using the Trial Control actions. For example From **Light On** To **Light Off** (depending on what name the Light On/Off actions have in the Trial Control Settings).
4. Insert the **Nest** box in the Data profile sequence.
5. If you want to analyze different periods and have the results in the same table, create more **Results** boxes as shown in the picture below. To add a **Result** box, in the **Components** pane click the button next to **Result**. In each sequence, add a **Nest** box that specifies a different Trial Control State.



6. Choose the dependent variables and run analysis (see page 113).

TIP See also the sample experiment **DanioVision with 96 wells XT** which you can download from our web site. There you can find the Data profile mentioned here.

Analyze data excluding periods of inactivity

In this example we want to exclude the interval in which a larva was *not* moving.

1. Choose **Analysis** > **Data Profile** > **New**. Give the new Data profile a a name and click **OK**.
2. In the **Components** pane, under **Nesting**, click the button next to **Movement**.
3. In the **Movement** tab, you can set the following:
   - **Averaging interval** (range 1 - 1000) – This is the number of samples across which changes in speed are calculated to determine whether the subject is moving or not. In order to reduce the sensitivity of the Movement variable to brief changes in velocity, the velocity data can be smoothed by taking the running average of the last *n* samples. Enter the averaging interval *n* or leave 1 if you do not want to smooth the velocity data.
   - **Start velocity** – The velocity above which the subject is considered to be moving.

- **Stop velocity** – The velocity below which displacements of the subject's body points are no longer attributed to locomotion but to system noise, body wobble or pivoting on the spot.

4. Under **Calculate nesting for**, select **Not moving**.
5. Click **OK**. Insert the **Nest** box in the appropriate position between the **Start** and the **Result** box.
6. Choose the dependent variables and run analysis (see page 113).

For more information on the Nesting functions, see **Analyze Track Segments** in the EthoVision XT Help.

Analyze the intervals around the onset of the white light

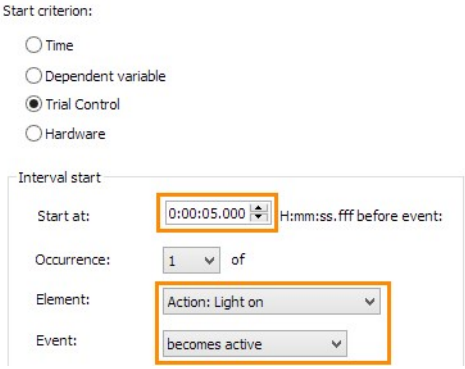
In this example, we want to analyze two intervals:

- From 5 seconds before the onset of the White light stimulus, to the onset of the stimulus.
- From the onset of the White light stimulus, to 5 seconds after the onset of the stimulus.

The aim is to compare activity, speed and other parameters between the two intervals.

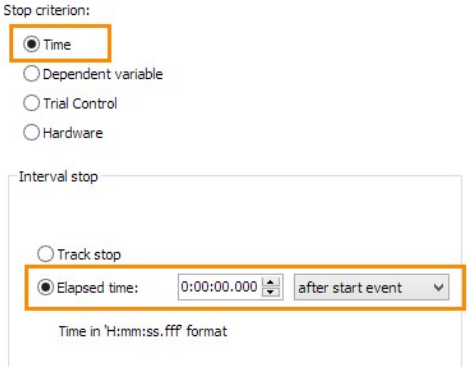
1. Choose **Analysis** > **Data Profile** > **New**. Give the new Data profile a a name and click **OK**.
2. In the **Components** pane, under **Nesting**, click the button next to **Free interval**.
3. Do the following:

   Under S**tart criterion**, select Trial Control. Choose the options as in the picture below.



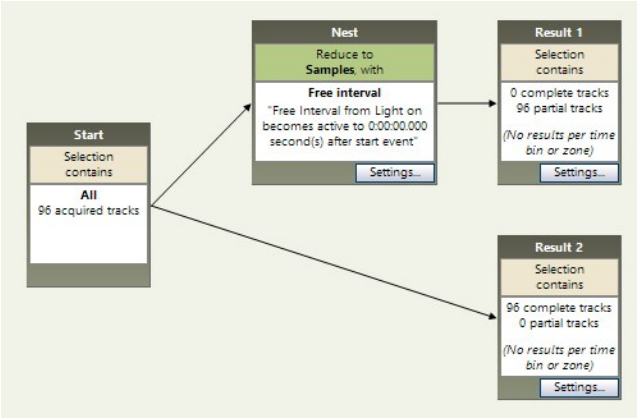
Under **Stop criterion**, select **Time.** Next to **Elapsed time** leave **0:00:00.000 after start event** selected.

NOTE "Start event" here refers to the White light selected under Interval start.

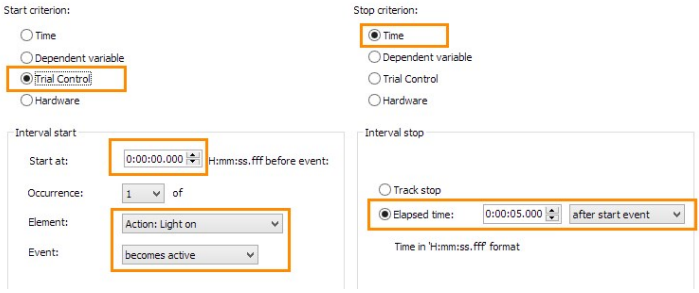


Click **OK** and insert the **Nest** box in the sequence.

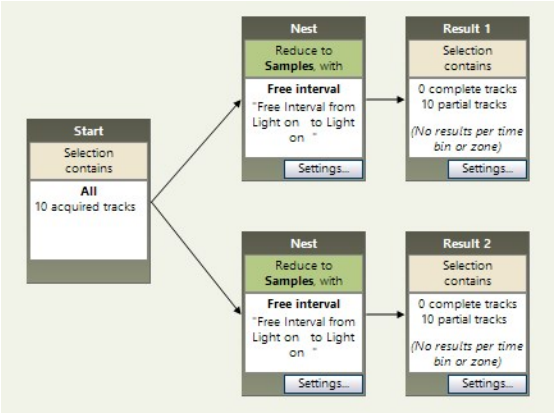
4. To create the second interval, choose first **Result** under **Common elements**. Click the middle of the **Start** box and drag to the **Result 2** box; this will connect the two boxes.



5. Repeat the steps 2-3 to create a new **Nest** box, this time for the interval "from White light to 5-s after that". In the settings, choose the criteria for the second interval: from "Trial Control - 0 seconds before Action: Light on" to "Time - Elapsed time 5 seconds after start event".



Click **OK** and insert the **Nest** box in the sequence.



6. Choose the dependent variables and run analysis (see page 113).

TIP Click **Settings** in the Result boxes and rename them, to for example "5-s Before white light" and "5-s after white light". Those names will be shown in your results.

14 Analyze data

ANALYSIS PROFILES AND OPTIONS

Analysis Profiles in the template experiment

- **Distance, Time & Movement** - Contains the dependent variables Distance moved, Velocity and Movement.
- **Path Shape** - Contains the dependent variables Heading, Turn angle, Angular velocity and Meander.
- **Rotation** - Contains two instances of the dependent variable Rotation, to count the number of clockwise and counterclockwise rotations.

For more information on these and other dependent variables, see **Dependent Variables in Detail** in the EthoVision XT Help. Also see **Analysis of Trial Control data** in the Reference Manual - Trial and Hardware Control in EthoVision XT.

Analyze intervals

Make sure you have defined a Data profile which specifies the intervals you are interested in (see page 106). Next, in the Analysis profile choose your variables (e.g. Distance moved). Then visualize the data (see an example in Figure 15) and run analysis (see page 115).

Activity analysis

You can also use the dependent variable **Activity state**. This variable is only available when you selected **Activity analysis** in the Experiment Settings (see page 46 and page 66).

With Activity state you can calculate how long and how frequent your subject has been in different activity states. These states depend on the total pixel change within the arena between a sample and the previous sample. It is important that all defined arenas have exactly the same size. The number of states (two to four) and their thresholds are user-defined.

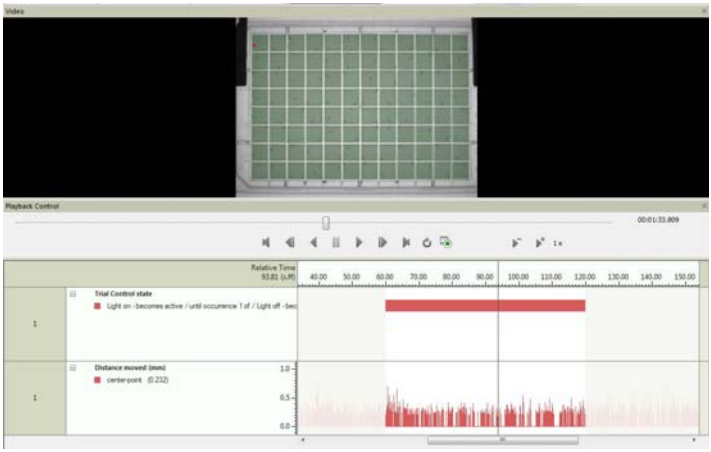


Figure 15 *Example of visualization of data after nesting over a Trial Control State. Here the samples of Arena 1 are displayed in full color when the white light was on. Only those samples are subject to analysis. The first plot is of the variable* **Trial Control State** *(defined as: From Light on to Light off; it illustrates the effect of nesting), and the second is of* **Distance moved** *defined in the Analysis profile.*

Analyze convulsions

To analyze convulsions, you can use several variables, such as Velocity, Activity and Mobility. If you use Mobility, start with setting a *High Mobility Threshold* to a high value like 95%, and an averaging interval of 2. Compare the scores of Highly Mobile with those of convulsions obtained manually.

CALCULATE THE STATISTICS

The instructions below describe the general procedure of selecting a dependent variable and calculating statistics for that variable.

1. Choose **Analysis** > **Analysis Profile**. In the **Analysis Profiles** window, select **New** and type a name for the new profile, then click **OK**.
2. Click the **Add** button next to the dependent variable you want to use for analysis (for an overview, see **Dependent Variables in Detail** in the EthoVision XT Help).
3. In the window that appears, select the properties of the dependent variable.

   In the **Trial Statistics** tab, choose the statistics you want to calculate per trial for that variable. If you run multiple trials, select additional statistics in the **Group Statistics** tab.
4. Repeat steps 2-3 to add more dependent variables.
5. Choose **Analysis** > **Results** > **Statistics & Charts**. Choose the Data Profile from the list on the tool bar and click the **Calculate** button.
6. The results appears on the screen. The **Trial Statistics** tab shows the statistics per trial. The **Group Statistics & Charts** tab shows the statistics and charts from the summarized results of all trials. See **Calculate Statistics** in the EthoVision XT Help for how to read and customize your results.
7. **OPTIONAL** Click the **Layout** button to modify the layout of the results table.

Batch calculations

It is also possible to carry out multiple analyses with different filters, data profiles and analysis profiles. To do so click the **Batch** button.

Make your selection from the lists under **Select the profile combinations to calculate** and click **Add**. When done, click **Calculate**. To view a specific analysis result, select the options from the lists on the toolbar. For more information on batch statistics calculation, see **Batch statistics calculation** in the EthoVision XT Help.

A Open the DanioVision casing

TOOLS YOU NEED

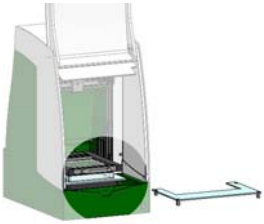
A Phillips or (preferably) a Pozidrive screwdriver, size 2.

Do not use electric screwdrivers!

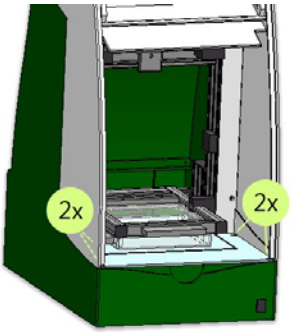
BENCH PLATE

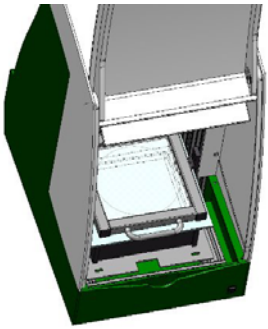
Remove the bench plate, for example, to install the Tapping Device and other, custom hardware you want to place inside the DanioVision Observation Chamber.

Also when opening the top casing (page 117) it is handy to first remove the bench plate.



1. Remove the four screws as indicated in the picture below.
2. Remove the bench plate.

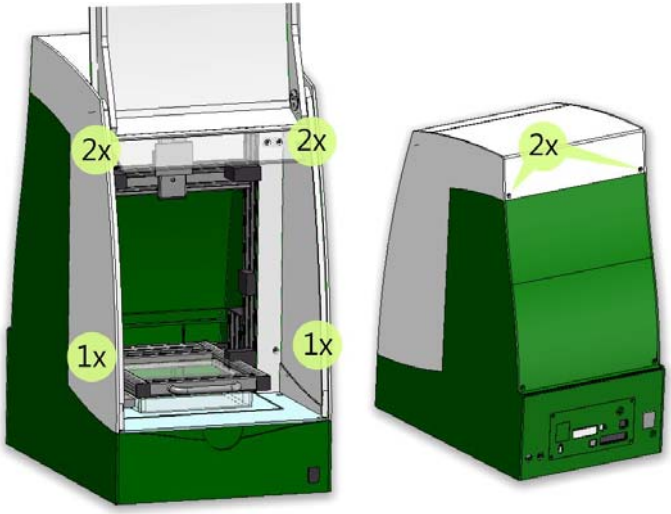




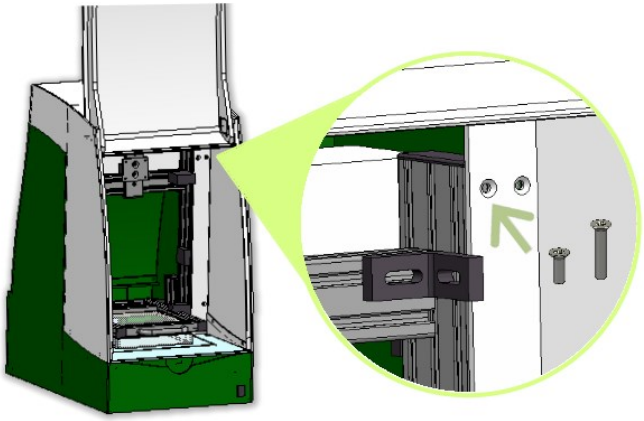
TOP CASING

Remove the top casing, for example, when you need to replace the camera.

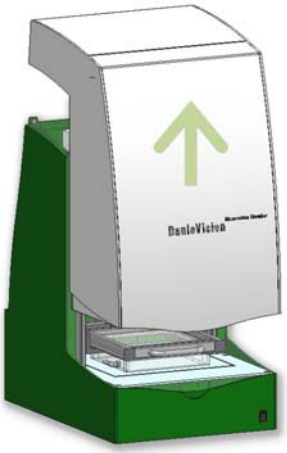
1. **OPTIONAL** Remove the bench plate first (page 116), this makes the following steps easier.
2. Remove the eight screws as indicated in the picture below.



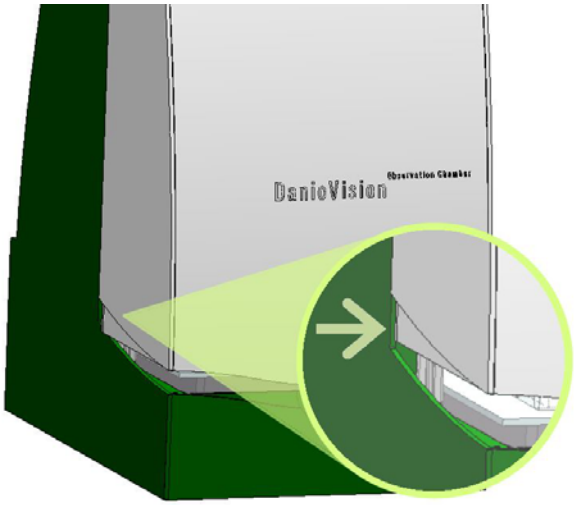
Note that the screws located more internally are shorter than the others. These are attached to the aluminium frame.



3. Close the lid and lift the top casing.



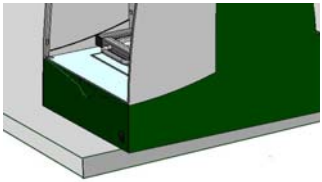
4. When putting the top casing back in place, make sure that you insert the sides into the bottom casing as indicated in the picture below. Then, tighten all the screws.

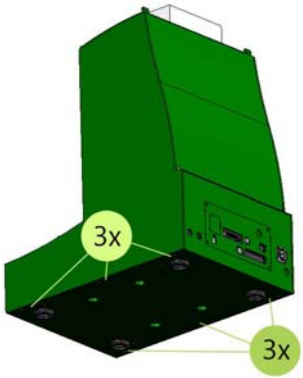


SIDES AND BACK CASING

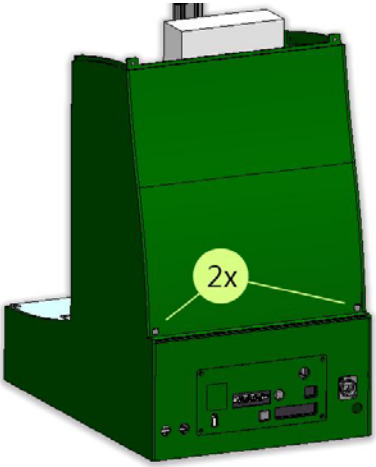
Remove the sides and back casing to access the connector box, wirings and internal tubings. In most cases you do not need to do so.

1. Follow the instructions on page 117 to remove the top casing.
2. Put the DanioVision Observation Chamber just over at the edge of the table and remove the three screws from the bottom plate. Repeat this step for the other side.





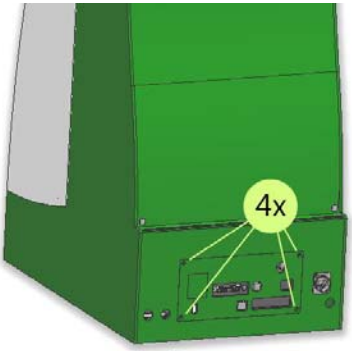
3. Remove the two screws at the back as indicated in the picture below.



4. You can now lift the casing.

CONNECTOR PANEL

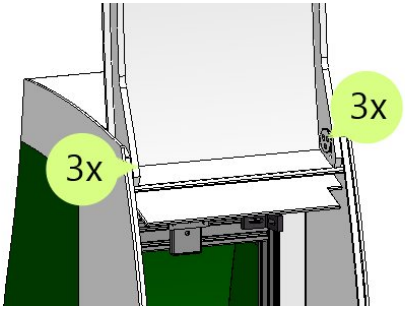
To remove the connector panel at the back of the observation chamber, remove the four screws as indicated in the picture below.



ADJUSTING LID FRICTION

You can adjust the friction of the Observation chamber lid.

If the lid falls down easily or opens with much friction, it can be adjusted by loosening or tightening the three screws per hinge.

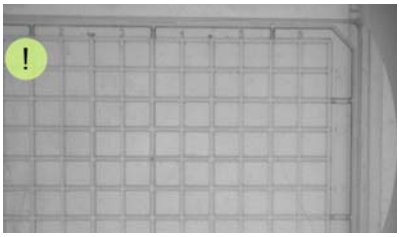


B Fine camera adjustments

Each DanioVision system is thoroughly checked and set to its optimal configuration. However, transport and handling of the system may sometimes cause slight changes in the camera position relative to the well plate, resulting in a sub-optimal video image. This section helps you restore the original settings. To open the DanioVision casing, see page 116.

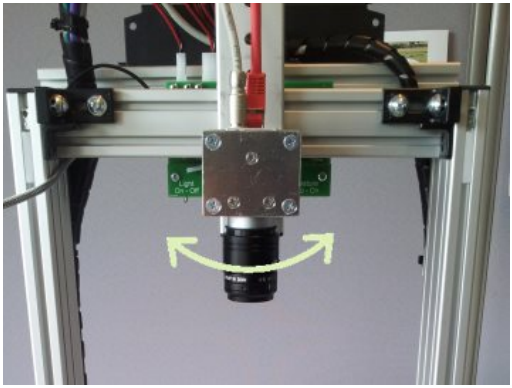
CENTERING THE WELL PLATE HORIZONTALLY

If the well plate is not centered horizontally:



1. Loosen the washer located behind the camera bracket, under the camera profile.

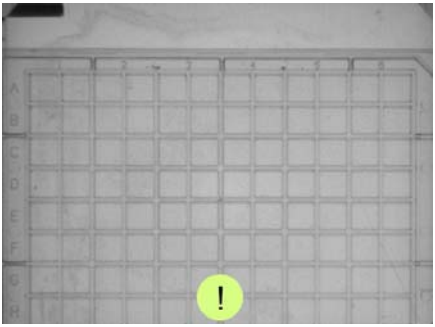
2. Turn the camera in the direction required until the well plate is in the middle of the camera view.



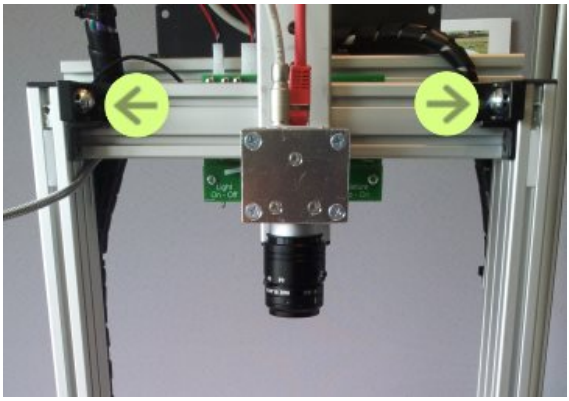
3. Tighten the washer to fix the camera in the new position.

CENTERING THE WELL PLATE VERTICALLY

If the camera is rotated forward or backward in its vertical plane, the video image may look like this:



1. Loosen the two bolts at the sides of the camera profile.

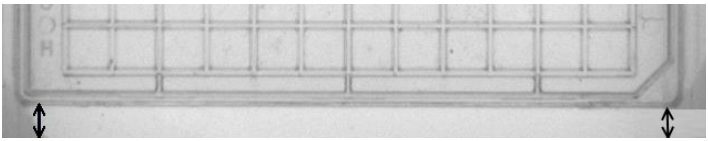


2. Rotate the camera bracket forward or backward until the well plate is centered vertically in the video image.

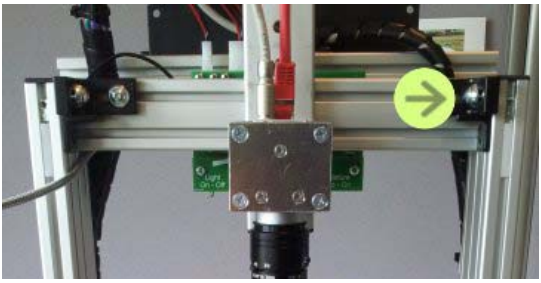
3. Tighten the bolts.

IF THE WELL PLATE LOOKS ROTATED

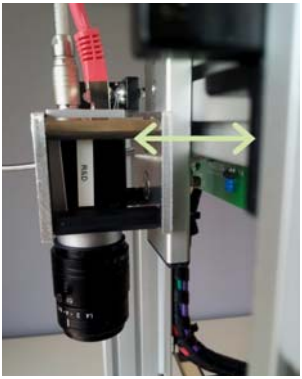
If the margin of the well parallel is not parallel to the margin of the video image:



1. Open the DanioVision Observation Chamber and locate one of the bolts at the side of the camera profile (either left or right):



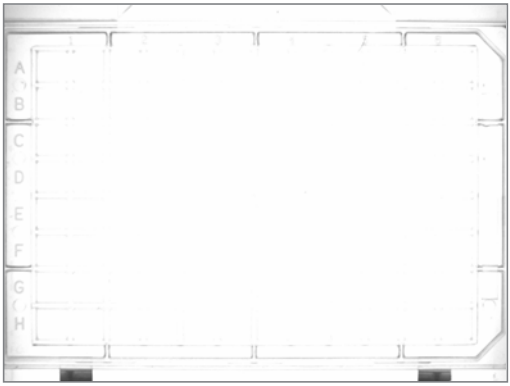
2. Loose the bolt using a 3-mm Allen key.
3. Gently move the camera bracket forward or backward until the well plate is in the correct orientation. When ready, tighten the bolt.



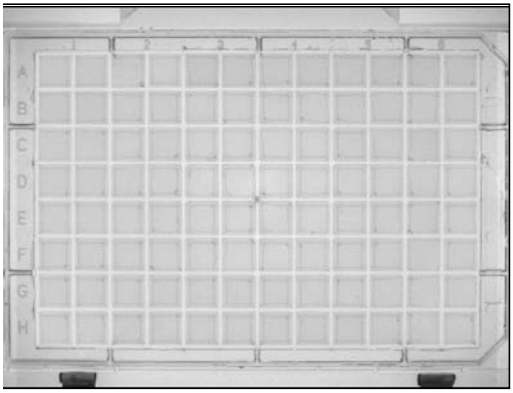
EXAMPLE OF A GOOD VIDEO IMAGE

Check that the well plate fills the video image as much as possible, with the letters A-H and numbers 1-8 well visible.

Set the camera lens to maximum aperture. The image is overexposed when no water is present.



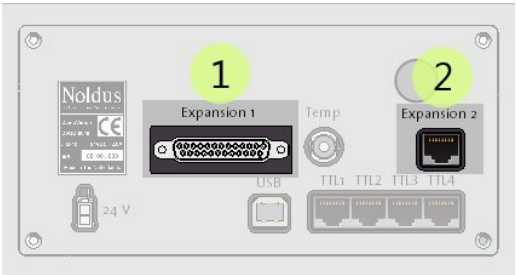
Below an example of a good video image when water is present:



Tighten the three screws on the camera lens once the image is optimized.

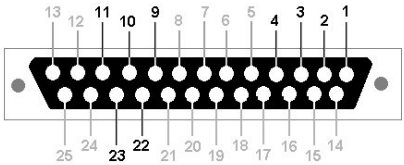
C Additional hardware

DanioVision offers additional connectors to attach and control custom hardware. These are **Expansion 1** and **Expansion 2** located on the back panel.



EXPANSION 1

Expansion 1 is a female 25-pin DB connector. It provides four additional **TTL out** lines to control external hardware and input/output lines to trigger the DanioVision camera (for example at a specific event). Numbers in black indicate pins currently available.

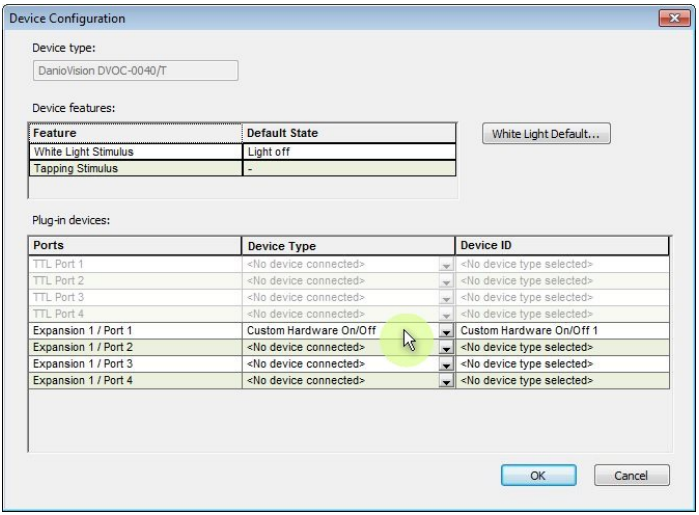


Pin number	Description
1	TTL-out port 1
2	TTL-out port 2
3	TTL-out port 3

4	TTL-out port 4
5 - 8	Not available
9	Common emitter for TTL-out ports
10	Common emitter for TTL-out ports
11	I/O output camera
12-21	Not available
22	GND I/O camera
23	I/O input camera
24-25	Not available

TTL-out ports are open collector outputs with a common emitter on pin 9 and 10. Voltage range 0 to 5 V.

Specify the custom hardware in the corresponding row of the **Device Configuration** window. For details, see **EXPERIMENT SETTINGS**.

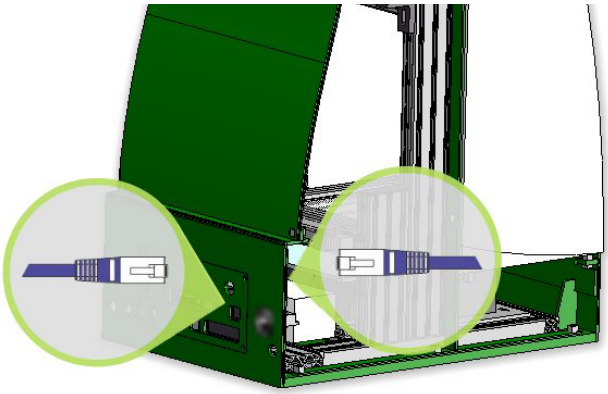


For more technical details and how to use these connections, contact Noldus Information Technology.

EXPANSION 2

Expansion 2 is a 8-pin modular RJ45 feed through connector.

You can use it to connect internal add-on hardware and get external signals in the DanioVision Observation Chamber.



D System specifications

DANIOVISION DVOC-0041

Power supply

- Power requirements: 24 Volt DC, 2.0Amp (max)
- Mains adapter: input 100-240 Volt AC, output 24 Volt DC, 3.0Amp

Camera

- Brand, type: 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

Lens

- 12 mm Megapixel, C-mount, F1.4, includes IR pass filter
- Optional lens: 8 mm Megapixel, C-mount, F1.4, includes IR pass filter

Video performance (H x V pixels)

- Default: 1280 x 960 at 30 fps (in combination with 12 mm lens)
- Optional:
  - 640 x 480 at 60 fps (in combination with 12 mm lens)
  - 800 x 600 at 60 fps (in combination with 8 mm lens)

Maximum frame rates as measured in 96 well plate setup.

Backlight unit

- Combined infrared (IR) and white light array
- Infrared wavelength (typical): 950 nm

According to published research, zebrafish are sensitive to infrared light, though not above 910 nm. See Shcherbakov *et al*. 2013, *PLoS ONE* **8**(5): e64429. The IR light provided by DanioVision is therefore unlikely to affect the fish's behavior.

- White light color temperature (typical): 5500°K
- White light level controllable via USB (EthoVision XT). Intensity range: 0 to ±10000 lux (light level measured directly at the bottom of the water basin).

  With the *standard* White Light settings, the light intensities of the white light are approximately:

Percentage	Steps	Intensity (lux)
5	207	139
25	1000	2500
50	2100	5300
75	3000	8000
100	4095	10500

NOTE If you set the *Low Light* switch to **L** (low), the highest light level (100%) is around 20 lux. See **CONFIGURE THE DANIOVISION WHITE LIGHT** on page 36 for more information.

NOTE Light intensity in DVOC-0041 can be set higher than in DVOC-0040. Here the reference values if you have DVOC-0040:

Percentage	Steps	Intensity (lux)
1%	40	70
25%	1025	1450
50%	2050	2950
75%	3000	4400
100%	4096	5450

You may want to replicate light conditions that you set in DanioVision DVOC-0040 in a new experiment with DanioVision DVOC-0041. For example, if you set light intensity to 50% in

DanioVision DVOC-0040, this gave 2950 lux. The percentage to be set in DanioVision DVOC-0041 is (2950/5300)*50 = approx. 28%.

Multi basin

- Water inlet, water outlet and overflow
- Basin size 158 x 132 mm
- Supports ANSI, SBS compatible micro plates (L =127.76± 0.5 mm (5 1/32" ± 1/64"), W=85.48 ± 0.5 mm (3 23/64" ± 1/64") with maximum height of 27mm (1 1/16")
- Supports Petri dishes up to 90 mm in diameter
- Temperature sensor supporting the use of Noldus DVTCU (Temperature Control Unit)

Water connections

- Water inlet/outlet: tube fitting 8 mm
- Water overflow: tube fitting 10 mm

Tubes included

- 1.5 m Clear PVC Hose, 8 mm OD, 5 mm ID for water inlet (green color marked)
- 1.5 m Clear PVC Hose, 8 mm OD, 5 mm ID for water outlet (yellow color marked)
- 1.5 m Clear PVC Hose, 10 mm OD, 8 mm ID for water overflow (red color marked)

PC interface

- USB-2 compatible

External I/O

- TTL input/output (4x modular RJ45 connector):
  - 4 x 2 TTL input
  - 4 x 2 TTL output

- TTL expansion port 1:
  - 4 x TTL output (open collector output)
  - 1 x input camera
  - 1 x output camera
- TTL expansion port 2 (1x modular RJ45 connector:
  - 8-pin feed through

System start-up time (cold start)

- ~ 15 minutes

Dimensions

- 61 x 32 x 46 cm (24 1/64" x 12 19/32" x 18 7/64") (h x w x d) in closed position
- Door opening 36 x 29 cm (14 11/64" x 11 27/64") (h x w)

Weight

- 15 kg excluding power supply, external tubes and cables

Cleaning the DanioVision Observation Chamber

We recommend to use a propriety glass-cleaning fluid to clean the outside of the DanioVision Observation Chamber.

Declaration of conformity

See page 136.

DANIOVISION TAPPING DEVICE

Power

- Power requirements: 24 Volt DC, 0.4Amp (max)

Features

- Controlled from EthoVision XT 10.1 or later with selectable stimulus intensity (1-8)
- Delivers a discrete 'tap' to the well plate holder
- Maximum tapping rate of ±3 taps per second

Connectors

- Molex 2 pin – Power (24V)
- Molex 4 pin – From Tapping Device to control box
- Multi connector (blue) – For TTL control signal

Dimensions

- Tapping Device (including aluminum bracket): 6 x 24 x 7.5 cm (2 23/64" x 9 29/64" x 2 61/64") (h x w x d)
- Control box: 2.5 x 10 x 6.5 cm (63/64" x 3 15/16" x 2 9/16") (h x w x d)

Weight

- ~ 300g (Tapping Device, bracket, control box)
- If not pre-assembled in the DVOC-0041, the package includes mounting material

Compatible DanioVision Chambers

- DVOC-0040, DVOC-0041

DANIOVISION TOPLIGHT UNIT

White light

> 30.000 lux at 25cm distance (well plate), 1584 lm

Color temperature 4000K

Array of 408 LED's

Max. voltage 18 Volt

Red light

192 lm, wavelength 623 nm

Array of 252 RGB LED's

Max. voltage 24 Volt

Green light

384 lm, wavelength 525 nm

Array of 252 RGB LED's

Max. voltage 24 Volt

Blue light

240 lm, wavelength 470 nm

Array of 252 RGB LED's

Max. voltage 24 Volt

Unit Dimensions

270 x 220 x 30mm (10 $^{5}/_{8}$" x 8 $^{21}/_{32}$" x 1 $^{3}/_{16}$")

DECLARATION OF CONFORMITY

CE

EC declaration of conformity

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

Declares that the following line of products:

DanioVision Observation Chamber DVOC-0041

Fulfills all relevant provisions of the EC EMC directive 2014/30/EU.
According the harmonized standards:

EN 61326-1 (2013)	**Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 1: General requirements**

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**

The signatory on behalf of the manufacturer:

Date: July 15 2020

Name : Jeroen Kemerink
Vice President Research & Development

DECLARATION OF CONFORMITY

CE

Name of company: CINCON ELECTRONICS CO., LTD.
Address: No. 8-1 Fu Kung RD. Fu Hsing Park, Fu Hsing Hsiang, Chang Hua Hsien, Taiwan, R.O.C.

Declares that the product

Adapter

TRH50A120; TRH50A150; TRH50A180; TRH50A190;
TRH50A240; TRH50A280; TRH50A360; TRH50A480;
TRH70A120; TRH70A150; TRH70A180; TRH70A190;
TRH70A240; TRH70A280; TRH70A360; TRH70A480;

referred to this declaration conforms with the standard(s) or directive(s) as far as applicable:

Product Safety Standard	:	EN60950-1	2006+A11+A1+A12+A2
EMC Standards	:	EN55022	2010/AC: 2011 Class B
		EN55032	2012 +AC:2013
		EN55024	2010
		EN61204-3	2000
		EN61000-6-1	2007
		EN61000-6-3	2007+A1: 2011+AC: 2012
		EN61000-3-2	2014
		EN61000-3-3	2013
Directives	:	Low Voltage Directive	2014/35/EU
		EMC Directives	2014/30/EU
		ErP Directives	2009/125/EU
		RoHS Directive	2011/65/EU

This product must be used within other equipment and must not operated as a stand alone product.

The company named above will keep on file for review the following technical documentation:

- Technical drawings
- Other technical documentation

Manufacturer Signature: Johnson cheng

Date: Mar. 03 2017

Name: Johnson Cheng / President