Panlab
An Affiliate of Harvard Bioscience, Inc.

USER MANUAL

SMARTIO 2.5.01

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Welcome

Congratulations on joining the community of users worldwide who rely on Harvard Bioscience products to perform preclinical physiologic research. Thank you for your interest in Harvard Bioscience products. We are committed to providing you with quality products and services.
This manual will help you get to know your SMARTIO 2.5 software for your video tracking and laser optogenetic system. The structure of the manual was designed to sequentially guide you through setting up your SMARTIO software.

WHAT YOU WILL BE LEARNING

- Install and set up your video tracking and laser optogenetic system.
- Start and configure a new experiment.
- Acquire, analyze, and visualize data.

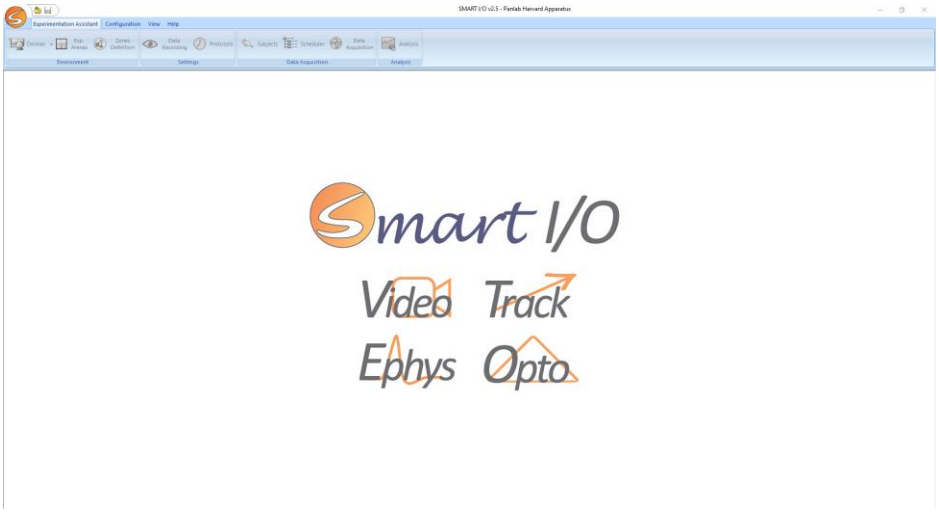
Scientific Background

SMARTIO is **the specific** evolution of the SMART 3.0 video-tracking software adapted for combining the video recording and video tracking functions of the SMART 3.0 with neural recording and stimulation (electrical and/or optogenetics). **The version 2.5 includes several improvements:**

- The possibility to record videos and from up to 16 image sources at the same time, thanks to the integration of SMARTIO 2.5 with Record-IT! Media 1.0.03.
- The possibility to acquire tracking data from up to 8 image sources at the same time.
- The possibility to integrate the tracking data with neural recording and stimulation (electrical and/or optogenetics) in up to 8 image sources at the same time.

Application Overview

Commercially, the SMARTIO functions are available through the SMARTIOBASIC package, including SMARTIO license and customized (CS), global activity (GA) and TriWise (TW) extensions, and the SMARTIOSUPER packages, including everything included in the SMARTIOBASIC package plus the Multiple Arenas (MA) extension.



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The SMARTIOBASIC and SMARTIOSUPER provides the following specific functions:

Video recording (up to 16 image sources).

Video tracking (up to 8 image sources).

Combined behavior and neural recording (Ephys) (up to 8 image sources).

Combined behavior and Optogenetics (Opto) (up to 8 image sources).

Please find below a list of equipment that can be combined with the SMARTIO system.

Multichannel Systems (MCS) wireless neural recording system (https://www.multichannelsystems.com/products/wireless-systems)

Third-party stimulator (ex: laser optogenetics stimulator)

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1. SMARTIO 2.5 AND SMART 3.0

The SMARTIO platform uses many of the functions provided by the SMART Video Tracking V3.0. The combination of the classical video recording/tracking process with optogenetic stimulation (laser) has implied a structural change in the application making it independent from the existing SMART video Tracking V3.0.
The experimental file and related files generated by the application are provided with a new extension: *.smep followed by a letter identifying each file.

File	Extension
Experimental file	*.smepe
Configuration file	* smepq
Zone files	*.smepz

	**Important**: The experimental files generated by SMARTIO are not compatible (cannot be opened) with SMART Video-Tracking 3.0 versions or inferiors and vice-versa.
	SMART and SMARTIO need 2 different licenses (separate USB Dongle USB Key).

Similar to SMART Video-Tracking 3.0, SMARTIO structure is internally composed of a platform that can be associated to experimental modules and extensions. The experimental modules provide specific tools to fit the specifications of the experimental paradigm. These tools include predefined zone templates, stop conditions and advanced analysis reports and calculations.
Combined with one or more modules, the extensions provide additional capacities/performance to the video-tracking system.

- **SMARTIO CS**

This is the standard experimental module associated with the SMARTIO platform.

- **SMARTIO GA**

SMARTIO-GA is the Global Activity extension to extend the data acquisition process not only to track the position of the animal but also to detect animal movements even without animal's displacement.

- **SMARTIO TW**

SMARTIO-TW enables advanced animal detection/tracking capabilities (other than the standard center-of-mass tracking):

- the TriWise® technology which enables SMARTIO to automatically track the subject's head, center and tail-base. This information is essential to refine the zone transition and permanence calculations, to enable the automatic detection of rearing, rotation and stretching and to calculate other advanced analysis parameters.
- the Color detection technology which enables SMARTIO to track animals marked with a color. This information is essential for tracking a specific part of the animal body or for a 100% reliable identification of the subjects in a Social Interaction test.

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- **SMARTIO MA**

SMARTIO-MA is the Multiple Arenas extension. SMARTIO-MA makes it possible to simultaneously track subjects independently in multiple separated arenas, boosting thus the productivity of your lab. Only your computer's performance limits SMARTIO!

Modules and extensions included in the SMARTIOBASIC and SMARTIOSUPER packages are listed in the following table:

	SMARTIOBASIC	SMARTIOSUPER
SMARTIO CS	•	•
SMARTIO GA	•	•
SMARTIO TW	•	•
SMARTIO MA		•

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2. INSTALLATION OVERVIEW

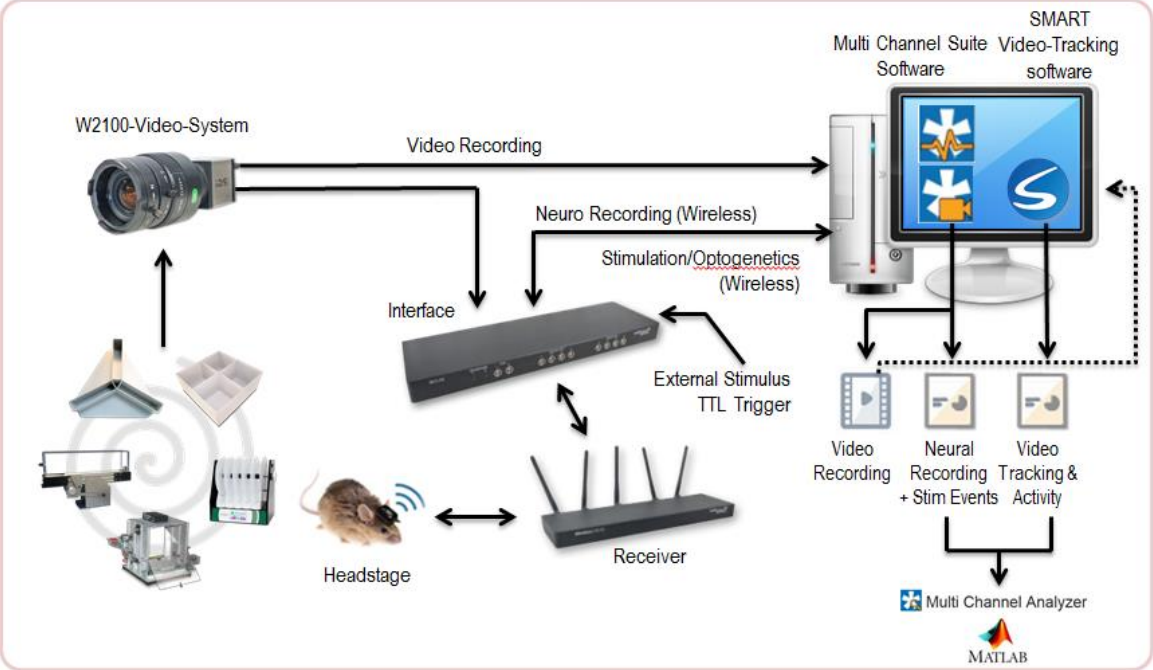
2.1. COMPATIBILITY WITH MCS *IN VIVO* ELECTROPHYSIOLOGY SYSTEM

SMARTIO can also be used with the Multichannel System MCS wireless neural recording and stimulation system.

multichannel systems

- o W2100 neural recording and stimulation system
- o Multi Channel Suite software

Web:
https://www.multichannelsystems.com/products/wireless-systems#products).
https://www.multichannelsystems.com/products/multi-channel-suite



In this configuration, the systems are used as following:

- The MCS system records both the video of the experiment and the neural data at the same time.
- The video file is then analyzed off-line by SMARTIO to get the behavioral data report.
- The neural data, video file and behavioral data reports are consolidated in Multi Channel Analyzer.
- Further analysis and correlation can then be carried out in other statistical software such as Matlab if needed.

2.2. COMPATIBLE LASER OPTOGENETIC SYSTEMS

SMARTIO is also able to interact with the Panlab Linkbox01 interface units (Legacy and/or High-Speed versions) to provide 2 output TTL signals able to trigger an external device such as an optogenetics laser stimulator.
Specific adapter cables would be needed to connect the Panlab Linkbox01 interface to the optogenetics laser stimulator. The adapter cables are not provided with the SMARTIO system because these adapters would depend on the optogenetics laser stimulator device used for the experiment. Panlab technical support should be contacted to discuss this point before purchasing the SMARTIO software or Panlab Linkbox01 interface.

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2.3. SYSTEM REQUIREMENTS

The computer must fulfill the following specifications:

- Intel Core™ i5-6700 for other applications (Celeron processor not supported).
- 8 GB RAM or superior.
- 1 TB hard drive or superior (minimum 500 GB).
- Display: 1280 x 720 pixels and 32-bit true color. Screen text size must be set at 96 DPI (100%).
- USB ports
- One free USB 2.0 port would be used for the software License and installation tools USB key
- One free USB 3.0 port is needed for the connection of the camera (direct USB connection or through an analog/digital converter)
- The Teleswitch unit requires an additional free USB 2.0 port to work. Please refer to chapter 16.4 - TELESWITCH SETTINGS for more details on how to connect and configure the Teleswitch device
- One free USB 2.0 would be needed when SMARTIO is combined with an external Optogenetics laser device that would require the connection of the Panlab Linkbox01.

We strongly recommend the use of a desktop PC to acquire data. The use of a laptop should be only limited to the analysis of the acquired data or to acquire data with low demand of resources. In this case, please be sure to set your laptop in “High performance” mode, disabling the Energy saving mode.

Supported operating systems:

- Microsoft® Windows® 11 64-bit.

Video sources:

- Live image sources supported: see chapter 7 DEVICES
- Digital video files: SMARTIO is provided with an embedded digital video file recorder. SMARTIO is also fully compatible with the range of RECORD-IT!® digital video recording solutions provided by Panlab Harvard Apparatus, including the Record-it! Media software.

In case the digital video files were not recorded with RECORD-IT!® technology, check that the video would be compatible with SMARTIO. The corresponding CODEC used to record the video file must be installed in the SMARTIO computer to process the specific file format. Please refer to chapter 3.9 - CODECS AND SUPPORTED VIDEO FILES for more details.

Third-party software required:

- Microsoft Excel® 2010 or higher (only for reports and data exported in Excel format).

Microsoft® Office 32bit is not compatible with reports generated with SMARTIO. Please check that the version installed is 64 bits.

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2.4. INSTALLING THE SOFTWARE

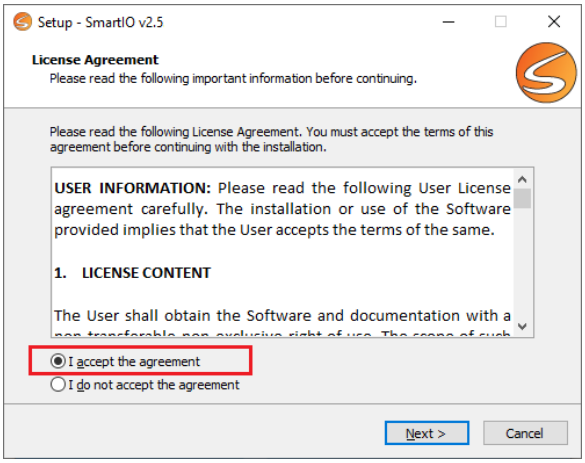
SMARTIO software and license are delivered within a single USB flash key. The key contains the software installation tool, this User's Manual in PDF format and other components required to work in specific conditions.

⚠	Due to security reasons of the Windows® operating system, you will need to have the administrative rights to install the software and other components. Please contact your IT staff before installing the software.

To install SMARTIO 2.5, follow the next steps:

1. Plug the USB flash key in a free USB 2.0 port of your computer and wait until Windows® installs it as a new removable drive.
2. Access the new removable drive detected and execute the PANLAB.EXE file.
3. Click on the "Install SMART I/O v2.5" option.

4. An installation wizard will be shown. Read carefully the "License Agreement" statement and select the "I accept the agreement" to continue the installation of SMARTIO. Then click the [Next] button to start the installation.



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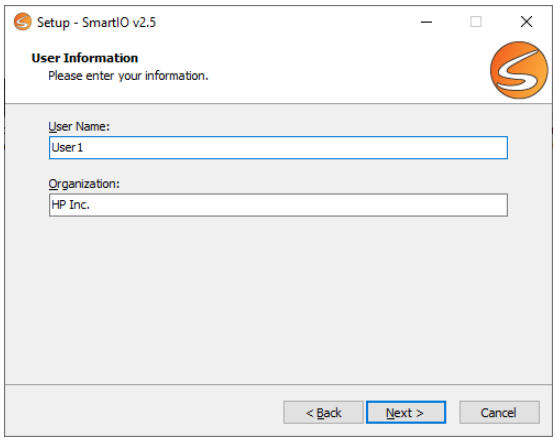
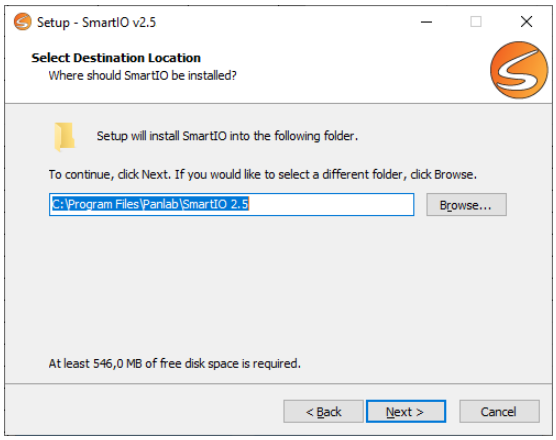
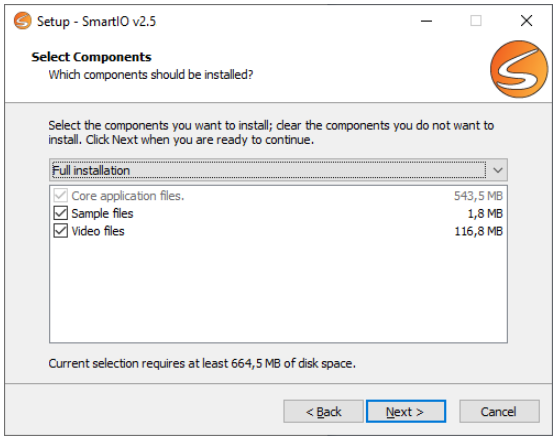
5. Enter the name of the user and the company in the correct field. Click [Next] button to continue.



6. During the installation, the software will be installed in a new folder called [Panlab\SmartIO v2.5] created, by default, under the "Program Files" folder of your hard disk. However, it is possible to choose a different location to install the software. This is independent of the data folder, which will be defined later. Click the [Next] button to continue.



7. Select the type of installation you need:



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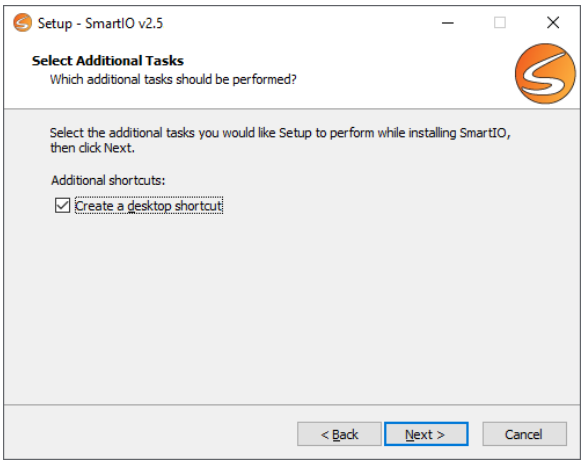
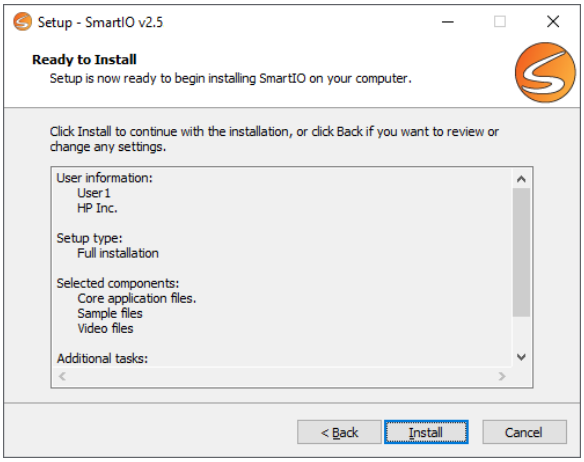
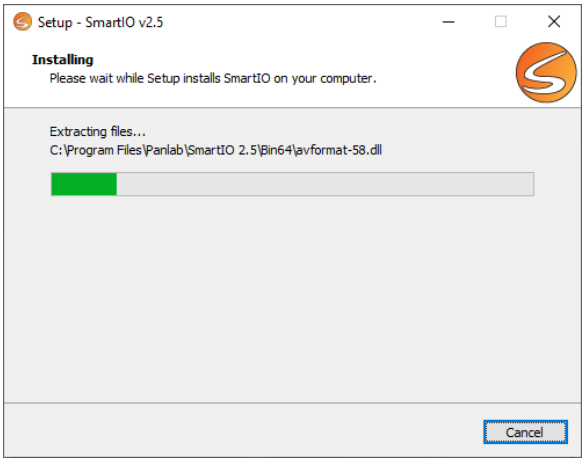
FULL	Install SMARTIO and a set of video sample files.
COMPACT	Install only SMARTIO
CUSTOM	Install SMARTIO and allows you to select whether to install video sample files or experiment samples to your hard disk.

8. Click the [Next] button to continue and select the Create a desktop icon option from the Select Additional Tasks window.



9. Click the [Next] button to continue. In the Ready to install window, click the [Install] button.





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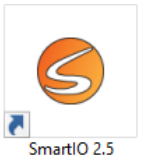
10. Click the “Finish” button to end the installation process.

A new shortcut will be shown on your desktop once the Installation process is finished.



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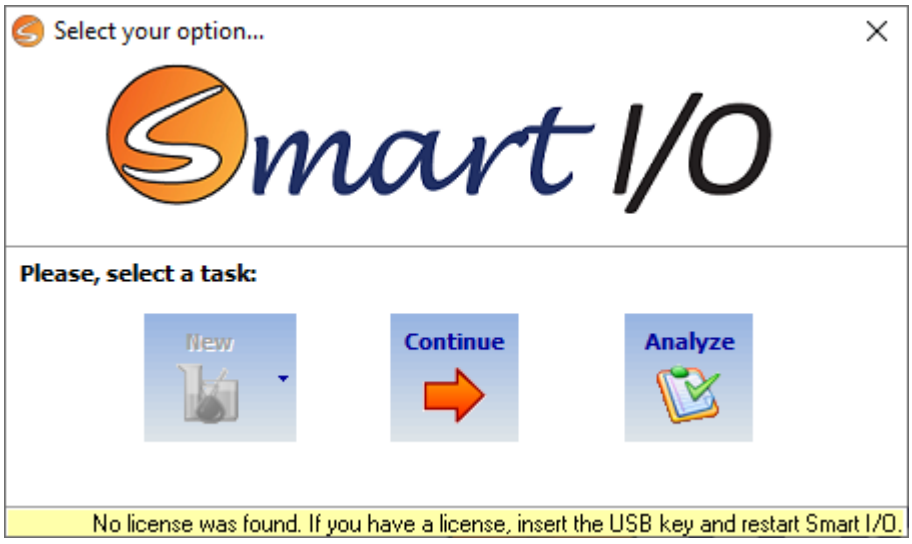
2.5. THE USB FLASH KEY

The USB flash key also serves as a software protection key, used to prevent software from unlawful use. Only one key is provided per purchased license, so it is not possible to record data on more than one computer at a time with one single key.

⚠	Please DO NOT rename the USB unit (its name should always be PBLICENSE).
	Please DO NOT remove or modify any file stored within the USB flash key, especially the regkey.dat file which stores the information regarding your license of use.

Please check that the key is plugged in before SMARTIO is started. If no protection key is present, data acquisition will not be possible.



2.6. INSTALLING THE REMOTE-CONTROL UNIT

If a wireless remote-control unit (a.k.a. Teleswitch) was acquired, please refer to the chapter 16.4 - TELESWITCH SETTINGS for more details on how to install and use it.

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3. INSTALLING THE IMAGE SOURCE

SMARTIO processes the sequence of images (frames) coming from a source, synchronizes the image with electrophysiological data and extracts the information related to the animal behavior (position, speed, events, etc.).

SMARTIO can process images coming from two types of sources:

- Live image, using the external image sources provided by the camera or from live image simulator provided with the system.
- Digital video files.

3.1. LIVE IMAGE SOURCES

Live image sources are external devices that provide a sequence of images at the same time in which the animal is moving within the arena. SMARTIO supports image sources with a framerate of up to 40 frames per second (fps) depending on the type of the image source. Each frame is generated by converting the color of the real scene into a matrix of digital values called pixels. SMARTIO analyzes each pixel of each frame to get all the information and provides it to you in a more convenient manner.

Live image source	Cost	Quality	Flexibility
Digital Camera	Medium- High	High	Medium
Analog-digital converter	Low	Medium	Medium
Webcam	Low	Medium-High	Low

SMARTIO also provides some simulated image sources, so that the application can be used in live source mode without the need of connecting a camera (see chapter 7.1 - Live image selection).

Camera considerations

SMARTIO can be used with a wide variety of standard analog cameras or user-defined image sources (Infrared cameras, camcorder, WIA-compliant USB camera, webcams, etc.). The choice of the camera depends on the requirements of your experimental setup.

Live Image Source	Max. Frame Rate
Digital Camera	40 fps
Analog-digital video converter	25 fps
Webcam	16 fps

The functions of SMARTIO strictly depend on the number and type of cameras used in each experiment:

	VIDEO	TRACK	EPHYS/OPTO	
Webcam	Up to 4 cameras	Up to 4 cameras	Not recommended	(see chapter 3.2)
Digital USB 3.0 Color and MC	Up to 16 cameras	Up to 8 cameras	Up to 8 cameras	(see chapter 3.3)
Digital USB 3.0 NIR	Up to 6 cameras	Up to 6 cameras	Up to 6 cameras	(see chapter 3.4)
Digital USB 2.0 Color and MC	Up to 16 cameras	Up to 8 cameras	Up to 8 cameras	(see chapter 3.5)

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Digital USB 2.0 NIR	1 camera	1 camera	1 camera	(see chapter 3.5)
Network cameras	Up to 16 cameras	Up to 8 cameras	Not recommended	(see chapter 3.5)

- **Frame rate**

A suitable frame rate for rat and mice tracking is 16 frames/sec (16 fps). SMARTIO increases this rate up to 40 fps (depending on the live image device used) so that the requirements of many of your experimental projects are fulfilled more than enough. However, both the camera and the recording unit used with the system should allow playing/recording the experiment with this rate. The standard image sources and recorders provided together with SMARTIO have been thoroughly tested by Panlab Harvard Apparatus to assure that they fulfill this requirement.

A higher frame rate can be achieved by recording a video with a higher frame rate and then processing it with SMARTIO. For more details about important factors to be considered while recording digital video files, please refer to chapter 16.2 - RECORDING SETTINGS and chapter 7.1 - IMAGE SOURCES).

- **Resolution and Angle of Vision (lens)**

The quality of the detection of the subject to be tracked will depend on the resolution of the camera, the maximal angle of vision of the lens installed and the distance between the camera and the experimental area. The subject must be "big" enough (and lighting conditions should be quite good to reduce the use of filters) to be detected and tracked. The standard camera provided by Panlab is associated with a standard lens that have maximal angle of vision of 90 degrees (focal length of 3 mm) radial of the camera. This angle of vision is suitable for most of the standard experiment conditions as it allows receiving the image of a 4 x 4 m area in a room with 2.5 m ceiling height. SMARTIO does not work with tangential distortion and the radial of the camera. For this reason, we recommend the camera to be as far as possible from the arena in a zenithal position, avoiding the use of wide-angle lenses.

- **Color / B&W / Infra-red**

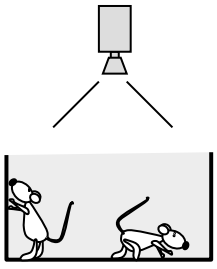
Standard analog cameras provided with SMARTIO are in color, but this is not mandatory for the experiment. However, the compatibility among all the devices in your setup must be assured so please contact your dealer to select the best option for your specific needs. It is very important to optimize the contrast between the subject and the experimental background to ensure optimal detection and tracking. Many times, in standard experiments involving mazes, a better contrast is obtained with B&W cameras. Infrared cameras are recommended when working with very low lighting conditions or in complete darkness.

- **Lens adjustments**

When working with a camcorder, it is very important to deactivate any automatic adjustment (aperture, zoom, focus) because the lens settings must remain unchanged during the whole tracking process.

- **Camera positioning**

The camera must be fixed above the experimental area in a zenithal position ensuring that it will stay immobile throughout the whole experiment. Any movement of the camera during the experiment may strongly impact the tracking process. The light obstructer, the zoom and focus of the camera must be set so to obtain an image as the more defined as possible. In case of using digital webcams, disable any automatic adjustment (such as auto-focus or lighting auto-adjustment). All these settings must remain unchanged during the tracking process.



It is strongly recommended to record the image of the experiment in a video digital file using the RECORD-IT! solutions provided by Panlab Harvard Apparatus. These options provide an

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opportunity to open the video of the experiment again afterwards and to track the animal with other detection settings.

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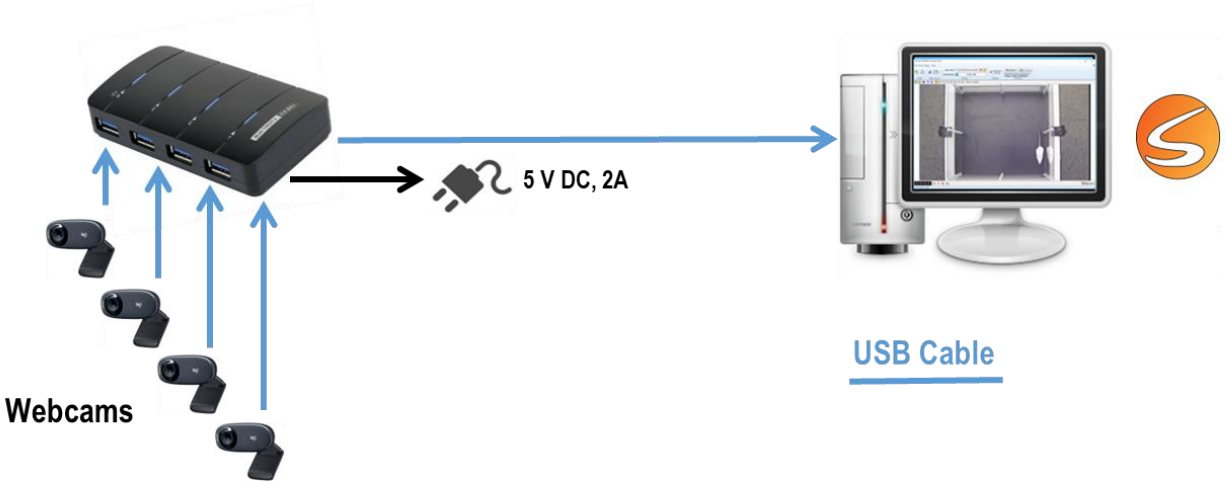
3.2. WEBCAM

⚠	Webcams should be used only for recording or video tracking without TTL triggering (Ephys and Opto functions), due to a possible delay between the animal's movement and the image source.

Standard USB webcams are one of the commonly used live image sources. They offer a reasonable trade-off between image quality and cost. However, due to technical limitations of the USB communication protocol, webcams cannot be used with long distances between the computer and the arenas. SMARTIO can record from up to 4 webcams simultaneously at 16 fps as maximum frame rate. If more than one webcam is to be connected, we recommend use of a specific 4-port USB Hub (Renkforce; 4 Port USB 3.0 HUB).
From Panlab-Harvard Apparatus, we recommend the use of the Logitech HD C9xx series of webcams.
Please refer to the manufacturer instructions to install the drivers of the webcam and avoid installing the generic drivers that Windows may try to install by default. Other standard USB webcams may be installed following a similar procedure.



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3.3. DIGITAL USB 3.0 COLOR AND MONOCHROME CAMERAS

Even though SMARTIO can work with several models of digital cameras (provided that the correct driver is correctly installed), we only ensure total compatibility with the digital USB 3.0 cameras manufactured by Basler.

⚠	Connecting a USB 2.0 camera to a USB 3.0 port will not increase the performance of the camera. Similarly, connecting a USB 3.0 camera to a USB 2.0 port will make the camera work as a 2.0 camera.

Depending on the configuration purchased we provide 3 USB cable length options with our cameras: 3 m, 5 m or 8 m (5 m cable with a 3 m extension cable).

Using a USB Hub to expand a USB port to 4 ports, it will be possible to connect up to 4 Digital USB 3.0 cameras simultaneously using SMARTIO. On the other hand, to connect more than 4 cameras, an expansion card (PCIe) will be necessary. For example, one PCIe connected to 4 USB Hub will allow to connect up to 16 Digital USB 3.0 cameras simultaneously. In this case, please follow the manufacturer instructions to install it. Please be aware that you will need to power the PCIe connecting it to a cable from the power unit within the PC, so it would be better to use a slot near the power unit. Ask your IT Department if you need further assistance.

⚠	Up to 16 Digital USB 3.0 cameras can be used for video recording only. For video tracking, with or without TTL triggering (Ephys and Opto functions), the maximum number of cameras that can be used simultaneously is 8.

Table 1 reports the maximum frame rate allowed for each camera model, depending on the number of cameras simultaneously connected:

Table 1: USB 3.0 Color and Monochrome cameras

REFERENCE	SENSOR	USB	RESOLUTION	FPS per number of cameras			
				1	2-4†	5-8*†	9-16*† (only recording)
CAMDC3COLOR	Color	3.0	1280X960	25 fps	20 fps	-	-
			640X480	25 fps	20 fps	16 fps	16 fps
CAMDC3BW	Monochrome	3.0	1280X960	25 fps	20 fps	-	-
			640X480	25 fps	20 fps	16 fps	16 fps

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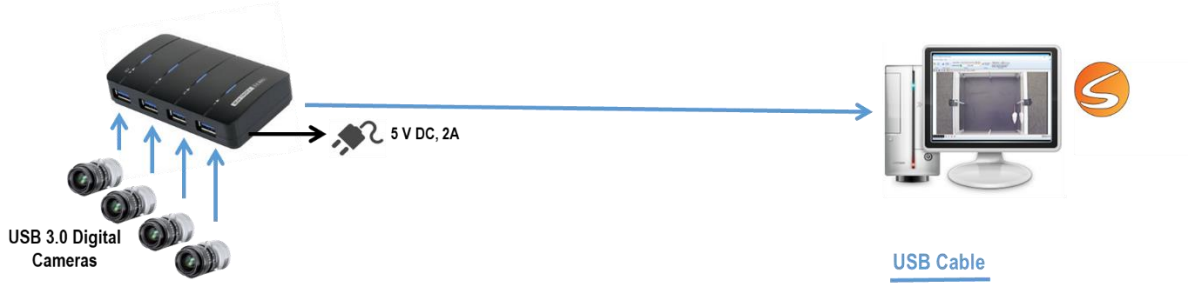
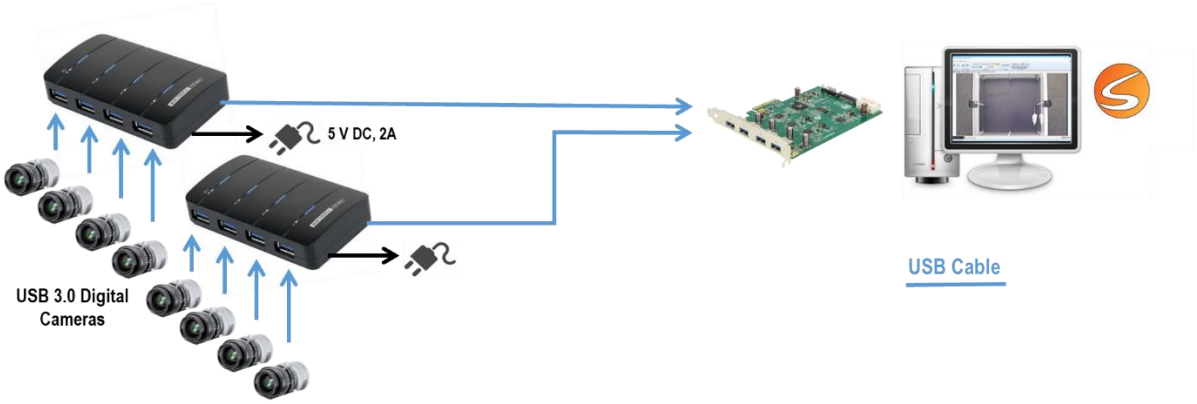
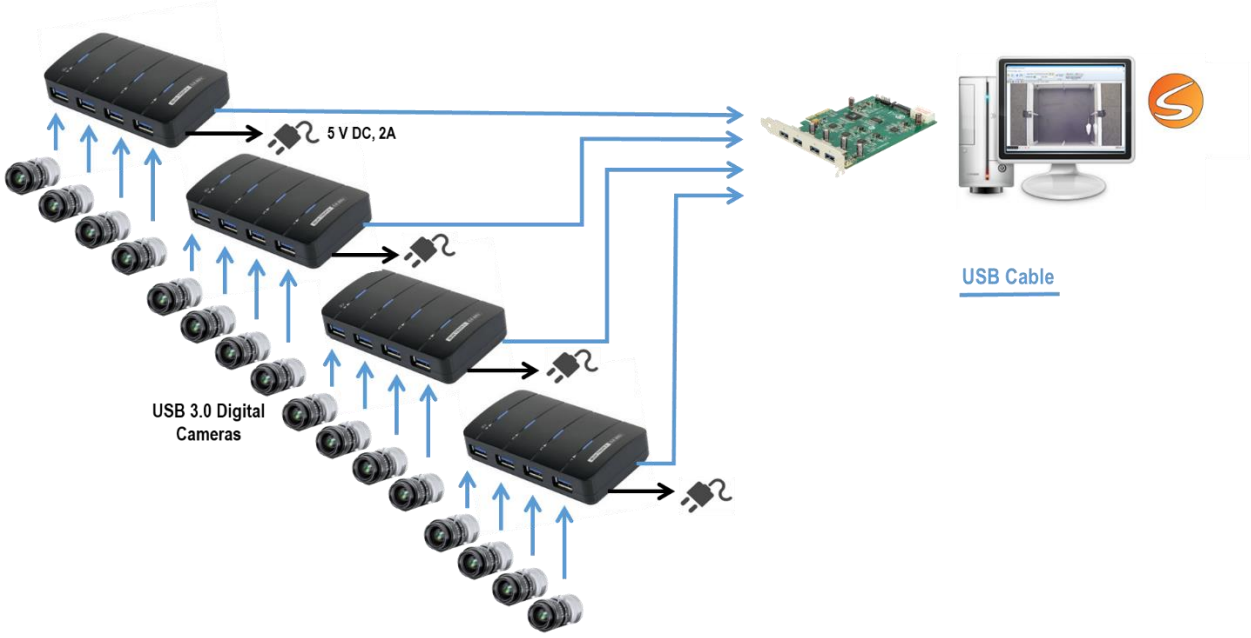
†Need USB 3.0 Hub
*Need USB 3.0 PCIe







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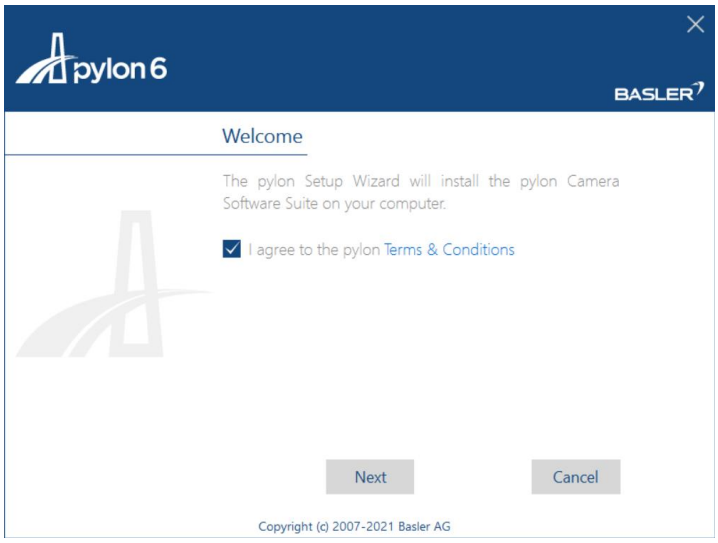
Installing Pylon Viewer

1. Unpack the camera.

⚠	IMPORTANT: Do not plug the camera into any USB port before step 7 of this installation guide.
	IMPORTANT: Uninstall any previous version of Pylon Viewer present on the PC before proceeding

2. Please make sure to have administrative privileges for the computer where the device will be installed. Contact your IT staff to ensure you have administrative rights before continuing with this procedure.
3. Plug the installation key of SMARTIO into a USB port and launch the installation tool (PANLAB.EXE).
4. Select the **Hardware Drivers** option.

5. Enter in the **Digital Cameras\Basler Digital 3.0 - Basler GigE Vision** folder and execute the file.
6. Read and accept the **Terms & Conditions**. Then click on **Next**.



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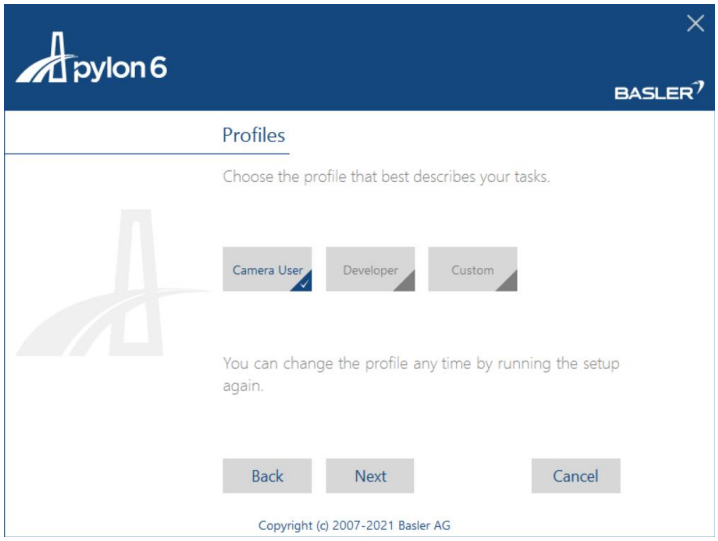
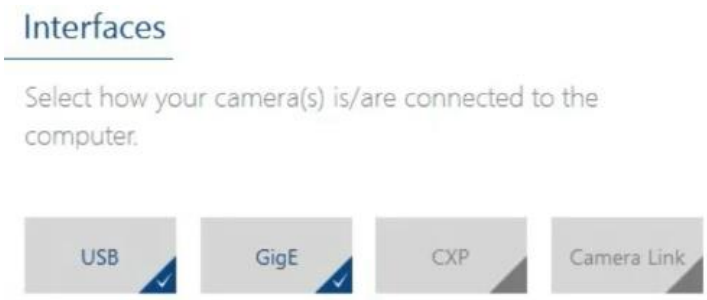
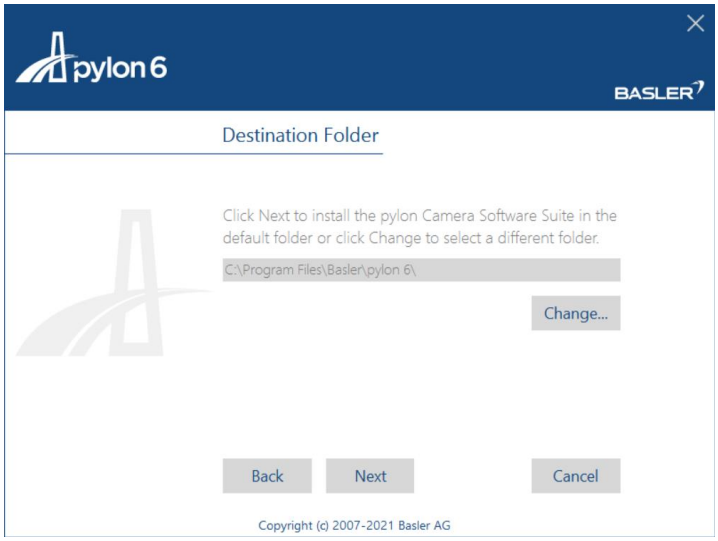
7. Select the **Camera User** profile.



8. In the Interfaces window, please be sure to only select **USB** and **GigE**. To avoid lagging problems during the use of the camera in SMARTIO, do not select CXP or Camera Link. Click on **Next**.



9. Specify the destination folder and click on **Next.**



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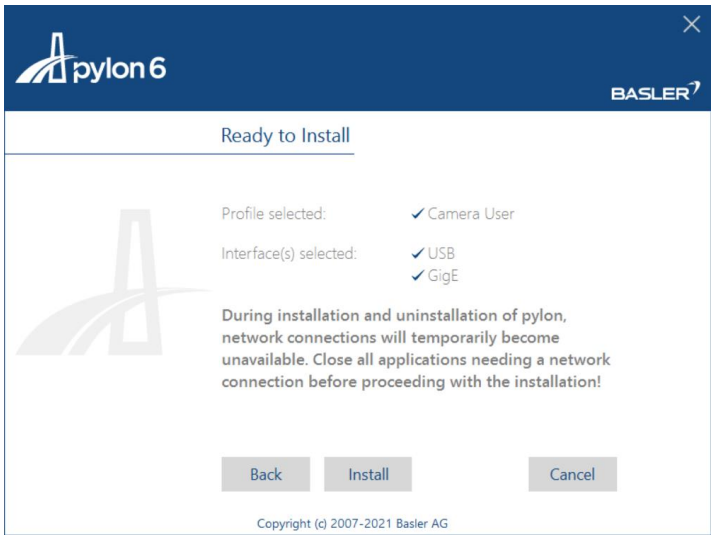
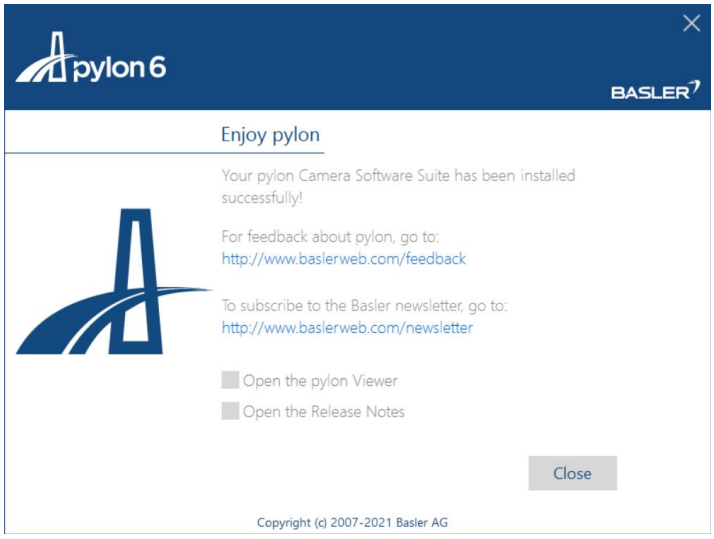
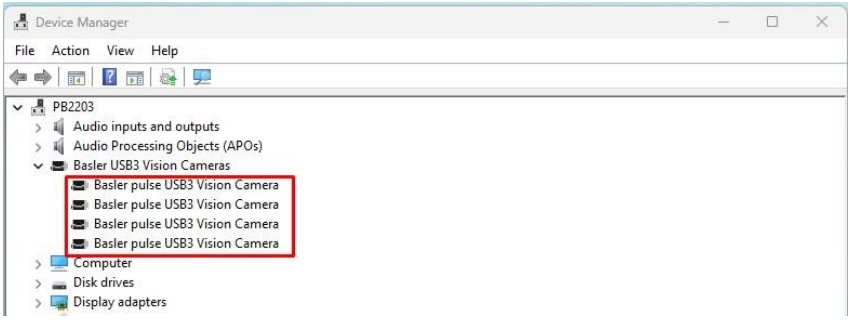
10. Click on **Install** to proceed with the installation.



11. Click on the **Close** button to finish the installation. Restarting the computer is advised.



12. Plug the device into a free USB 3.0 port.
13. Windows 11 will automatically install the cameras. If working with Windows 10, wait for the Windows Device installation assistant to launch and follow the default steps until finishing.
14. In Device Manager, check that the cameras are detected.



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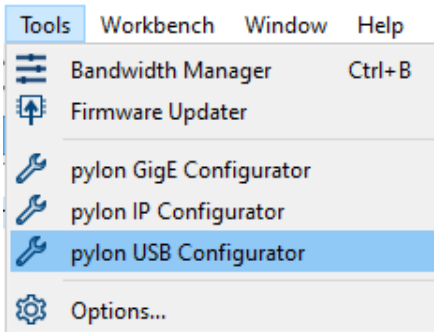
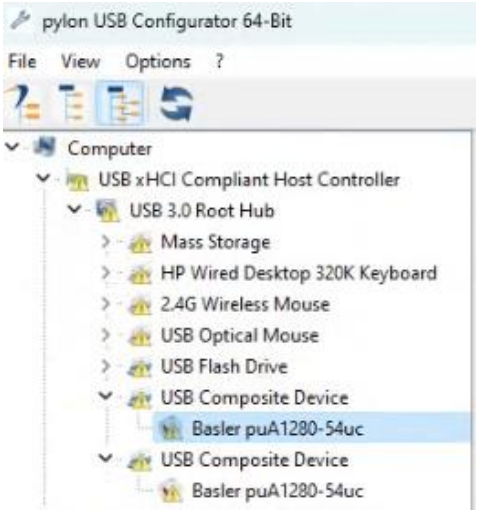
Configuring Basler USB digital cameras with Pylon Viewer

Configuring a new camera model

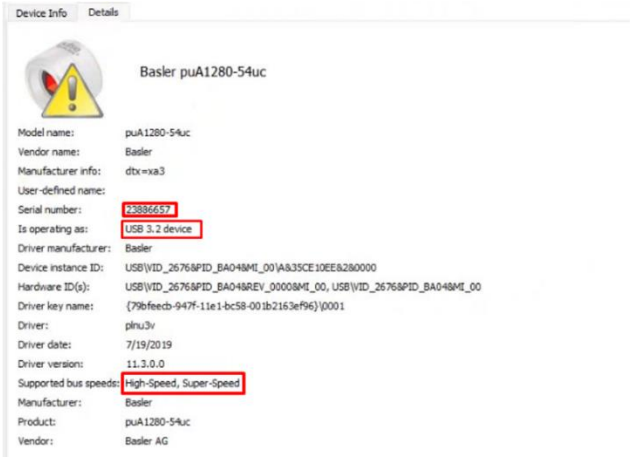
1. Select **Tools > Pylon USB Configurator**



2. Identify the cameras connected: the path may change whether the camera is connected directly to the motherboard, through a USB Hub or an additional PCIe card.



3. Click on the camera and go to the **Details** table.
4. Check that the Serial Number of the camera is correct, that the **Is operative at:** field shows **USB 3.n** device and that **Supported bus speeds:** indicates **High-Speed, Super-Speed**.



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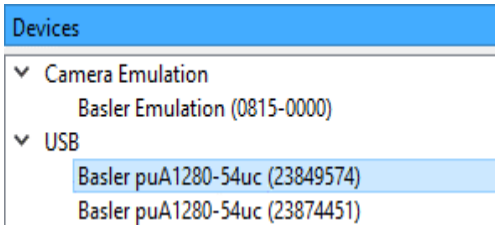
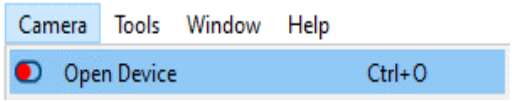
⚠	If the **Is operative at:** field shows **USB 2.n device**, the speed of the bus would not be enough to support the performance of the camera(s). Remove the USB cable from the USB 2 port and connect it to a USB 3 and restart from step 12 of section “Installing Pylon Viewer”.

5. Click on **File >** Exit to close the USB configurator

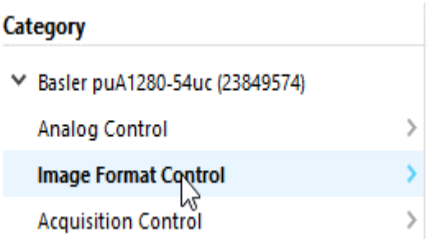
6. Under the **Devices** section identify the camera by its serial number



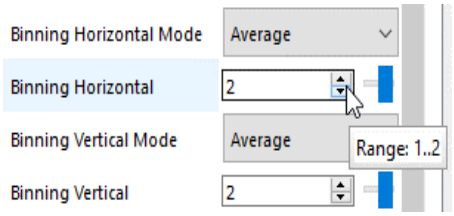
7. Select **Camera > Open Device**



8. Select **Category > Image Format Control**

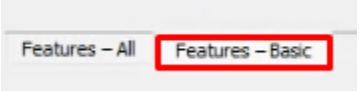


9. Insert the value **2** in **Binning Horizontal** and **Binning Vertical**



10. Click on  on the top main menu to open the preview of the camera

11. Click on the **Features – Basic** tab shown at the bottom left of the Pylon application main window.



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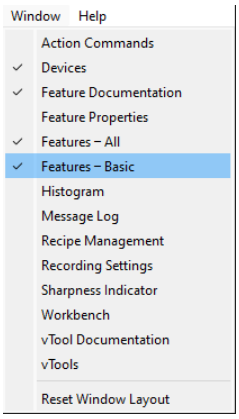
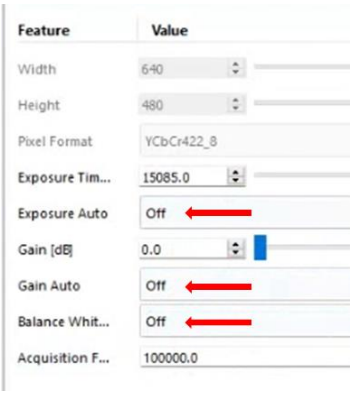
If the **Features – Basic** tab is not visible, click on the **Window** tab at the top of the task bar to show it



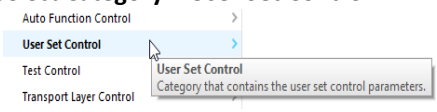
12. Set **Exposure Auto, Gain Auto** and **Balance Whites** on **OFF**



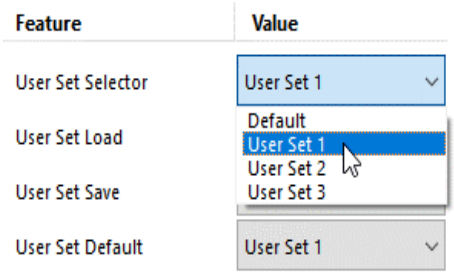
13. Set the optimal **Exposure Time** and **Gain [dB]** to have a clear and definde image

14. Click on  on the top menu to stop the preview

15. Select **Category > User Set Control**



16. Select **User Set Selector > User Set 1** and **User Set Default > User Set 1**



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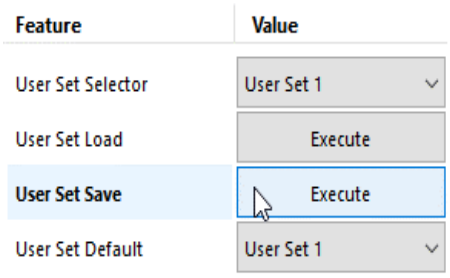
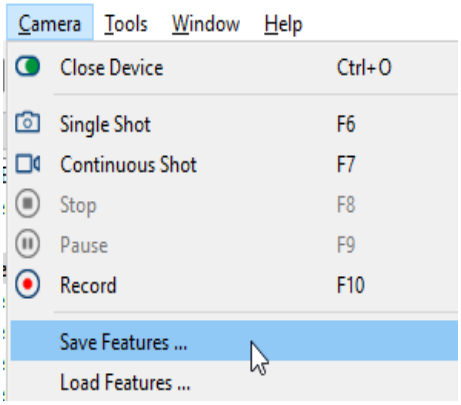
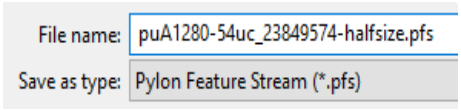
17. Select **User Set Save > Execute** to save the configuration



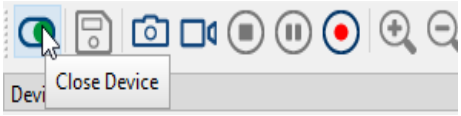
⚠	Different settings may be saved in different User Sets if the same camera is intended to be used with different light conditions. In SMARTIO it will be possible to select the correct setting when defining the image settings (see chapter 7.1 - USB digital camera image source)

18. Select **Camera > Save Features** and indicate name and path of the configuration file





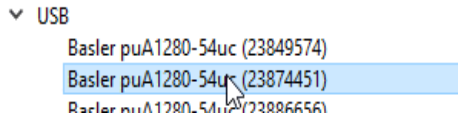
19. Select **Camera > Close Device** to exit



<u>Configuring a camera of an existing model</u>

If using multiple cameras of the same model, follow the next steps to apply the same configuration to all the cameras.

1. Identify the camera by its serial number



2. Select **Camera > Open Device**

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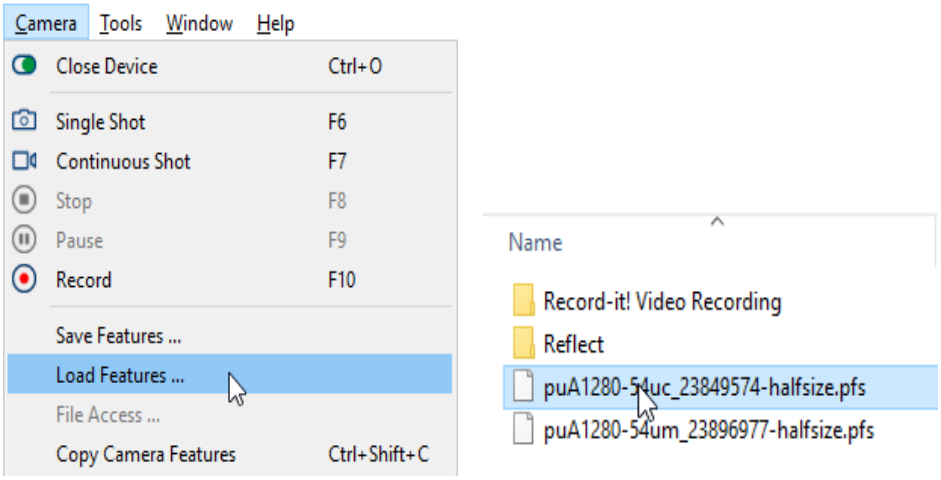
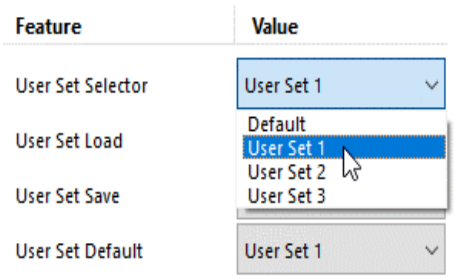
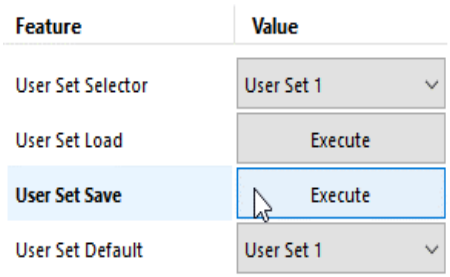
3. Select **Camera > Load Features …** to load a pre-exisiting configuration file



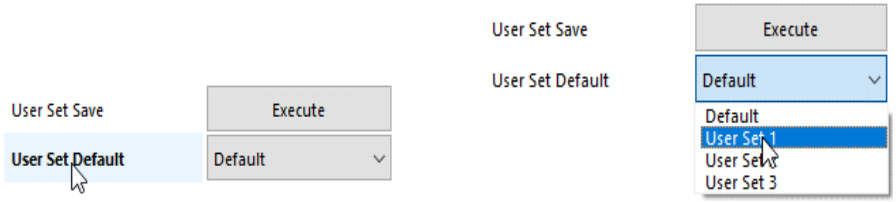
4. Select **User Set Selector > User Set 1**



5. Select **User Set Save > Execute** to save the configuration



6. Select **User Set Default > User Set 1** to define this configuration as default



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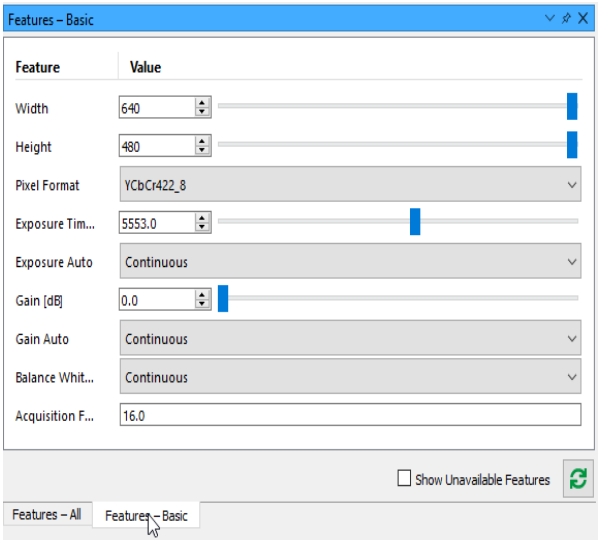
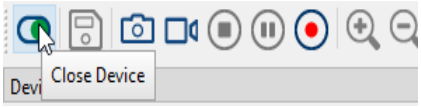
7. Select the tab **Window -> Features - Basic** to visualize a summary of the selected configuration



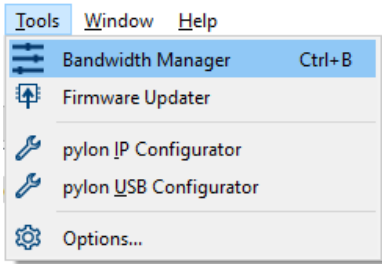
8. Select **Camera > Close Device** to exit



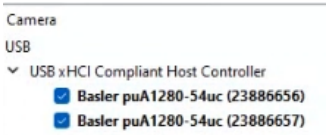
Configuring the correct bandwidth/frame rate for each camera

The factory configuration of the cameras is set by default to high resolutions. This setting needs to be lowered for optimal use with our video solutions. The following step is very important to ensure the performance of the video recording process (frame rate/resolution).

1. Once configured all the cameras connected to the PC, open **Tools > Bandwidth Manager**



2. Select all the cameras:



3. The bandwidth of the USB Controller (or the Hub) and the bandwidth per each camera are shown in the column Bandwidth (MB/s). The sum of all the bandwidths of the cameras must be lower than the total bandwidth of the USB Controller. In this case, 163 MB/s per two cameras is 326 MB/s total, which is lower than 450 MB/s.

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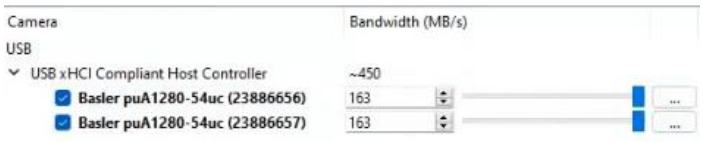
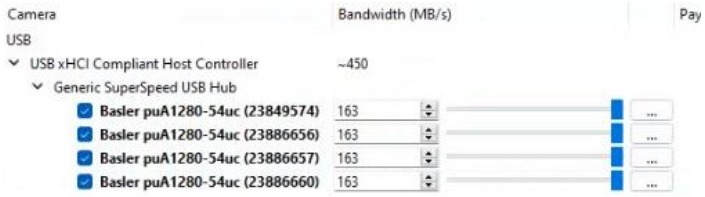
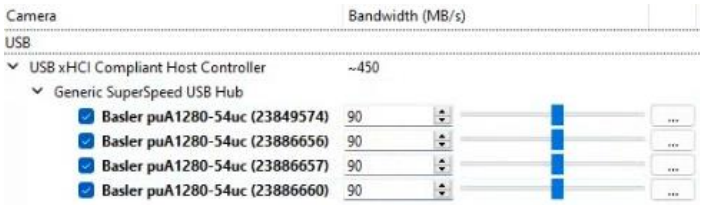


If the sum of the bandwidth of all the cameras exceeds the bandwidth of the USB controller, divide this last value for the total number of cameras + 1.
In this example, the four cameras use a total bandwidth of
163 MB/s * 4 = 652 MB/s
which is higher than 450 MB/s managed by the USB controller.

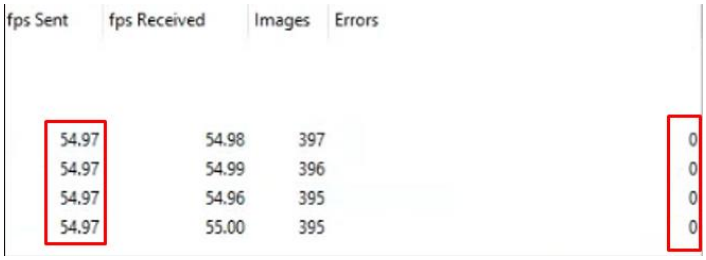


Divide 450 MB/s by (4+1) = 5 cameras (total 90 MB/s) and modify the bandwidth per each camera.



4. Click on **Start Analysis**

5. Check the value under the column **fps Sent** is higher than the values reported in Table 1 (for the number of cameras connected). Check that no errors are shown under the column **Errors**.



6. Click on **Stop Analysis** and then on **Close** to exit the Bandwidth Manager.

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3.4. DIGITAL USB 3.0 NIR CAMERAS

SMARTIO works with a wide variety of digital cameras (provided that the relevant driver is correctly installed), but we only ensure total compatibility with the digital USB 3.0 NIR cameras manufactured by IDS.

Panlab-Harvard Apparatus can only provide after-sales support on the SMARTIO camera management if used with the models recommended in this User Manual.

Depending on the configuration purchased we provide 3 USB cable length options with our cameras: 3, 5 or 8 m.

Using a USB Hub to expand a USB port to 4 ports, it will be possible to connect up to 4 Digital USB 3.0 cameras simultaneously using SMARTIO. On the other hand, in order to connect more than 4 cameras (up to 6), an expansion card (PCIe) will be necessary. In this case, please follow the manufacturer instructions to install it. Please be aware that you will need to power the PCIe connecting it to a cable from the power unit within the PC, so it would be better to use a slot near the power unit. Ask your IT Department if you need further assistance.

Table 2 reports the maximum frame rate allowed for each camera model depending on the number of cameras:

Table 2: USB 3.0 NIR cameras

REFERENCE	SENSOR	USB	RESOLUTION	FPS per number of cameras			
				1	2-4†	5-6*†	7-16
CAMDC3NIR	NIR	3.0	640x512	25 fps	16 fps	16 fps	-

†Need USB 3.0 Hub

*Need USB 3.0 PCIe

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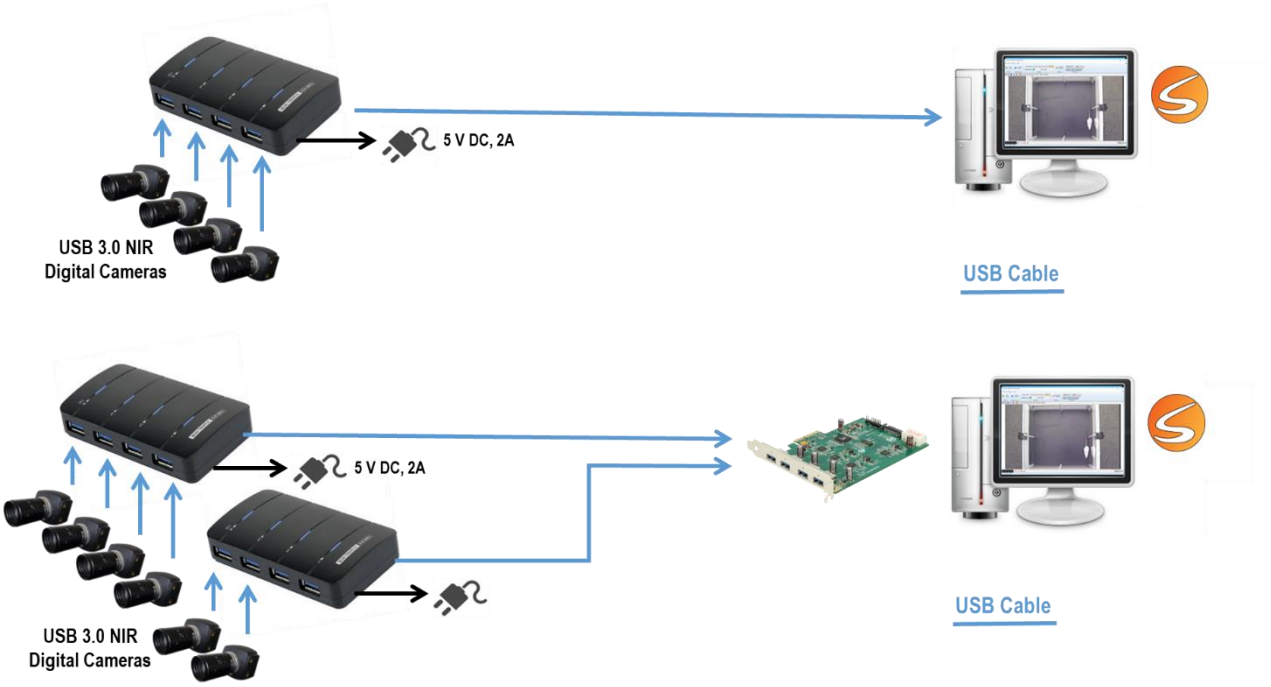
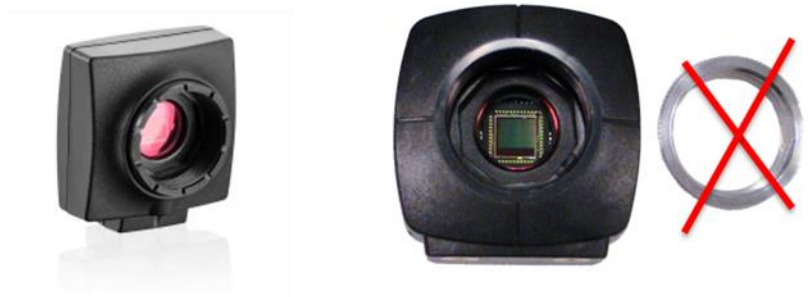


Installing the drivers of the IDS USB digital cameras

To install an IDS digital camera in Windows® systems, please follow the next steps:



1. Unpack the camera, remove the O-ring from the objective and mount the lens. If the O-ring is not removed, the image received from the camera may be blurry. This ring may be black or silver in color.

⚠	Do not plug the camera into any USB port before step 7 of this installation guide.
	Please make sure to have administrative privileges for the computer where the device will be installed. Contact your IT staff to ensure you have administrative rights before continuing with this procedure.

2. Plug the installation key of SMARTIO into a USB port and launch the installation tool (PANLAB.EXE).

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3. Select the **Hardware Drivers** option.

4. Enter in the **Digital Cameras -> IDS Digital Camera** folder and execute the file. Then follow all the steps with the default options.
5. Click the **Finish** button to finish the installation. Restarting your computer is advised.
6. Plug the device to a free USB port.
7. Wait for the Windows Device installation assistant to launch and follow the default steps until finishing.

Configuring IDS USB 3.0 digital cameras with IDS Camera Manager

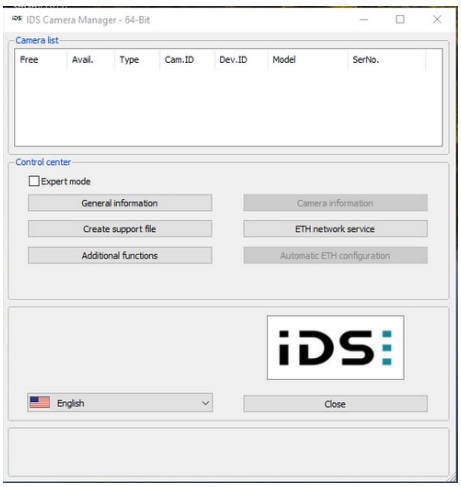
When working with the IDS digital USB cameras, several parameters need to be set for optimal video recording process:

- Internal camera ID (especially if working with a multiple camera setup)
- Frame rate and frame size, this will depend on the available USB communication bandwidth.

Set the internal ID of the IDS camera

Follow the next steps:

1. Open the “IDS Camera Manager” (the shortcut should be available from the desktop).



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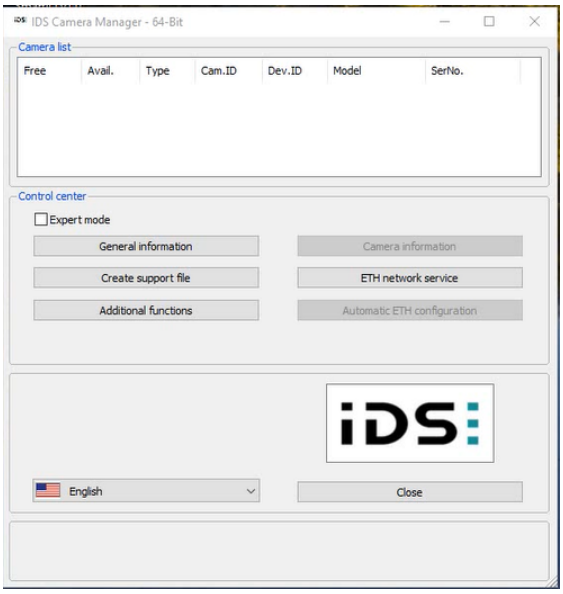
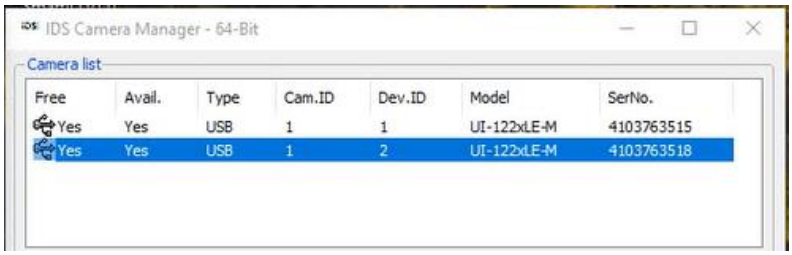
2. Connect the first camera. If a USB HUB is needed to connect multiple cameras to the computer, make sure that you connect the camera first to the USB Hub and then USB HUB to the computer. The camera will be automatically detected by the IDS Camera Manager and displayed in the Camera list.



3. The IDS Camera Manager displays the camera Serial Number (SerNo.) as well as the Camera ID (Cam.ID) and Device ID (Dev.ID). The camera will be automatically assigned as ID 1.

⚠	We recommend the user place an identification label on the camera to prevent confusion between the individual cameras used in the same setup.

4. Connect the second camera. The camera will be automatically detected by the IDS Camera Manager and displayed in the Camera list.



5. The IDS Camera Manager displays the camera Serial Number (SerNo.) as well as the Camera ID (Cam.ID) and Device ID (Dev.ID). The IDS Camera Manager always sets the Camera ID (Cam.ID) to 1. The Cam.ID should be changed to 2.

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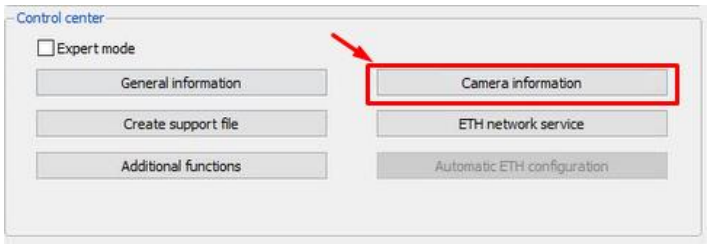
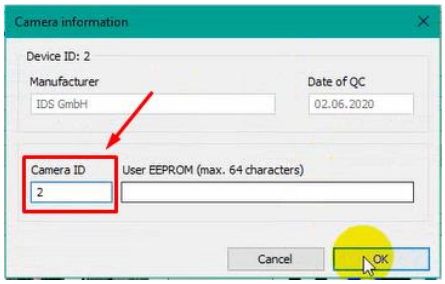
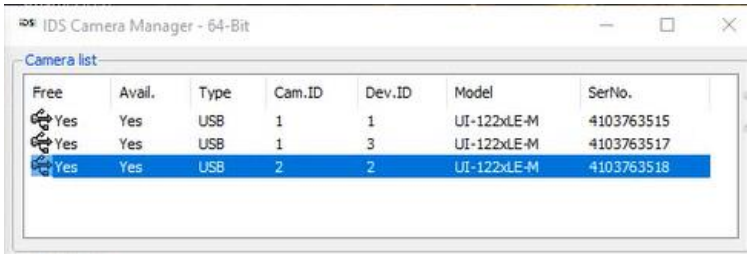
6. Click the Camera Information button in the Control center section and change the Camera ID to 2, then press the OK button.

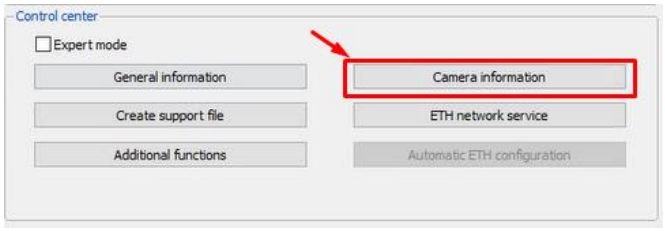


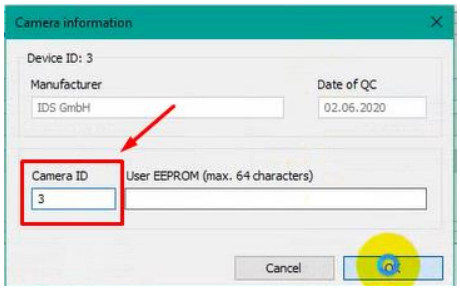


7. Connect the third camera. The camera will be automatically detected by the IDS Camera Manager and displayed in the Camera list.



8. The IDS Camera Manager displays the camera Serial Number (SerNo.) as well as the Camera ID (Cam.ID) and Device ID (Dev.ID).
9. The IDS Camera Manager always sets the Camera ID (Cam.ID) to 1. The Cam.ID should be changed to 3.
10. Click the Camera Information button in the Control center section and change the Camera ID to 3, then press the OK button.





11. Connect the next camera and repeat the step until all the cameras have been connected and set with an appropriate ID.
12. Make sure that all the cameras have a different camera and device ID numbers.

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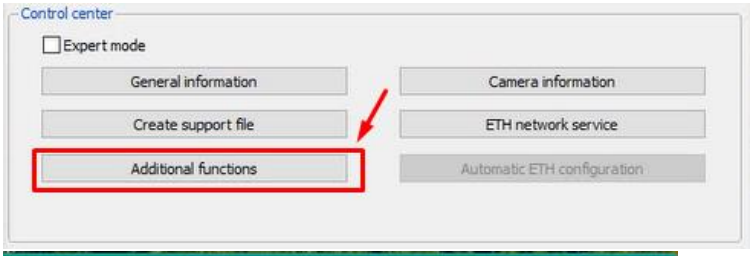
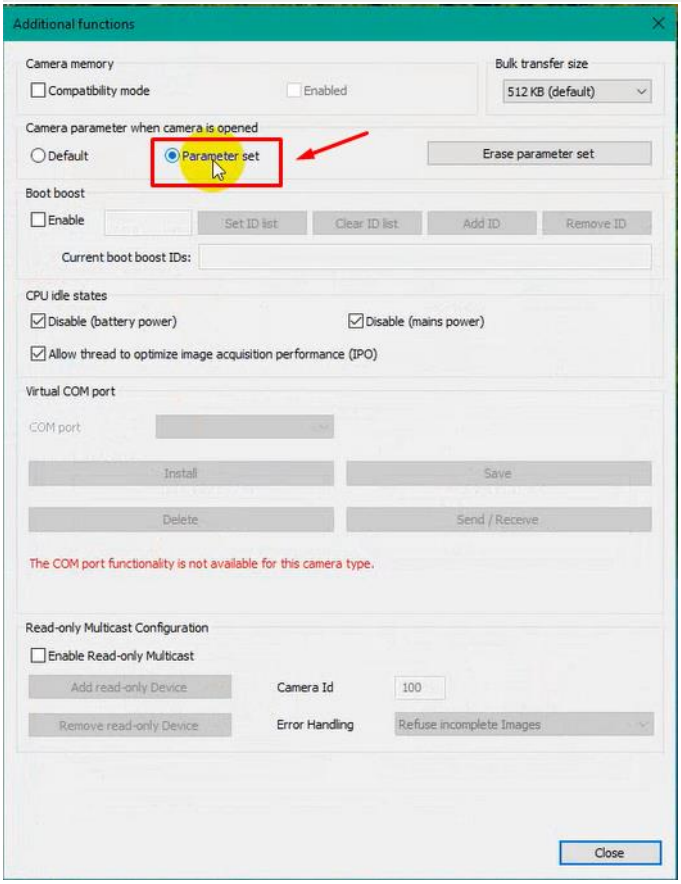
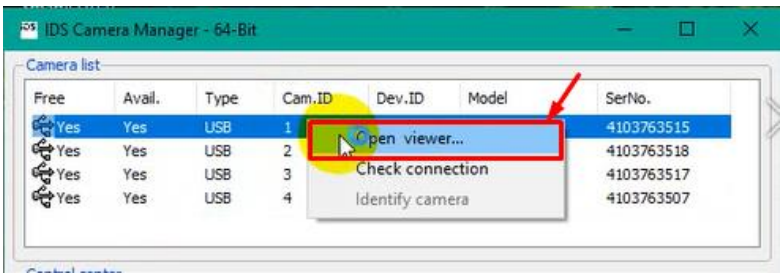
Setting the optimal frame rate that will be used for the video recording

1. Click the Additional functions button in the Control center section, choose the Parameter Set option and press the Close button to Exit.





2. Select Camera 1 in the table, right-click and then choose the “Open viewer...” option in the list.



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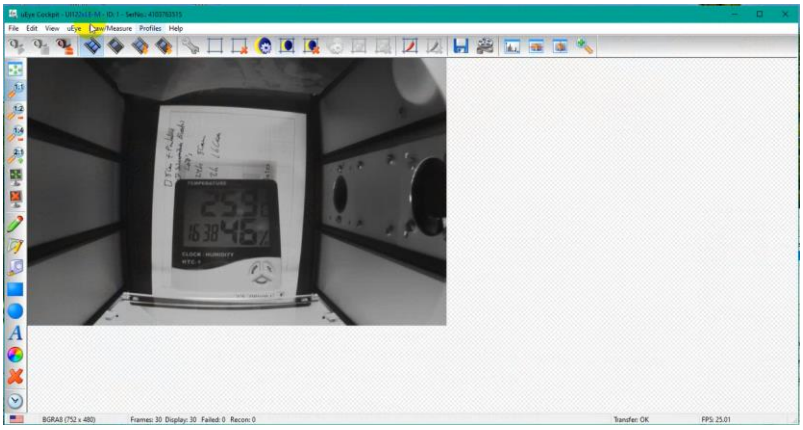
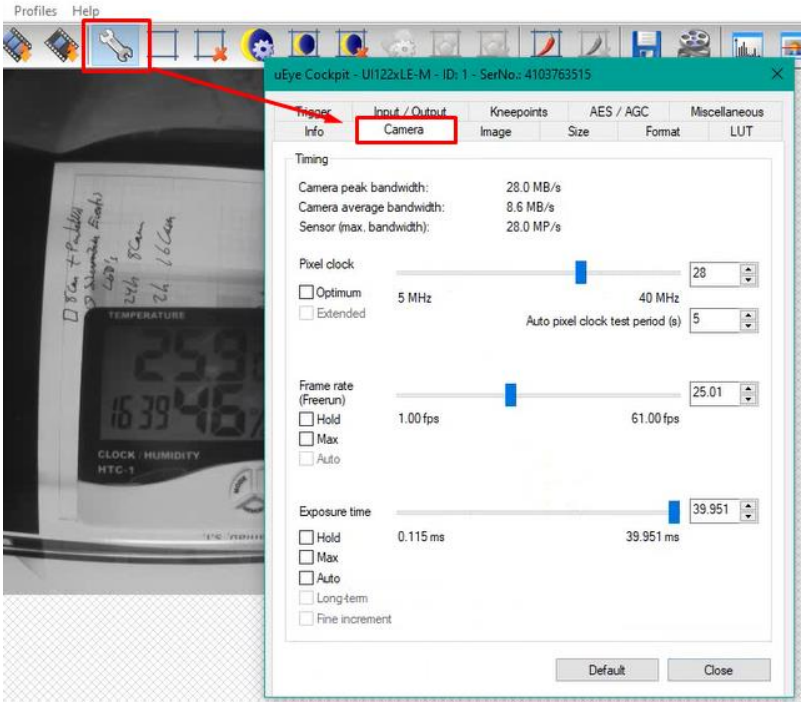
3. The IDS Camera Manager will provide a view of the images coming from the camera.



4. Click on the **Camera properties** button and display the **Camera** Tab.



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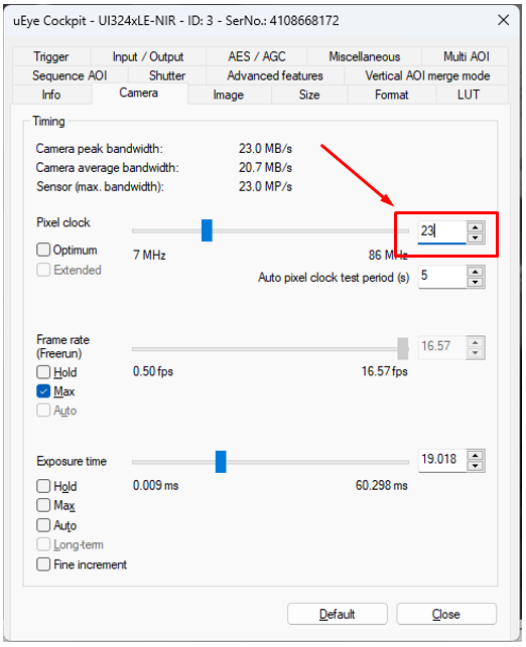
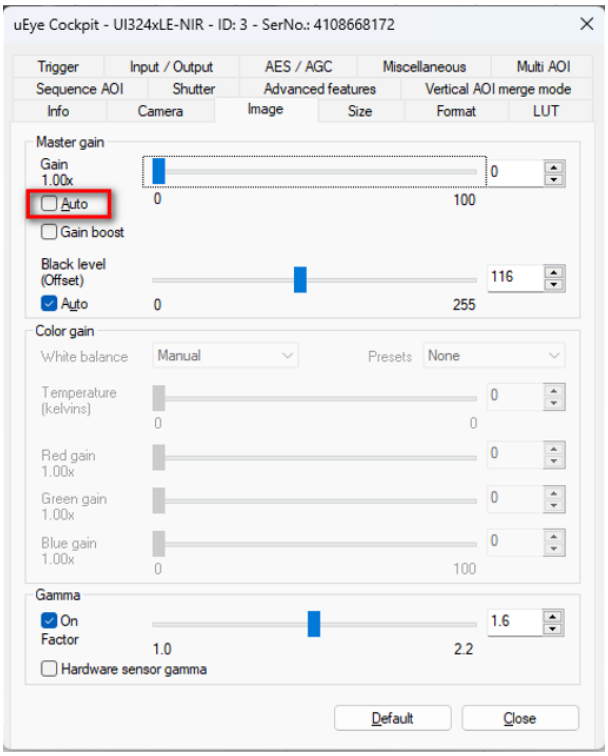
5. Set the **Pixel Clock** to 23. The values shown in the **Frame rate** section of the screen will update. Check the **Max** option to display the maximum frame rate that can be used for each cameras: here 16.57 fps.



6. Go to **Image** Tab and uncheck “Auto” in the “Master gain” section.



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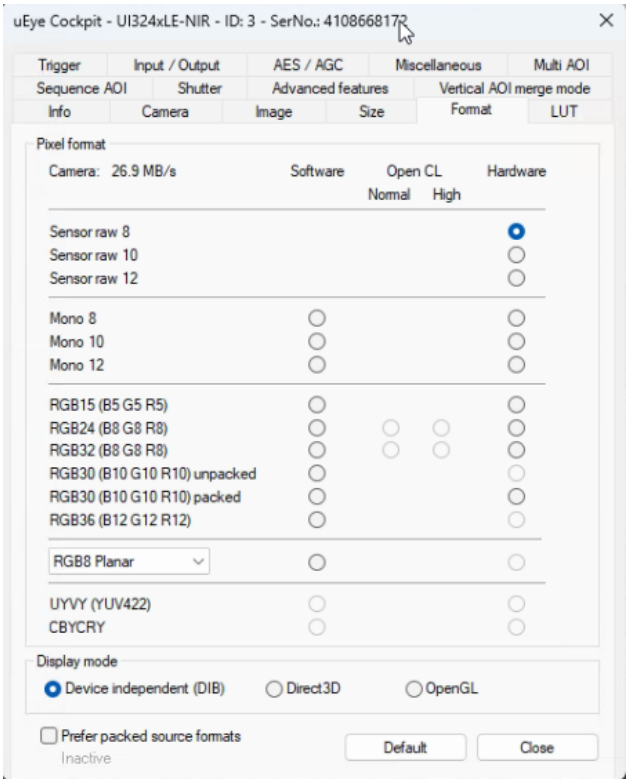
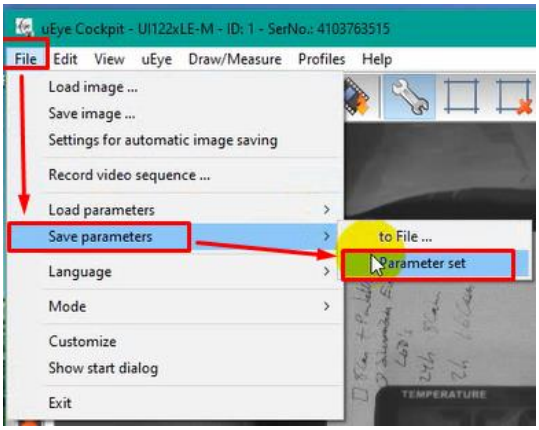
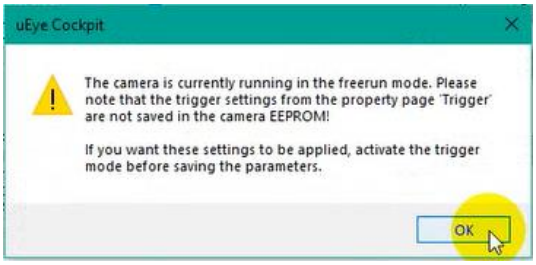
7. Go to **Format** Tab and select “Sensor raw 8”

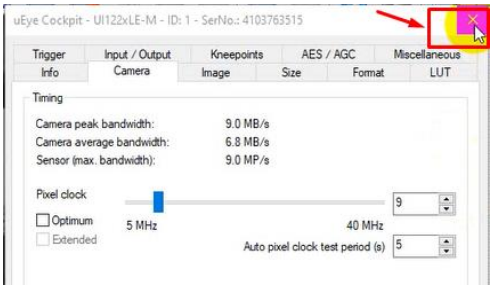


8. Save the **Parameter set** by selecting the **Save Parameters/Parameter set** option of the **File** main menu.





9. Close the uEye Cockpit panel of the Camera 1.



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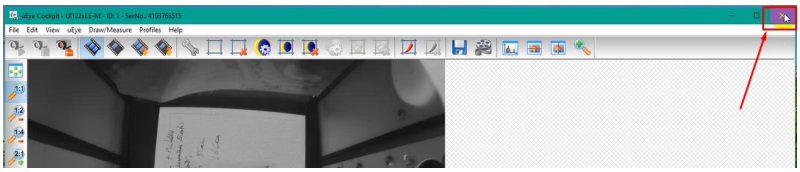
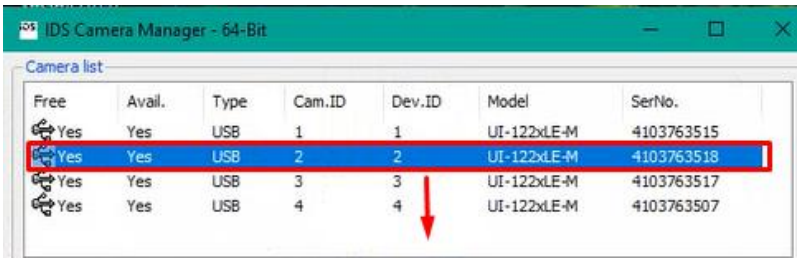
10. Close the Camera 1 Viewer

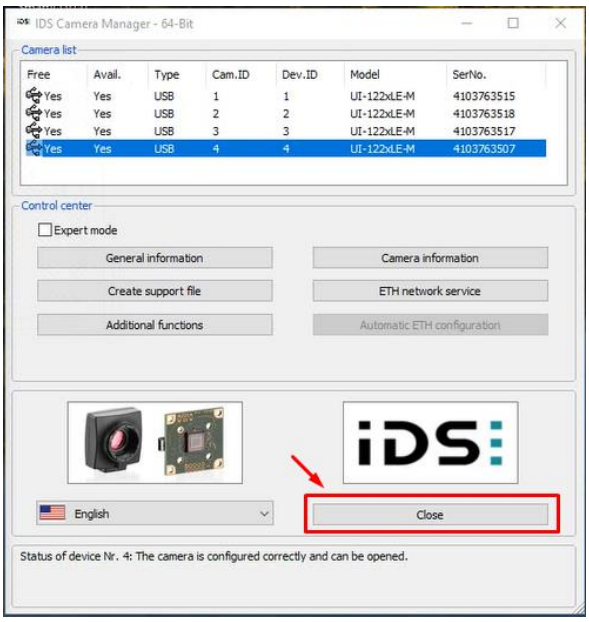


11. Repeat the same operation with all cameras:



- Select Camera 2 in the table, right-click and then choose the **Open viewer...** option in the list.
- Set the **Pixel Clock** to 23. The values shown in the **Frame rate** section of the screen will update. Check the **Max** option to display the maximum frame rate that can be used for each cameras: here 16.57 fps.
- Go to **Image** Tab and uncheck “Auto” in the “Master gain” section.
- Go to **Format** Tab and select “Sensor raw 8”
- Save the **Parameter set** by selecting the **Save Parameters/Parameter set** option of the **File** main menu.
- Close the uEye Cockpit panel of the Camera 2.
- Close the Camera2 Viewer.
- Select Camera 3 in the table, click right and then choose the **Open viewer...** option in the list.
- Etc.

12. Close the IDS Camera Manager when all cameras are set



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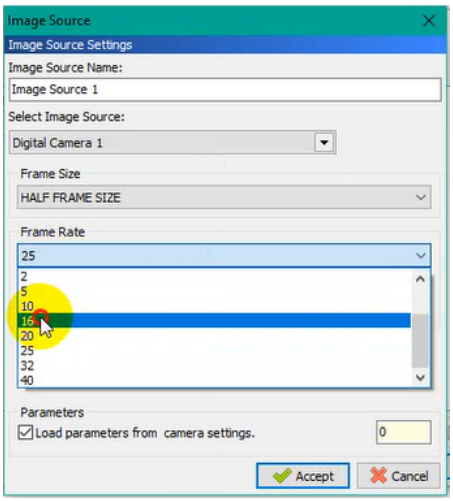
Set the optimal frame rate in when defining the cameras

Select a frame rate value equal to or lower than the one selected in step 5 on page 45 (16 FPS in this case).



	The selected frame value should always be lower or equal to the value selected in uEye.
	If the selected frame rate is greater than the value selected in uEye, some problems may occur with the display of the camera and video recording (performance issues, incoherent video file duration, …).

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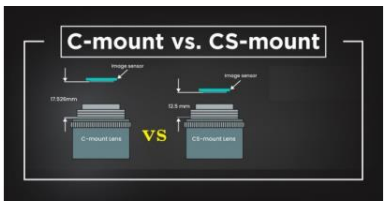
3.5. DIGITAL USB 2.0 CAMERAS

SMARTIO works with a wide variety of digital cameras (provided that the relevant driver is correctly installed), but we only ensure total compatibility with the digital USB 2.0 cameras manufactured by IDS and The Imaging Source (TIS). Panlab-Harvard Apparatus can only provide after-sales support on the SMARTIO camera management if used with the models recommended in this User Manual.
Depending on the configuration purchased we provide 3 USB cable length options with our cameras: 3, 5 or 8 m.

Installing TIS Digital USB 2.0 Cameras

SMARTIO can manage one TIS Digital USB 2.0 camera at a time. Once plugged in, the camera is recognized by Windows, but it will be necessary to install the drivers in order to manage it. To install a TIS digital camera in Windows® systems, please follow the next steps:

1. Unpack the camera and remove the CS/C lens adapter ring supplied with the camera before mounting the lens.



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⚠	Please make sure to have administrative privileges for the computer where the device will be installed. Contact your IT staff to ensure you have administrative rights before continuing with this procedure.

2. Plug the device to a free USB 2.0 or USB 3.0 port. Once plugged in, Windows recognizes that there is a connected device, but it will be necessary to install the drivers in order to manage the camera.
3. Please make sure to have administrative privileges on the computer on which the device will be installed. Contact your IT staff to confirm this issue before continuing with this procedure.
4. Plug the installation key of SMARTIO into a USB 2.0 port and launch the installation tool (PANLAB.EXE).
5. Select the "**Hardware Drivers**" option.

6. Enter the "**Digital Cameras -> TIS USB 2.0 Digital Camera**" folder and execute the .exe file. Then follow all the steps with the default options.
7. Click the 'Finish' button to finish the installation.
8. Wait for the Windows Device installation assistant to launch and follow the default steps until finishing.

Installing IDS Digital USB 2.0 Cameras (Legacy mode)

Using a USB Hub to expand a USB port to 4 ports, it will be possible to connect up to 4 Digital USB 2.0 cameras simultaneously using RECORD-IT! MEDIA. On the other hand, in order to connect more than 4 cameras, an expansion card (PCIe) will be necessary. For example, one PCIe connected to 4 USB Hub will allow to connect up to 16 Digital USB 2.0 cameras simultaneously using RECORD!-IT MEDIA. In this case, please follow the manufacturer instructions to install it. Please be aware that you will need to power the PCIe connecting it to a cable from the power unit within the PC, so it would be better to use a slot near the power unit. Ask your IT Department if you need further assistance.

⚠	Up to 16 Digital USB 2.0 cameras can be used for video recording only. For video tracking, with or without TTL triggering (Ephys and Opto functions), the maximum number of cameras that can be used simultaneously is 8.

Table 3Table 3 reports the maximum frame rate allowed for each camera model depending on the number of cameras:

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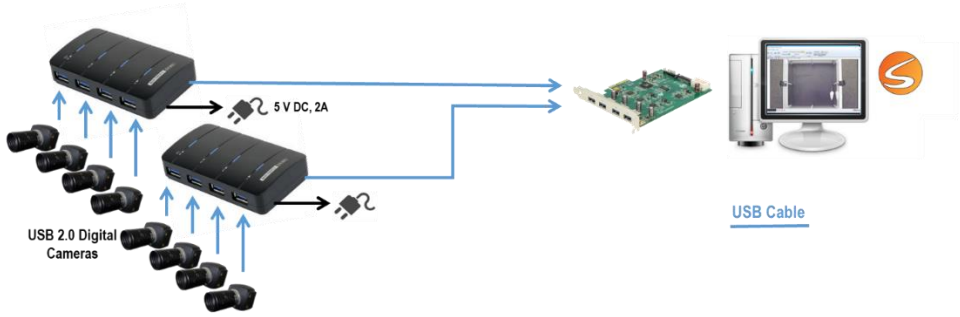
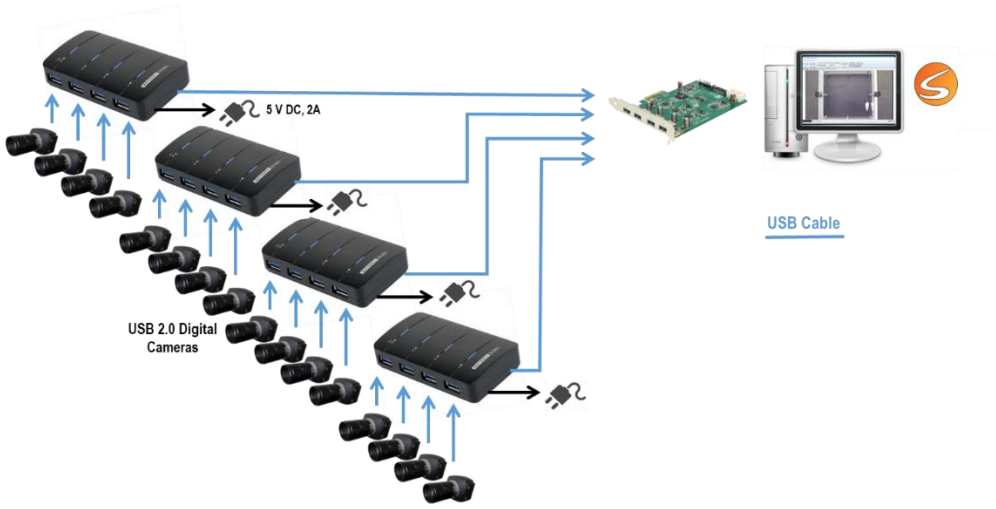
Table 3: USB 2.0 legacy cameras

REFERENCE	SENSOR	USB	RESOLUTION	FPS per number of cameras			
				1	2-4†	5-8*†	9-16*† (only recording)
CAMDCCOLOR$	Color	2.0	640X480	25 fps	25 fps	25 fps	15 fps
CAMDCBW$	Monochrome	2.0	640X480	25 fps	25 fps	25 fps	15 fps
CAMDCNIR$	NIR	2.0	640X480	25 fps	-	-	-

†Need USB Hub
*Need USB PCIe
$Legacy Model





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To install an IDS digital camera in Windows® systems, please follow the next steps:

1. Unpack the camera, remove the O-ring from the objective and mount the lens. If the O-ring is not removed, the image received from the camera may be blurry. This ring may be black or silver in color.

⚠	Do not plug the camera into any USB port before step 7 of this installation guide.
	Please make sure to have administrative privileges for the computer where the device will be installed. Contact your IT staff to ensure you have administrative rights before continuing with this procedure.

2. Plug the installation key of SMARTIO into a USB port and launch the installation tool (PANLAB.EXE).
3. Select the **Hardware Drivers** option.

4. Enter the "**Digital Cameras -> IDS Digital Camera**" folder and execute the correct .exe file (32 or 64 bit depending on the version of Windows). Then follow all the steps with the default options.
5. Click the **Finish** button to finish the installation. Restarting your computer is advised.
6. Plug the device to a free USB port.
7. Wait for the Windows Device installation assistant to launch and follow the default steps until finishing.

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Configuring IDS USB 2.0 digital cameras with IDS Camera Manager (Legacy mode)

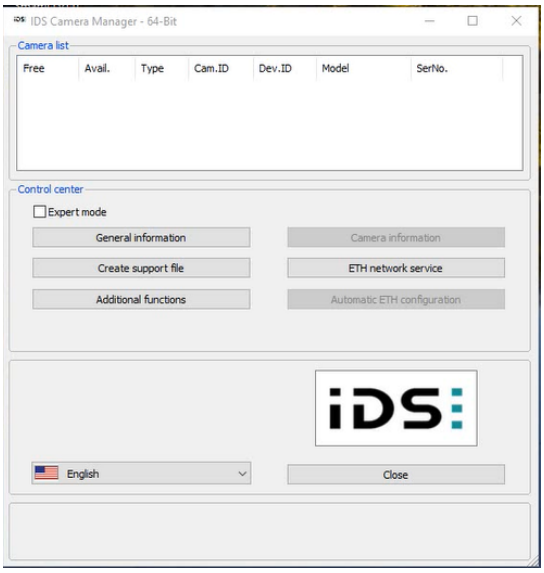
When working with the IDS digital USB cameras, several parameters need to be set for optimal video recording process:

- Internal camera ID (especially if working with a multiple camera setup)
- Frame rate and frame size, this will depend on the available USB communication bandwidth.

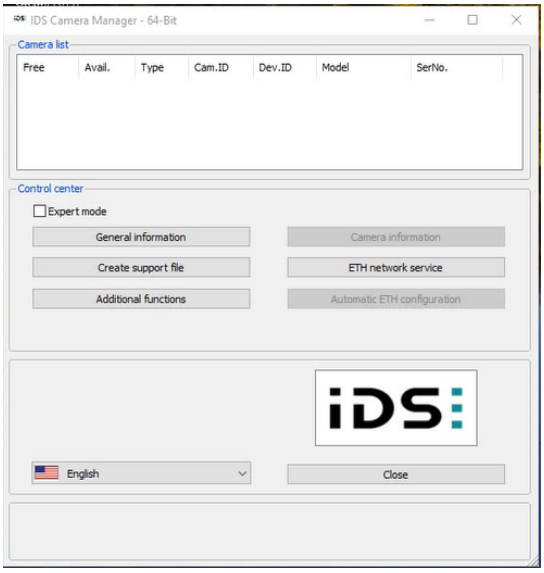
Set the internal ID of the IDS camera

Follow the next steps:

1. Open the “IDS Camera Manager” (the shortcut should be available from the desktop).



2. Connect the first camera. If a USB HUB is needed to connect multiple cameras to the computer, make sure that you connect the camera first to the USB Hub and then USB HUB to the computer. The camera will be automatically detected by the IDS Camera Manager and displayed in the Camera list.



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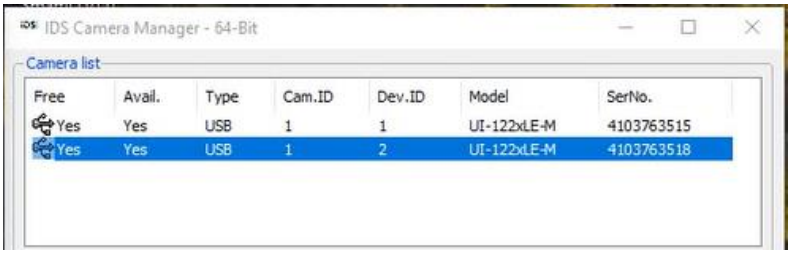
3. The IDS Camera Manager displays the camera Serial Number (SerNo.) as well as the Camera ID (Cam.ID) and Device ID (Dev.ID). The camera will be automatically assigned as ID 1.

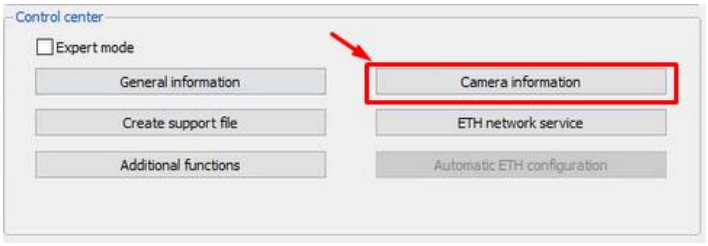
⚠	We recommend the user place an identification label on the camera to prevent confusion between the individual cameras used in the same setup.

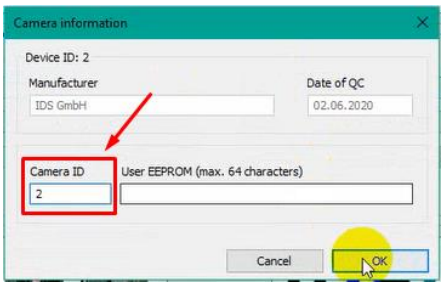
4. Connect the second camera. The camera will be automatically detected by the IDS Camera Manager and displayed in the Camera list.



5. The IDS Camera Manager displays the camera Serial Number (SerNo.) as well as the Camera ID (Cam.ID) and Device ID (Dev.ID). The IDS Camera Manager always sets the Camera ID (Cam.ID) to 1. The Cam.ID should be changed to 2.

6. Click the Camera Information button in the Control center section and change the Camera ID to 2, then press the OK button.





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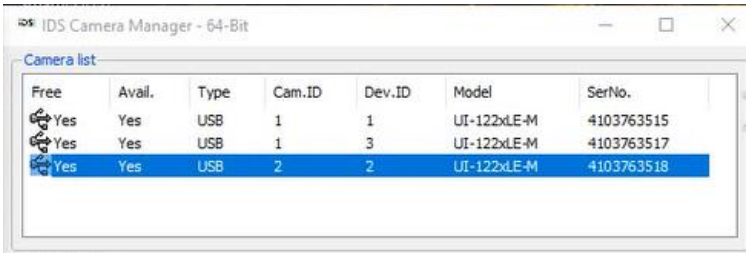
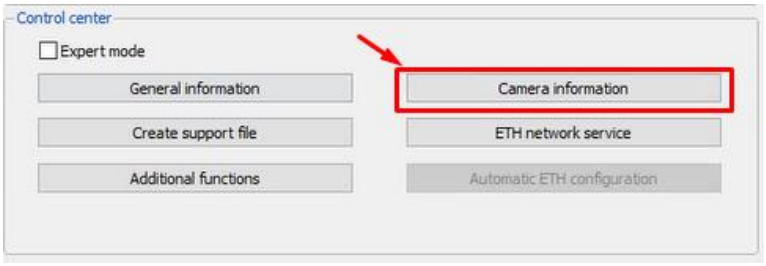
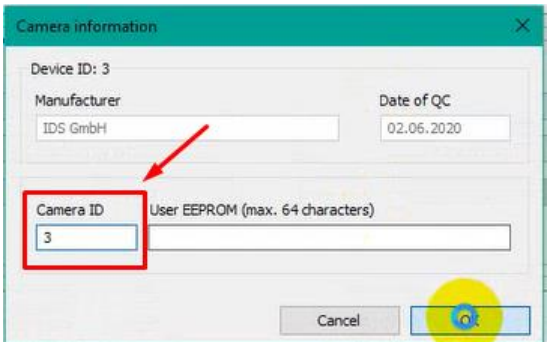
7. Connect the third camera. The camera will be automatically detected by the IDS Camera Manager and displayed in the Camera list.



8. The IDS Camera Manager displays the camera Serial Number (SerNo.) as well as the Camera ID (Cam.ID) and Device ID (Dev.ID).
9. The IDS Camera Manager always sets the Camera ID (Cam.ID) to 1. The Cam.ID should be changed to 3.
10. Click the Camera Information button in the Control center section and change the Camera ID to 3, then press the OK button.





11. Connect the next camera and repeat the step until all the cameras have been connected and set with an appropriate ID.
12. Make sure that all the cameras have a different camera and device ID numbers.

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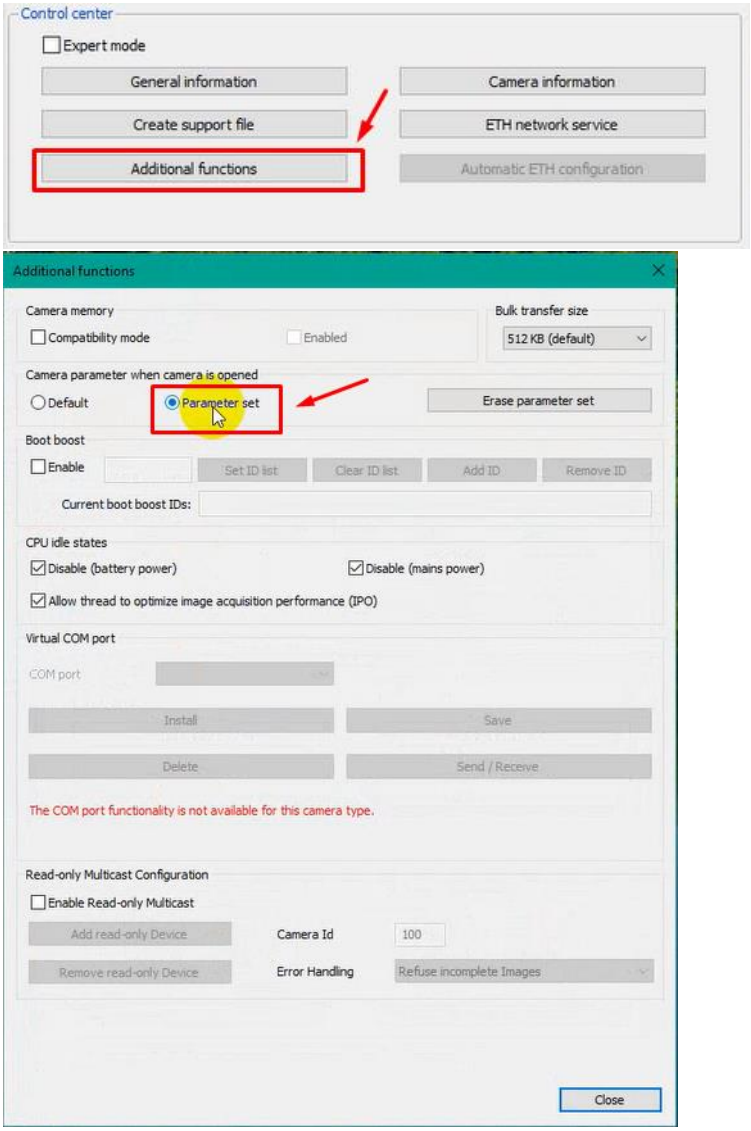
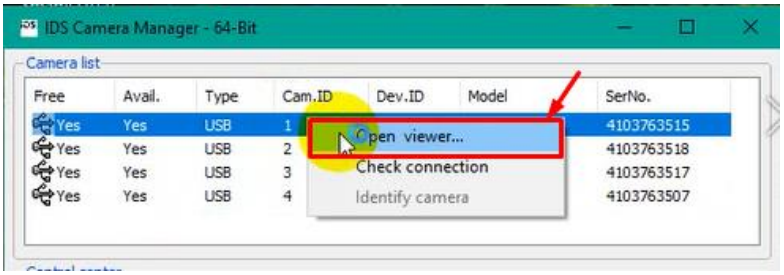
Setting the optimal frame rate that will be used for the video recording

1. Click the Additional functions button in the Control center section, choose the Parameter Set option and press the Close button to Exit.



2. Select Camera 1 in the table, right-click and then choose the “Open viewer...” option in the list.



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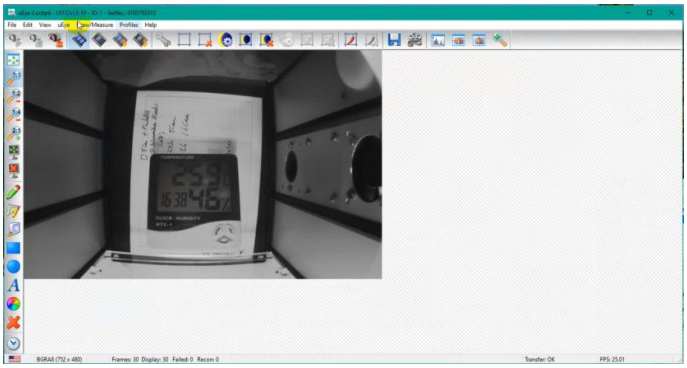
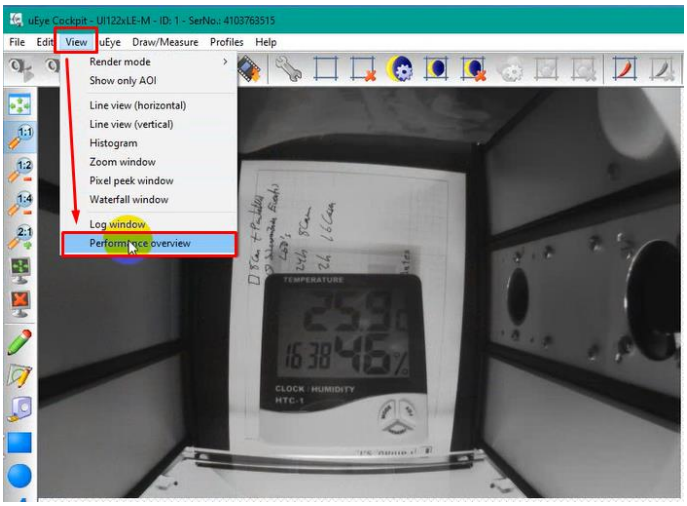
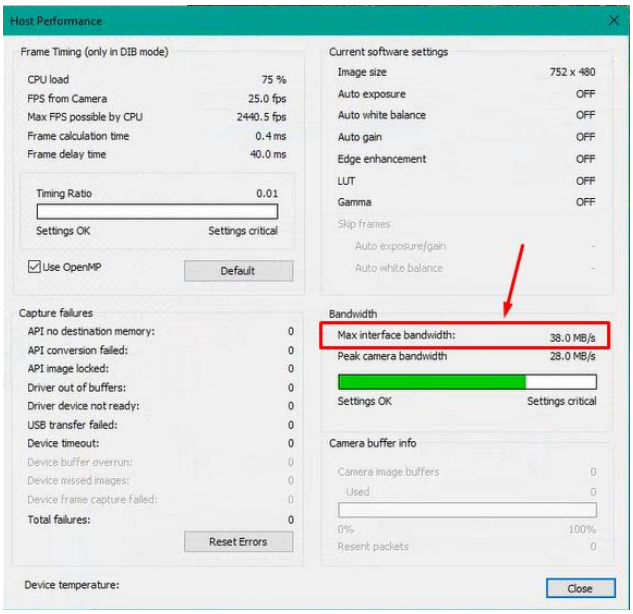
3. The IDS Camera Manager will provide a view of the images coming from the camera.



4. Click on the View main menu and select the Performance overview option and note the value of the Max Interface bandwidth.





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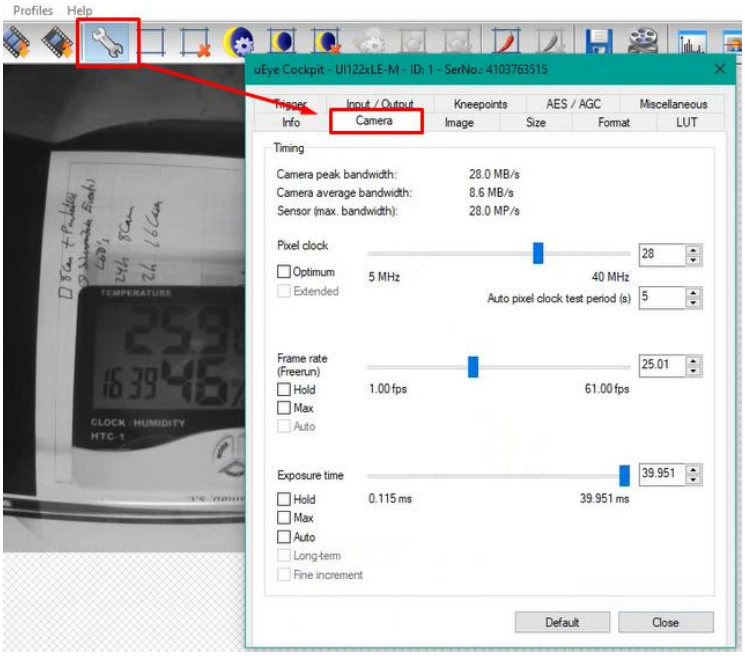
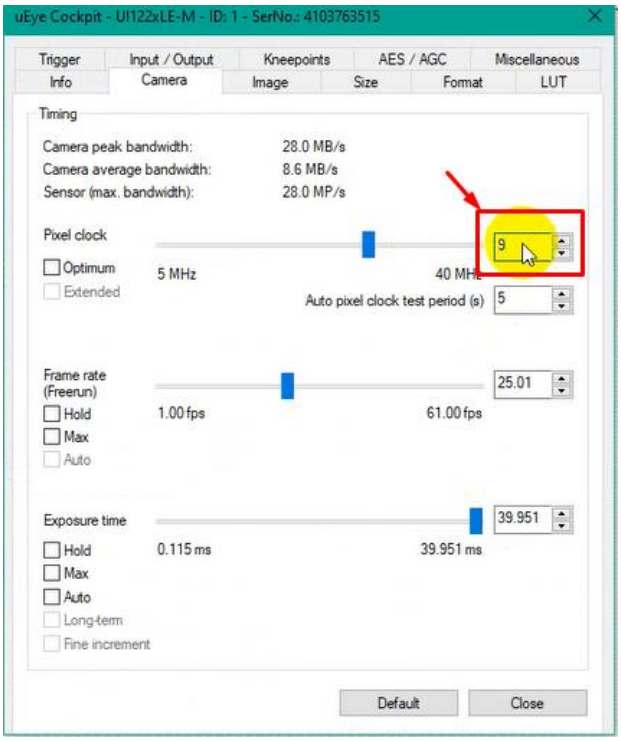
5. In this example the Max interface bandwidth value is 38.0 MB/s. This is the shared USB communication bandwidth available to all cameras connected to the same USB HUB or computer USB port.
6. If 4 cameras are connected to this USB HUB/computer USB port, then the available bandwidth available for each camera would be 38.0/4 = 9.5. Round this value to the lower value: 9 MB/s.
7. Click on the Camera properties button and display the Camera Tab.



8. Set the Pixel Clock to the calculated value (9 in this example):



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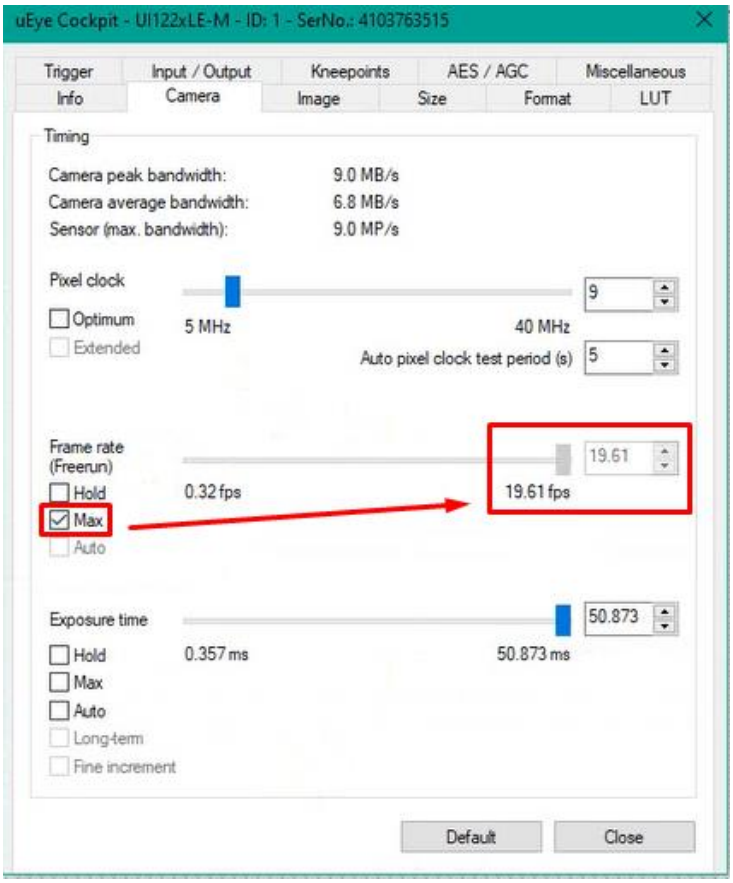
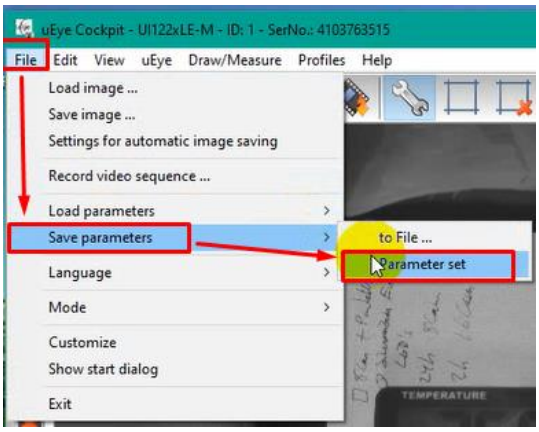
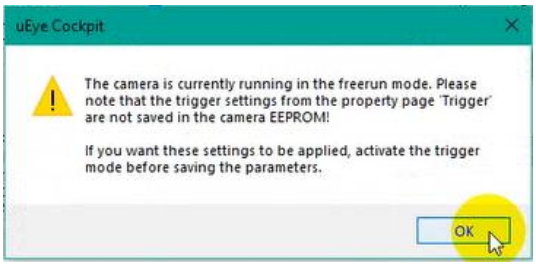
9. The values shown in the Frame rate section of the screen will update. Check the Max option to display the maximum frame rate that can be used for each camera: here 19 fps.



10. Save the Parameter set by selecting the Save Parameters/Parameter set option of the File main menu.





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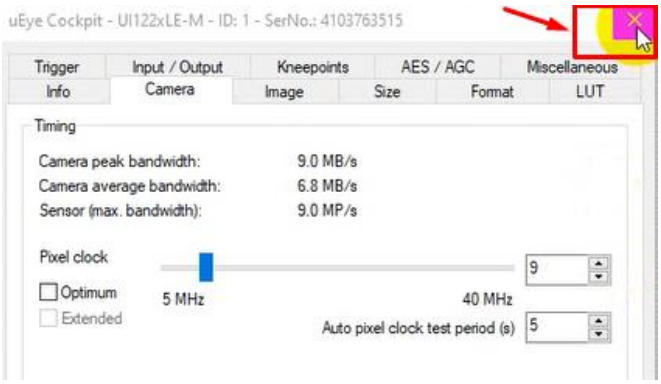
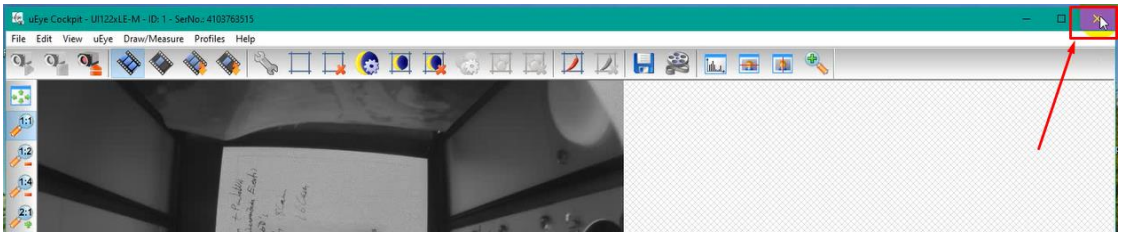
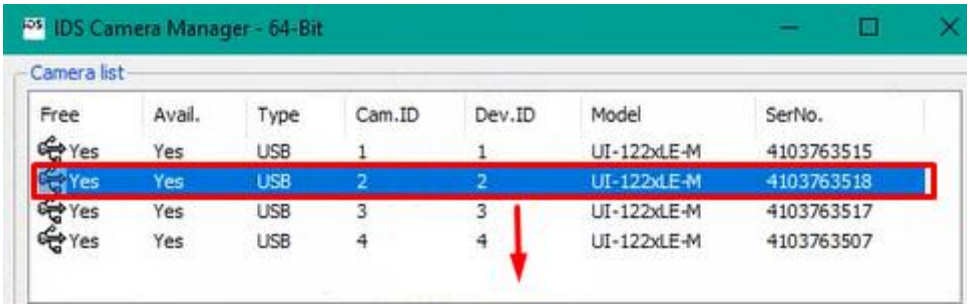
11. Close the uEye Cockpit panel of the Camera 1.



12. Close the Camera 1 Viewer



13. Repeat the same operation with all cameras:



- Select Camera 2 from the table, right-click and then choose the Open viewer... option on the list.
- Click on the Camera properties button and display the Camera Tab.
- Set the Pixel Clock to the calculated value (9 in this example).
- The values shown in the Frame rate section of the screen will update. Check the Max option to display the maximum frame rate that can be used for each camera: here 19 fps.
- Save the Parameter set by selecting the Save Parameters/Parameter set option of the File main menu.
- Close the uEye Cockpit panel of the Camera 2.
- Close the Camera 2 Viewer.
- Select Camera 3 on the table, click right and then choose the Open viewer... option in the list.

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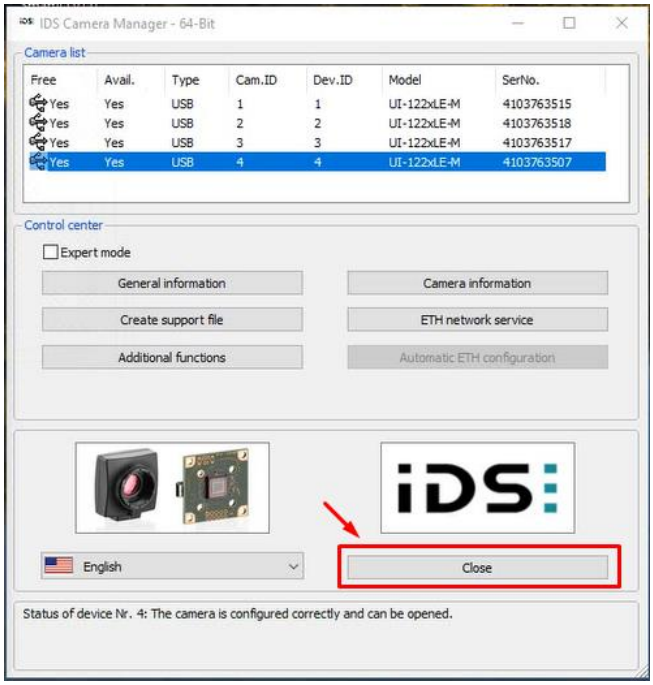
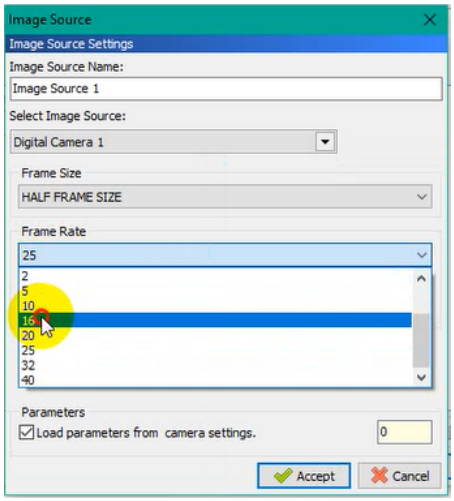
14. Close the IDS Camera Manager when all the cameras are set



Set the optimal frame rate in when defining the cameras

Select a frame rate value equal to or lower than the calculated MAX Frame Rate (from Step 6 above). The max frame rate calculated was 19 fps in our example, so 16 fps is the closest available that is equal or LOWER.



⚠	The selected frame value should always be lower or equal to the calculated optimal value.
	If the selected frame rate is greater than the value selected in uEye, some problems may occur with the display of the camera and video recording (performance issues, incoherent video file duration, …).

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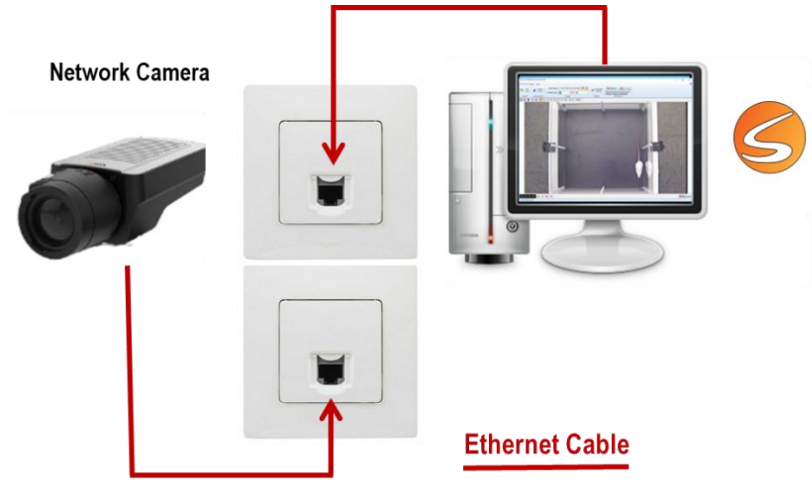
3.6. NETWORK CAMERAS

⚠	Network Cameras should be used only for recording or video tracking without TTL triggering (Ephys and Opto functions), due to a possible delay between the animal's movement and the image source.

SMARTIO is compatible with ONVIF and non-ONVIF Network cameras.

- For ONVIF cameras, SMARTIO provides direct access to the camera settings.
- For non-ONVIF cameras, please follow the manufacturer's set-up instructions prior to using SMARTIO. SMARTIO will only provide access to the camera images (RTSP Streaming), but not to its settings.

Most IP cameras can stream videos using a RTSP URL request. Contact the camera supplier/manufacturer to obtain the correct RTSP URL address for your camera model.
IP cameras are designed to be used on Ethernet networks. An IP address is needed to access the camera. Please, contact your IT to connect your IP camera(s) to your Ethernet network.



It is possible to connect up to 16 cameras simultaneously. However, be aware that you will need an Ethernet Switch POE to connect more than one camera at the same time.

The main requirement for ONVIF cameras is to have Profile S protocol.

The main requirement for non-ONVIF cameras is to fulfill the RTSP protocol.

Table 4 reports the maximum frame rate allowed depending on the number of cameras simultaneously connected:

Table 4: Network ONVIF Profile S cameras

MODEL	SENSOR	PROTOCOL	RESOLUTION	FPS per number of cameras			
				1	2-4	5-8$	9-16$
Tested with the Axis M1134 Network Camera	Color	ONVIF Profile S	1280X960	25 fps	20 fps	16 fps	16 fps

$ Need Ethernet Switch

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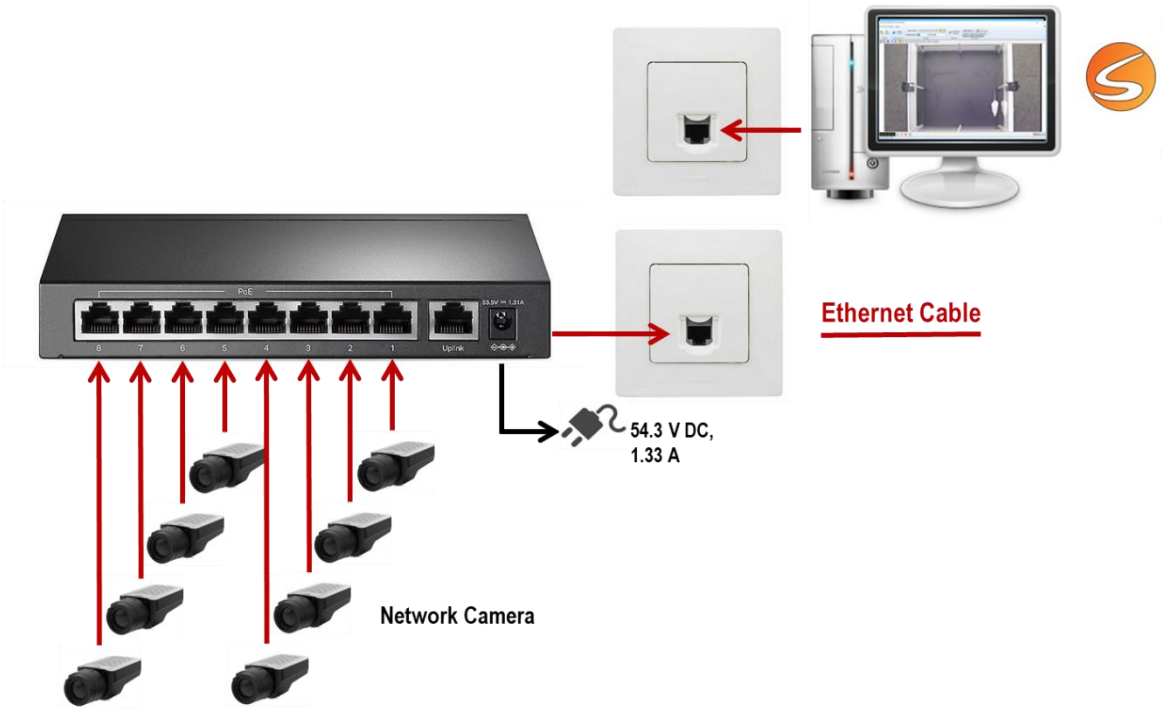
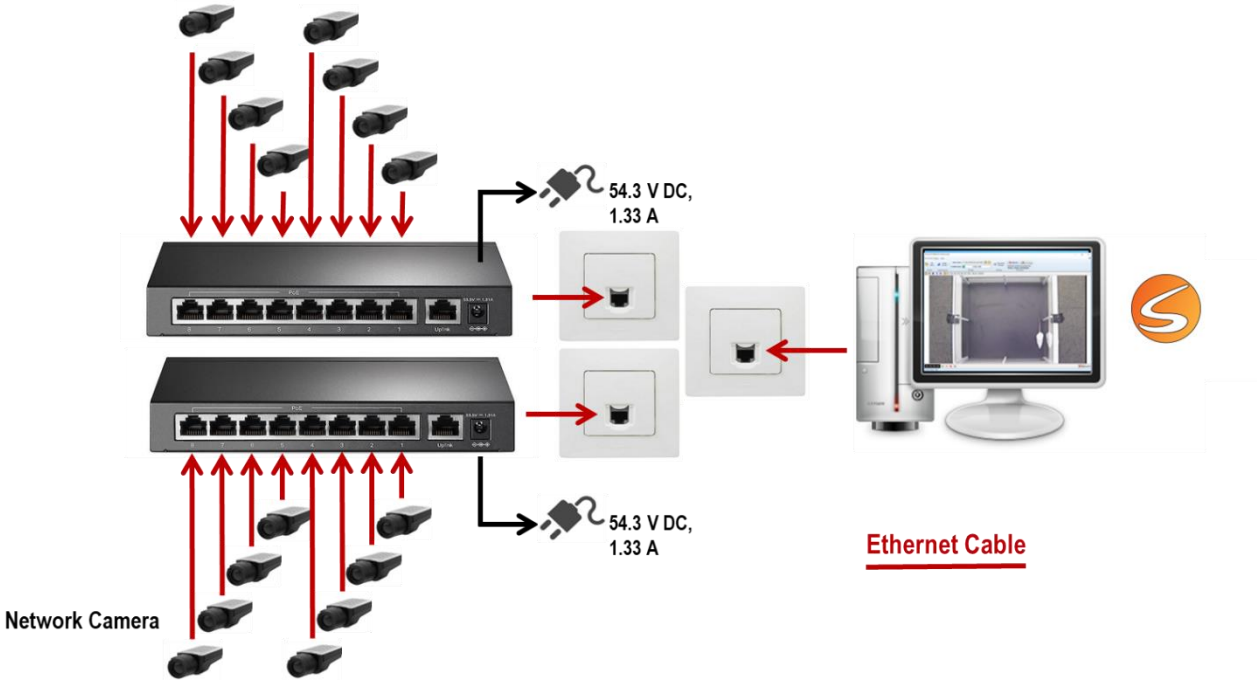

Ethernet Cable
54.3 V DC,
1.33 A
Network Camera


54.3 V DC,
1.33 A
54.3 V DC,
1.33 A
Ethernet Cable
Network Camera

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⚠	When working with IP Network cameras, the network infrastructure (LAN/WAN) and performance of your experimental facilities is an important point to be considered. If bandwidth bottlenecks occur, streaming video can show jitter, delay, or corruption. This could result in the video not getting recorded or showing aberrant duration

3.7. INSTALLING AN ANALOG-DIGITAL CONVERTER FOR ANALOG CAMERAS

Our analog-digital video converter (CONVANAUSB) enables the use of analog image technology by digitizing the incoming images of analog sources. The analog-digital video converter (CONVANAUSB) is an optional accessory that can be purchased with your SMARTIO system. Please contact your dealer to learn the advantages of this accessory and how to acquire it.

⚠	**Only one** analog-digital video converter (CONVANAUSB) can be plugged into the computer. This option cannot be used for multiple camera needs.

Before using the device, the corresponding drivers must be installed. Three different versions of the analog-digital video converter have been released, each one of them requiring different drivers. Please check which version you have in your system:

Version 1	Version 2	Version 3
		

Installing Version 1 of the Analog-Digital Converter

To install the device in Windows® systems, please follow the next steps:

1. IMPORTANT: Do not plug the device into any USB port before step no. 7 of this installation guide.
2. Please make sure to have administrative privileges for the computer where the device will be installed. Contact your IT staff to ensure you have administrative rights before continuing with this procedure.
3. Plug the installation key of SMARTIO into a USB 2.0 port and launch the installation tool (PANLAB.EXE).
4. Select the **Hardware Drivers** option.

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5. Enter the "**Analog Camera -> CONVANAUSB Drivers ->Version 1**" folder and execute the correct .exe file (32 or 64 bit depending on the version of Windows). Then follow all the steps with the default options
6. Click the **Finish** button to finish the installation. Restarting your computer is advised.
7. Plug the device into a free USB 2.0 port. Make sure to remove the yellow label from the USB end of the cable first.
8. Wait for the Windows Device installation assistant to launch and follow the default steps until finishing.

Installing Version 2 to 4 of the Analog-Digital Converter

To install the device in Windows® systems, please follow the next steps:

1. IMPORTANT: Do not plug the device into any USB port before step no. 7 of this installation guide.
2. Please make sure to have administrative privileges for the computer where the device will be installed. Contact your IT staff to ensure you have administrative rights before continuing with this procedure.
3. Plug the installation key of SMARTIO into a USB 2.0 port and launch the installation tool (PANLAB.EXE).
4. Select the **Hardware Drivers** option.

5. Enter the "**Analog Camera -> CONVANAUSB Drivers ->Version 2 to 4**" folder and execute the .exe file. Then follow all the steps with the default options.
6. Click the **Finish** button to finish the installation. Restarting your computer is advised.
7. Plug the device into a free USB 2.0 port. Make sure to remove the yellow label from the USB end of the cable first.
8. Wait for the Windows Device installation assistant to launch and follow the default steps until finishing.

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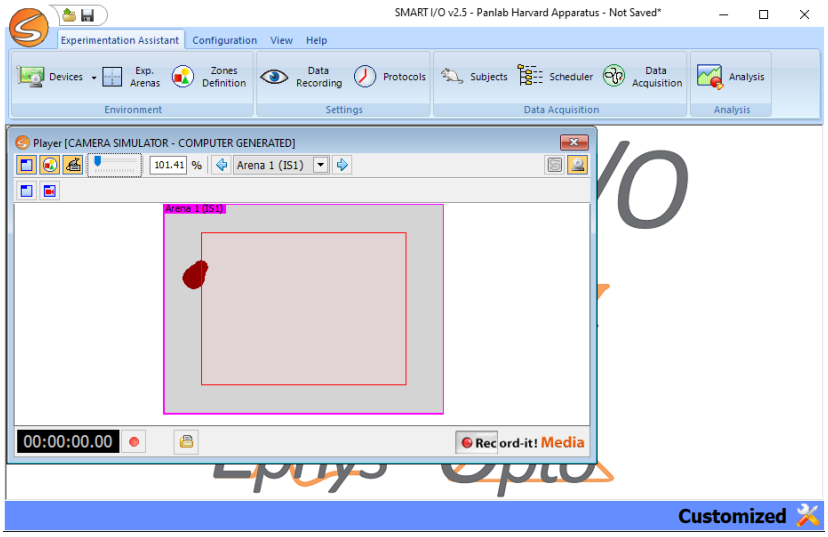
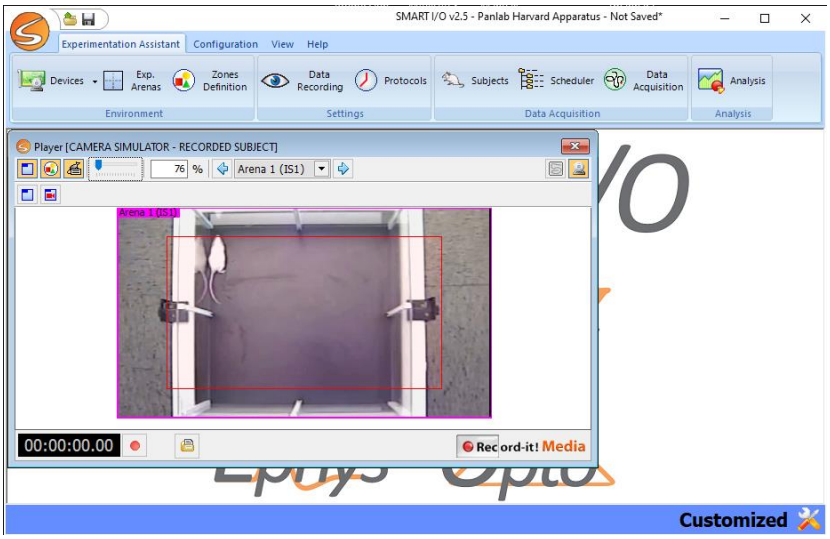
3.8. SIMULATED LIVE IMAGE SOURCE

Three additional simulated live image sources can be used in SMARTIO for demo purposes. A simulated live image is a “live image” that is not fed by an on-line camera but is digitally built on an already recorded video file played in a closed loop.

- **Camera Simulator - Computer generated:** SMARTIO provides the live image of a moving red dot on a grey background.



- **Camera Simulator - Recorded Subject**: SMARTIO provides the live image of a moving white rat on a black background.



- **Camera Simulator - Custom Recorded video**: SMARTIO provides a tool to select a user video file.

⚠	Recommended frame rate for maximum performance on 8 simulated cameras: 5 fps

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3.9. CODECS AND SUPPORTED VIDEO FILES

Digital video files can store large image sequences into relatively small files. To do that, digital recorders use the compression and codification methods that reduce the final size of the file.

	To be processed by SMARTIO, digital video files must be recorded at Constant Frame Rate (CFR), and their Frame Rate cannot be greater than the original one, which means that videos that have been accelerated using an external tool are not supported

Compression and codification processes are carried out by a special software component called CODEC (CO-mpressor/DEC-ompressor) that should be installed both on the recorder and in the reader devices. Each CODEC can deal with a variety of digital video formats, according to vendor specifications. For this reason, SMARTIO can manage digital video files in a variety of formats, provided the corresponding CODEC is already installed.

The CODECS used by SMARTIO are the **H.264/MPEG-4 AVC codec** (Xvid equivalent), providing file with the ***.mkv** extension.
When installing the SMARTIO software, the H.264/MPEG-4 AVC (Xvid) will be **automatically installed**.

Please note that Microsoft Windows® operating system includes CODECs for several standard video formats such as MPEG-4 or Indeo®. SMARTIO installation software also includes additional CODECs to process the video recorded using the H.264/MP4AVC codec and other formats. Installing additional CODECs will allow SMARTIO to open any digital video format, for example DIVX or SVCD.

	Most CODECs discard some of the original data to reduce the file size. Although a CODEC tries to remove only portions of the data that humans are not likely to note, if the compression level of a video file is too high, portions of the removed data will be easily notable, and accuracy will be lost. Therefore, when using CODECs to compress your video, there is a trade-off between quality and file size.
	PANLAB suggests the H.264/MPEG-4 AVC (Xvid) implementation provided within the software or, if possible, acquiring the commercial edition of the CODEC used.

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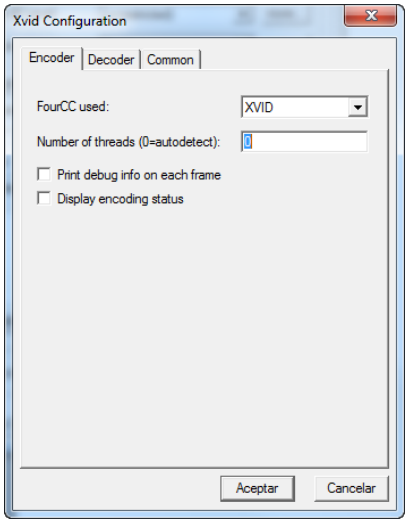
Installing the H.264/MPEG-4 AVC codec manually

This CODEC is installed automatically on the computer when the SMARTIO software is installed. In case a manual installation is required, please follow these steps:

1. Plug the installation key of **SMARTIO** into a USB 2.0 port and launch the installation tool (PANLAB.EXE).
2. Select the **Digital Video Codecs** option.

3. Execute the **Xvid Codec.exe** file. Then follow all the steps with the default options.
4. Click the **Finish** button to finish the installation.
5. Execute the **Configure Encoder** tool located at the Start > All programs > Xvid folder.
6. Press the **Other options…** button located at the bottom of the **Xvid Configuration** window.
7. Uncheck the option **Display encoding status** and press the **OK** button.



8. Then click on **OK** button in the **Xvid Configuration** window.

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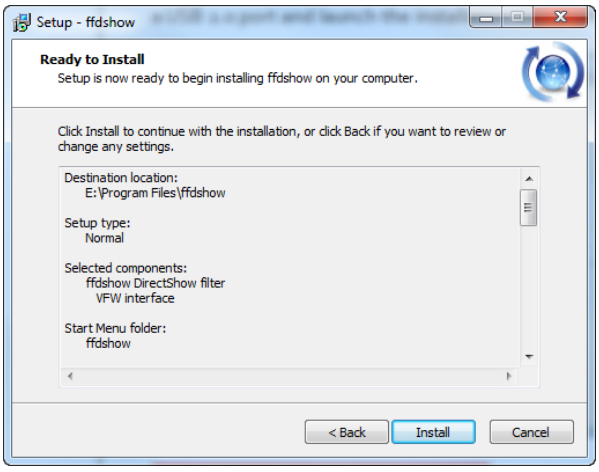
3.10. INSTALLING CODECS TO PROCESS EXTERNAL RECORD-IT! VIDEOS

To be able to properly open the video files generated through RECORD-IT! MEDIA solutions, an MPEG-4 video decoder must be installed on the SMARTIO computer. To install the required video codec, plug the installation key of SMARTIO into a USB 2.0 port and launch the installation tool (PANLAB.EXE).

1. Plug the installation key of SMARTIO into a USB 2.0 port and launch the installation tool (PANLAB.EXE).
2. Select the **Digital Video Codecs** option.

3. Enter the **Record-It! Support** folder and execute the "Ffdshow Codec.exe" file. Then follow all the steps with the default options until finishing the installation.



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3.11. SUPPORTED AND UNSUPPORTED VIDEO FILE FORMATS

SMARTIO supports all common digital video formats. It contains proprietary decoding systems that can decode most of the existing video formats. However, in some special cases, it is possible that the digital video file cannot be opened by RECORD-IT! MEDIA. Also, it is possible that it does not playback correctly or that it requires a special codec to be installed on the system.

Table 5 shows a subset of the most common formats supported by SMARTIO:

Table 5: File extensions supported by SMARTIO

Video format	Usual file extension
Audio Video Interleave	*.avi
Windows Media Video	*.wmv, *.asf
QuickTime	*.mov,*.qt
Moving Picture Experts Group	*.mpg, *.mpeg, *.mp4, *.m4v, *.3gp
Matroska Multimedia Container	*.mkv

The above-mentioned formats contain video streams that may be compressed with different codecs. SMARTIO supports the codecs specified in the following list. Additional codecs are supported if the corresponding codec is installed in the system.

8088flex TMV	LCL (LossLess Codec Library) MSZH
Amazing Studio PAF Video	LOCO
AMV Video	LucasArts SANM/Smush
ANSI/ASCII art	lossless MJPEG
Apple Intermediate Codec	Microsoft ATC Screen
Apple MJPEG-B	Microsoft Expression Encoder Screen
Apple ProRes	Microsoft RLE
Apple QuickDraw	Microsoft Screen 1
Asus v1	Microsoft Screen 2
Asus v2	Microsoft Video 1
ATI VCR1	Mimic
ATI VCR2	Miro VideoXL
Auravision Aura	MJPEG (Motion JPEG)
Auravision Aura 2	Mobotix MxPEG video
Autodesk Animator Flic video	Motion Pixels video
Autodesk RLE	MPEG-1 video
Avid 1:1 10-bit RGB Packer	MPEG-2 video
AVS (Audio Video Standard) video	MPEG-4 part 2
AYUV	MPEG-4 part 2 Microsoft variant version 1
Beam Software VB	MPEG-4 part 2 Microsoft variant version 2
Bink Video	MPEG-4 part 2 Microsoft variant version 3
Bitmap Brothers JV video	Nintendo Gamecube THP video
y41p Brooktree uncompressed 4:1:1 12-bit	NuppelVideo/RTjpeg

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CamStudio	On2 VP3
CD+G	On2 VP5
CDXL	On2 VP6
Chinese AVS video	On2 VP7
Discworld II BMV Video	VP8
Canopus Lossless Codec	VP9
Cinepak	Pinnacle TARGA CineWave YUV16
Cirrus Logic AccuPak	Prores
CPiA Video Format	Q-team QPEG
Creative YUV (CYUV)	QuickTime 8BPS video
Deluxe Paint Animation	QuickTime Animation (RLE) video
DNxHD	QuickTime Graphics (SMC)
Duck TrueMotion 1.0	QuickTime video (RPZA)
Duck TrueMotion 2.0	R10K AJA Kona 10-bit RGB Codec
DV (Digital Video)	R210 Quicktime Uncompressed RGB 10-bit
Dxtory capture format	Raw Video
Electronic Arts CMV video	RealVideo 1.0
Electronic Arts Madcow video	RealVideo 2.0
Electronic Arts TGV video	RealVideo 4.0
Electronic Arts TGQ video	Renderware TXD (TeXture Dictionary)
Electronic Arts TQI video	SGI RLE 8-bit
Escape 124	Sierra VMD video
Escape 130	Silicon Graphics Motion Video Compressor 1 (MVC1)
FFmpeg video codec #1	Silicon Graphics Motion Video Compressor 2 (MVC2)
Flash Screen Video v1	Smacker video
Flash Screen Video v2	SMPTE VC-1
Flash Video (FLV)	Sony PlayStation MDEC (Motion DECoder)
Forward Uncompressed	Sorenson Vector Quantizer 1
Fraps	Sorenson Vector Quantizer 3
Go2Webinar	Sunplus JPEG (SP5X)
H.261	TechSmith Screen Capture Codec
H.263 / H.263-1996	TechSmith Screen Capture Codec 2
H.263+ / H.263-1998 / H.263 version 2	Theora
H.264 / AVC / MPEG-4 AVC / MPEG-4 part 10	Ut Video
HEVC	v210 QuickTime uncompressed 4:2:2 10-bit
HNM version 4	v308 QuickTime uncompressed 4:4:4
HuffYUV	v408 QuickTime uncompressed 4:4:4:4
HuffYUV FFmpeg variant	v410 QuickTime uncompressed 4:4:4 10-bit
IBM Ultimotion	VBLE Lossless Codec
id Cinematic video	VMware Screen Codec / VMware Video
id RoQ video	Westwood Studios VQA (Vector Quantized Animation)

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IFF ILBM	Windows Media Image
IFF ByteRun1	Windows Media Video 7
Intel H.263	Windows Media Video 8
Intel Indeo 2	Wing Commander III / Xan
Intel Indeo 3	Wing Commander IV / Xan
Intel Indeo 4	Winnov WNV1
Intel Indeo 5	WMV7
Interplay MVE video	YAMAHA SMAF
J2K	Psygnosis YOP Video
Karl Morton's video codec	YUV4
Lagarith	ZeroCodec Lossless Video

SMARTIO does not support videos with DRM (Digital Rights Management) protection. Incompatibilities with **K-Lite Codec Pack** have also been detected and they may affect the acquisition process in ".avi" digital video files. It is strongly recommended to avoid installing this codec pack.

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4. INSTALLING THE LASER OPTOGENETIC SYSTEM

The use of an external laser optogenetic stimulator needs the Panlab Linkbox01 interface (Legacy or High-Speed version). See the Panlab Linkbox01HS User's Manual for more details about the installation of this device.

	Only the 2 first ports of the Linkbox would be available for the optogenetic TTL output trigger.

4.1. INSTALLING THE RS232/USB CONVERTER DRIVERS

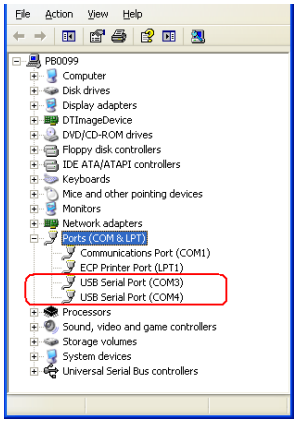
The connection of the Linkbox01 to the computer requires the use of an RS232/USB converter.

The RS232/USB adapter is needed for converting a USB port to a serial port valid for communications between hardware and software. It allows the use of two serial ports in your PC or laptop. We recommend the use of a specific model of adapter. We cannot guarantee the correct functioning of the system with any other USB-serial adapter. The adapter includes a spare extension cable.

RS232/USB converter for the Linkbox01HS

The connection of the Linkbox01HS (high-speed version) to the computer needs the use of the high-speed CONRS232USBHS converter (USB2-H1002).

1. Connect the adapter to the computer.
2. Windows 8 and 10 will automatically install the drivers. If working with a Windows 7 or inferior, please refer to the notice provided in the box of the adapter.
3. Once connected and installed, two serial ports will be shown into the [Device Manager] window. Usually, the numbers assigned by the system are sequential.



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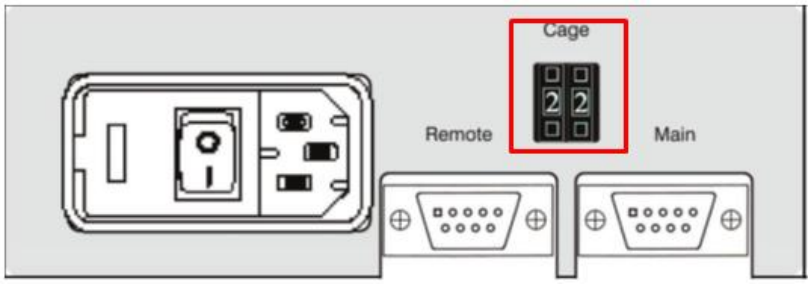
4.2. LINKBOX SETTINGS

An ID number must be set by the user on the rear panel of the Linkbox. Make sure that the ID number is set to 01 before beginning the experiment.



4.3. LASER OPTOGENETICS SETTINGS

The settings of the laser optogenetics stimulator would depend on the model and brand used for the experiment.
Specific adapter cables would be needed to connect the Panlab Linkbox interface to the optogenetics laser stimulator. The adapter cables are not provided with the SMARTIO system because these adapters would depend on the optogenetics laser stimulator device used for the experiment. Panlab technical support should be contacted to discuss this point before purchasing the SMARTIO software or Panlab Linkbox interface.

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5. VIDEO-TRACKING PREVIOUS CONSIDERATIONS

SMARTIO is provided with the very powerful and flexible SMART 3.0 video-tracking functions, which can be used in a wide variety of behavioral experiments. Below is given a list of steps that must be fulfilled to perform the experiments. The list must be considered as a general guideline given that the order of these steps can be changed depending on the user's experimental stage:

- Experimental area preparation
- Image source selection, settings and calibration
- Ephys/Opto Hardware and software device selection, settings and test
- Arena/Working area settings
- Zones definition
- Subject detection settings
- Experimental protocol/timing
- Experiment subject data base
- Project scheduler/planning
- Data acquisition (Video recording, tracking, global activity or manual scoring)
- Data analysis & report generation

5.1. HOW DOES A VIDEO-TRACKING WORK?

Classic video-tracking systems are designed to automate the typical observational task required by a scientific experiment. In behavioral experimentation, video-tracking systems automatically detect and register the position/displacement of a specific subject within a specific experimental area (arena).

The techniques used for such a purpose can be divided in two main modes: tracking and global activity (see chapter 12 - DATA RECORDING & DETECTION SETTINGS for more details about each technique used). A deeper understanding of the video-tracking basic techniques is very important to:

- choose the most appropriate technique for their desired studies.
- learn how the configurations (experimental conditions, software settings…) may affect the relevancy of the data obtained and the interpretation of the results.

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5.2. IMPLICATIONS FOR EXPERIMENT PREPARATION AND SETTINGS

Some important considerations must be considered when working with a video-tracking system.

Lighting conditions

Lighting is a fundamental environmental condition that should be finely controlled in order to obtain reliable data in SMARTIO. An ineffective lighting configuration may greatly reduce the possibility of obtaining a detectable and clear tracking of the animal. Lighting conditions can be adjusted by acting on the experimental area (light chosen, position, intensity) and by using some available functions in the SMARTIO software. Especially regarding the lighting conditions, an ideal setup should satisfy some key aspects.

- Work in a controlled environment

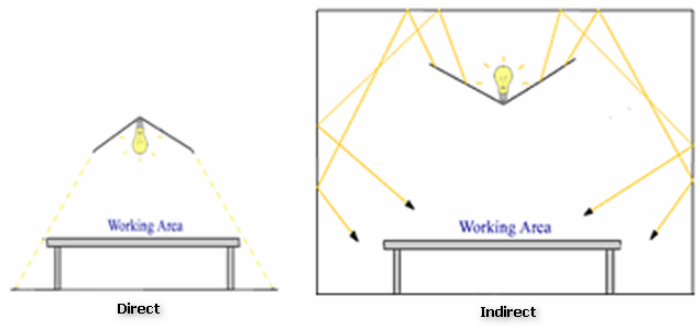
The lighting conditions must be specifically controlled with no interference from the uncontrolled sources of light (e.g., sunlight). As a consequence, the experimental room should be a space without windows, or a room as much protected as possible from the exterior light.

- Avoid fluorescent light

The day light conditions can be easily simulated with standard lamps or bulbs. Whenever possible, avoid using fluorescent lamps, as these are susceptible to changes in power supply frequency and offer light concentrated in the blue area of the spectrum.

- Use indirect illumination

Direct illumination can easily produce reflections on the experimental area and should be avoided as far as possible. Always use indirect illumination, i.e., illuminate the ceiling instead of directly illuminating the scenery.



Mazes & enclosures

SMARTIO can work with a wide range of different mazes and enclosures. It also can be used in any user-defined area. Mazes and experimental enclosures are available in different materials, sizes and colors. Please contact your dealer to look for the best solution for your experimental requirements. Here are some key aspects to be taken into account when choosing the correct enclosure.

- Material

An enclosure built with a material which does not retain odors after washing, such as Plexiglas, is recommended. The odors left by the previous animal placed into the apparatus may affect the outcome of the following subject. For this reason, wooden materials are highly not recommended.

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- Size

The sizes of commercially available enclosures are standard for adult mice (25-30g) and rats (250-300g). For young or old animals, depending on the dealers, it may be possible to order special enclosures with user-requested sizes. The perfect size for video-tracking studies is “when the experimental area can be seen entirely through the image of the camera”.

- Colors and finish

When using a video-tracking system, the better the contrast between the animal and the enclosure, the better the detection of the animal. Thus, a good contrast is a key feature to obtain high reliability and precision of the tracking measurements (distance covered, speed, displacement tracking). Therefore, the color and the finish of the enclosure are of great importance. It is preferred for the color to be matte, minimizing reflections due to the lighting as these can induce artifacts within the video and impact the tracking results. Exceptions do exist and are to be followed when the color of the background is not critical for the experiment as in:

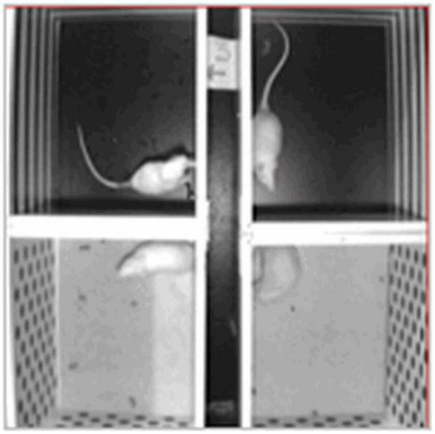
  - If only white animals will be used in the experiment, we recommend using an enclosure with black color floor.
  - If only black animals will be used in the experiment, we recommend an enclosure with light grey or white color floor
  - If animals with different color will be used in the same experiment (mixed-background mice for instance), we recommend an enclosure with light grey color.
  - IR-illumination through translucent floors.

When using IR-illumination through translucent floors, the room illumination can be set accordingly to meet the requirements for the behavioral aspect of the experiment regardless the IR-illumination, which needs to meet the requirements of the video tracking system. Since rodents do not perceive the IR-light, they are not disturbed by the IR-light settings; Conversely, since the camera lens is equipped with an IR-filter the tracking is not altered by the room light setting. Furthermore, the contrast of the animals' dark silhouettes on a bright background is very much suited for reliable tracking.

Image noise

Due to the nature of basic video-tracking systems, image noise can produce little variations of the center of mass that could imply errors in the estimation of the total distance travelled. This is a common phenomenon easily corrected by filters available in the Detection Settings and in the Analysis sections of SMARTIO.

- Special case: zones with different contrast – zones with shadows/reflections



Special care should be taken with the settings and interpretations of the results when working with configurations in which the contrast between the color of the floor and the color of the animal is very low, as it is the case in Black & White boxes or in some place preference boxes.

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In these specific conditions, the volume of pixel detected in the low-contrast compartment may be lower than the volume of pixels detected in the high-contrast compartment.

The meaning of all the pixels detected is considered for tracking the animal. The tracking in the zone with the lower number of pixels may produce more “vibrations” than the tracking in the good contrast zone in which a greater number of pixels is considered. Good contrast is necessary to avoid the false impression that the animal displayed higher locomotor activity in the low-contrast compartment.

Same problems may occur in experiments performed with direct illumination as this lighting is known to cause shadows or light reflections in the experimental area and artifacts.

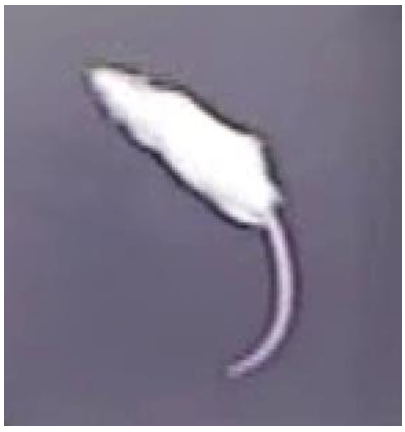
Solutions proposed:

- Improve lighting conditions to avoid any shadows and light reflection.
- Use the SMARTIO option allowing setting different brightness/contrast conditions in specific zones for that the volume of pixels detected will be the same in all the experimental areas or between each compartment (for details see chapter 12.2 - Lighting conditions for specific zones)
- Use the SMARTIO advanced options for filtering and smoothing in the Detection Settings and Analysis sections (for details see chapter 12.3 - DETECTION SETTINGS and chapter 20.4 - Filtering and smoothing techniques in SMARTIO).

Analog cameras interlaced video

The images provided by most analog cameras are interlaced. Interlacing videos is a technique for doubling the perceived frame rate of a video display without consuming extra bandwidth. The interlaced signal contains two fields of a video frame captured at two different times. Although this enhances motion perception to the viewer, this technique produces images which are not optimal for video tracking, especially in case the subject is moving fast horizontally across the image.

The following is an example of a normal image compared to an interlaced image:



Normal Image

Interlaced Image

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Solutions proposed:

- For all the devices supported by SMARTIO that use analog cameras (CONVANAUSB and the frame grabber board), there is available a deinterlacing filter that decodes interlaced images back to normal images. For information on how to activate this filter, please refer to chapter 7.1 - Analog camera image source).
- The use of digital cameras that do not interlace images.

Considerations about recording digital video files

SMARTIO is provided with embedded video digital recording capabilities which represent a useful tool for most of the experiments performed in Neuroscience research (short tests conducted in mazes).

For more advanced needs (long duration experiment, multiple camera needs…), SMARTIO is also fully compatible with the full line of external RECORD-IT! digital video recording solutions provided by Panlab Harvard Apparatus. Please contact your dealer for a detailed description of the benefits and advantages of using external RECORD-IT! options in combination with SMARTIO.

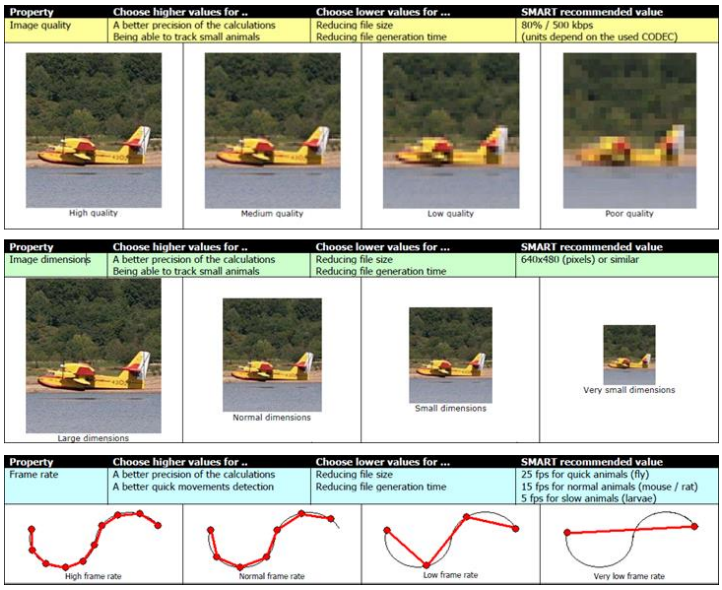
The following considerations must be considered when creating a digital video file using the embedded RECORD-IT! module of SMARTIO or using an external video recording unit.

- CODEC performance

  In case the digital video files are not recorded with RECORD-IT!, they must be compatible with SMARTIO. The corresponding CODEC used to record the video file must be installed on the SMARTIO computer to process the file format. Please refer to chapter 3.9 - CODECS AND SUPPORTED VIDEO FILES for more details.

  The performance of the CODEC used by the recorder device influences the properties of the created digital video file: (i) image quality, (ii) image dimension, (iii) frame rate and (iv) time needed to generate the digital video file.

  Choosing and configuring the CODECs adequately will then allow optimizing the tracking process by the SMARTIO video-tracking software.



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- Computer's performance

  If the digital video file is recorded within SMARTIO using the embedded module of RECORD-IT!, a much higher computer's performance is required. Please check that the specifications of your computer fulfill the system requirements described in the chapter 2.3 - SYSTEM REQUIREMENTS.

- Background snapshot

  In the standard detection modes used by SMARTIO (center-of-mass and TriWise) a snapshot of the experimental background (without animal) must be taken before starting tracking. This snapshot will serve as reference. The differences between the experimental image (with animal) and the reference image (without animal) will be materialized in pixel format (black dots) and processed by SMARTIO; the rest (which is identical to the reference image) will be ignored by SMARTIO (white).

  Given these considerations, it is highly advisable to have the first seconds (at least 1 s) of the digital video file recorded with the image of the experimental background recorded without animals. If this is not fulfilled, SMARTIO provides a Snapshot Editor tool to remove the animal and other artifacts from the image. Please refer to the chapter 12.3 - Snapshot editor for more details on this tool.

- Brightness & contrast

  Although SMARTIO offers the possibility to adjust brightness and contrast in a live image as well as in a digital video file, it is very important to set adequate lighting conditions before recording the experiment.

  Use the Test mode of SMARTIO to check your environmental conditions before recording the digital video files. Please refer to the chapter 12.3 - DETECTION SETTINGS for more information on how to use the Test mode in each detection mode provided by SMARTIO.

- Minimum video length

  At least 5 seconds of video must be recorded to allow SMARTIO to analyze it.

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6. BEGINNING WITH SMARTIO

6.1. STARTING ASSISTANT

Once SMARTIO is launched, a Starting Assistant tool is shown on the screen:

The Starting Assistant tool allows you to:

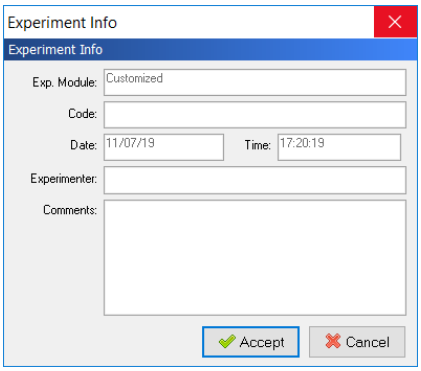
- Start a new experiment.
- Continue with a previously saved experiment.
- Analyze the data acquired in a previously saved experiment.

The Starting Assistant also shows the list of modules and extensions available in your license.

Starting a new experiment

To start a new experiment:

1. Select **New**.
2. Make double click on the icon of the protocol which the new experiment should follow (Alternatively you may also select the protocol first and then double click on the New task).
3. Enter details in the **Experiment Info** dialog.



4. Click on the **Accept** button.

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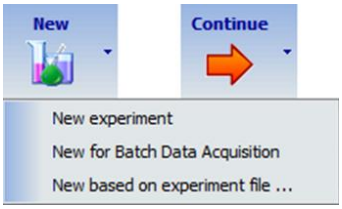
Starting a new experiment for Batch Data Acquisition

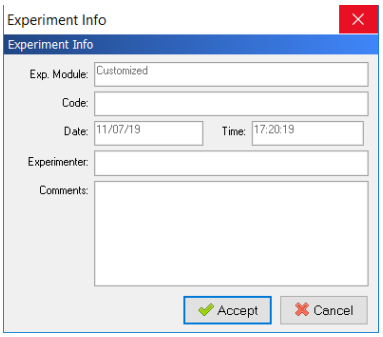
Batch Data Acquisition is a tool that allows to acquire data from multiple digital videos in sequence. This is very useful when many videos from different subjects have been recorded with the same parameters and conditions.



To start a new experiment for Batch Data Acquisition:

1. Click on the arrow located at the right side of the New button.
2. Select the **New for Batch Data Acquisition** menu option.

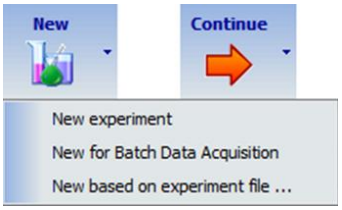
3. Enter details in the **Experiment Info** dialog.



4. Click on the **Accept** button.

Starting a new experiment based on an already existing experiment

A new experiment can be also created from an existing one so that most of the information and configurations can be reused:



5. Click on the arrow located at the right side of the **New** button.
6. Select the **New based on experiment file…** menu option.
7. Locate and select the file in which the base experiment is stored.
8. Enter details in the Experiment Info dialog.
9. Click on the **Accept** button.

A new experiment is created importing all the following elements from the base experiment:

- Image source
- Calibration
- Arenas
- Zones definition
- Detection settings
- Time settings
- Scheduler's phases and sessions

The subject list is not imported automatically but it can be imported manually later. Please note that the trials acquired in the original experiment are not imported in the new one.

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Continuing an experiment

To continue with an already existing experimental file:

1. Select **Continue**.
2. Locate and select the desired folder and experimental file.
3. Click on **Open** to load the experimental file.
   Most recently used experimental files are provided within the dropdown button of the **Continue** button.

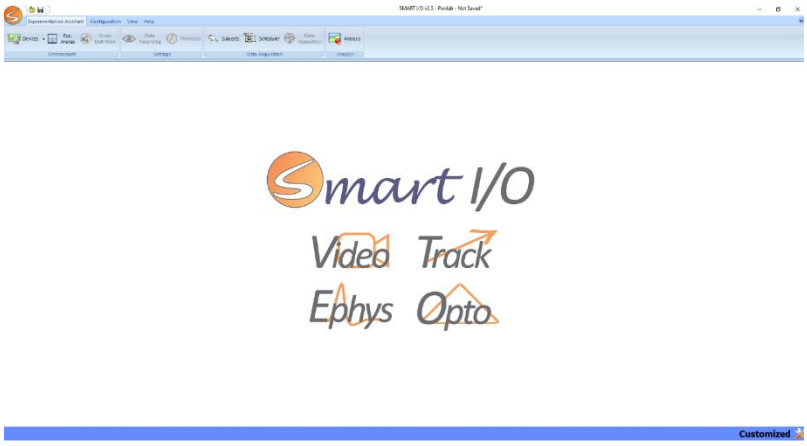
Analyzing an experiment

To analyze the session contained in an existing experimental file:

1. Select **Analyze**.
2. Locate and select the desired folder and experimental file.
3. Click on **Open** to load the experimental file.
4. The application will directly open the Analysis section.
   Most recently used experimental files are provided within the dropdown button of the **Analyze** button.

6.2. SMARTIO MAIN WINDOW

Once the new experiment is initiated, the main window of SMARTIO is shown with the following elements:



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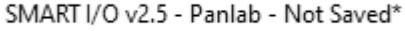
Title bar



The title bar shows the name and version of the application and the name of the experimental file open. As no experimental file has yet been opened, “Not Saved” is shown. The asterisk “*” indicates that modifications on the experiment file have still not been saved.

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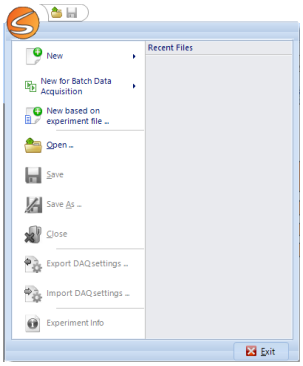
Main menu

The main menu is shown when the “S” button located at the top left corner of the window is clicked.



The main menu contains the options to:

New	Start a new experiment: similarly to what explained in chapter 6.1 - Starting a new experiment but using a dropdown menu to choose the experimental module.
New for Batch Data Acquisition	Start a new Batch Data Acquisition experiment: similarly to what explained in chapter 17.7 - BATCH DIGITAL VIDEO DATA ACQUISITION.
New based on experiment file ...	Start a new experiment based on the settings of an existing experiment similarly to what explained in chapter 6.1 -Starting a new experiment based on an already existing.
Open ...	**Open** a previously saved experiment: as explained in chapter 6.1 -. In addition, the “Recent Files” section gives you a shortcut to the experimental files most recently used.
Save	**Save** your experiment under the same experimental file name.
Save As ...	Save your experiment under a different experimental file name.
Close	**Close** the experiment.
Export DAQ settings ...	**Export** all the settings required for data acquisition to an external file. More information in chapter 6.2 - Importing and exporting data acquisition settings.
Import DAQ settings ...	**Import** all the settings required for data acquisition from an external file. More information in chapter 6.2 - Importing and exporting data acquisition settings.
Experiment Info	Access the **Experiment Info** window to review and/or edit the information.

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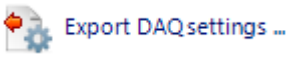
Importing and exporting data acquisition settings

All settings required to acquire data can be exported and imported to and from an external DAQ Settings file (.smepq). The settings stored within a DAQ Settings file are:

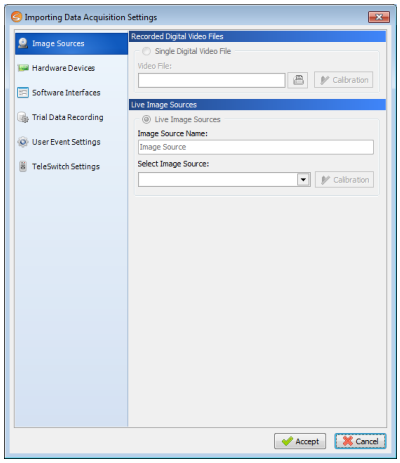
- Devices settings
- Trial Data Recording
- User Event Settings
- Tele switch Settings

To export the current settings of the experiment, select the option Export DAQ settings from the main menu of the main form. A standard dialog will be shown to choose the location and name of the file to be exported. By default, the dialog will show the folder configured as Configuration files in the Path Settings panel described in chapter 16.1 - PATH SETTINGS.



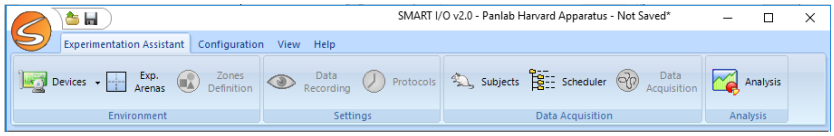
To import a settings file into the current experiment, select the option Import DAQ settings from the main menu of the main form. A standard dialog will be shown to choose the file to be imported. By default, the dialog will show the folder configured as Configuration files in the Path Settings panel described in chapter 16.1 - PATH SETTINGS.

Once a file is selected for importation, the Data Acquisition Settings panel will be shown. The Data Acquisitions Settings panel allows the reviewing and editing of the settings before they are imported into the experiment.



	The **Export DAQ Settings…** and **Import DAQ Settings…** button are disabled while the Zones Definition panel is open (see chapter 11 - ZONES DEFINITION).

Menu bar



The **Experimentation Assistant** bar has been designed to give you a quick way to access the main operations. Each button in the bar corresponds to a task within the typical experimentation process.

This bar is designed in a way that only the currently allowed tasks are active. The main tasks are ordered following a suggested order in which a new experiment may be set:

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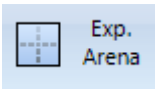
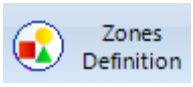
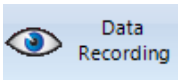
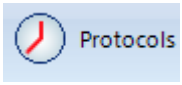
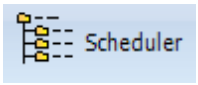
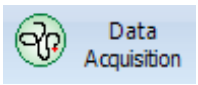
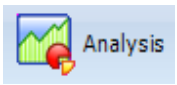
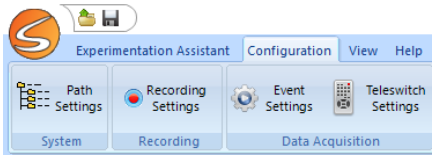
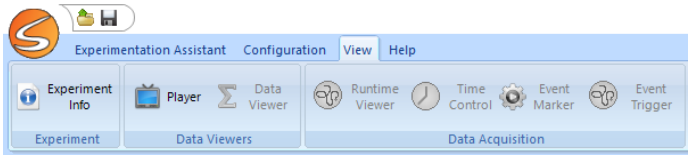
	To set the source of image and connected hardware/software
	To define the experimental area used in the experiment and the Element available in each arena.
	To determine the regions of interest (zones) in the experimental area.
	To adjust the parameters of data acquisition: tracking mode, detection settings etc.
	To set the time conditions and instructions of the trials.
	To manage the experimentation subject database.
	To define the experimentation plan (phases, sessions and trials).
	To execute the scheduled trials and acquire the data.
	To generate analysis reports of the finished trials.

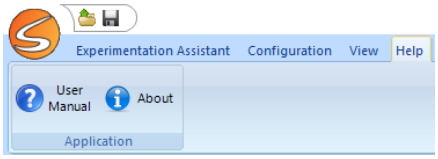
The **Configuration** bar collects additional options to set folder paths. manual events, recording and teleswitch.



Under the **View** bar it is possible to activate/deactivate the view of specific panels such as the info about the experiment, data viewers panels and data acquisition panels.



The **Help** bar shows an icon to access the user manual and one button to show the license information.



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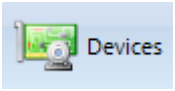
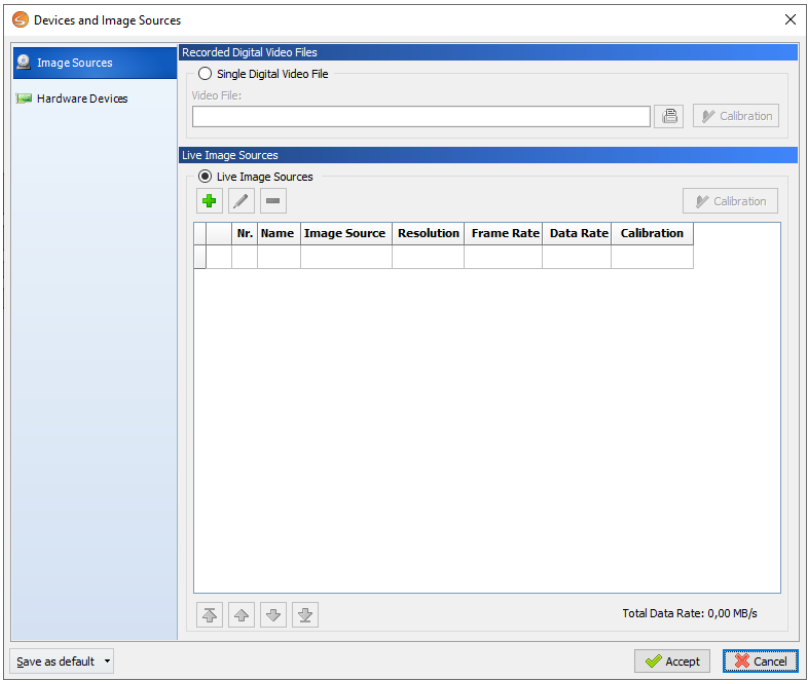
7. DEVICES



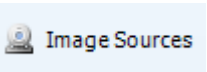
The **Devices** main button leads to an assistant panel for the choice and configuration of the image sources as well as for the choice and configuration of external hardware.



It is possible to open and close all the image sources and devices at the same time clicking on the arrow at the side of the **Devices** button

7.1. IMAGE SOURCES

The first step to follow before starting the experiment is selecting the image source. It is possible to select a recorded digital video file or use a live image source that provides images in real time.



To define an image source, press the Image Source button in the left part of the panel. SMARTIO provides the option to work with multiple live image sources or a single recorded digital video file.

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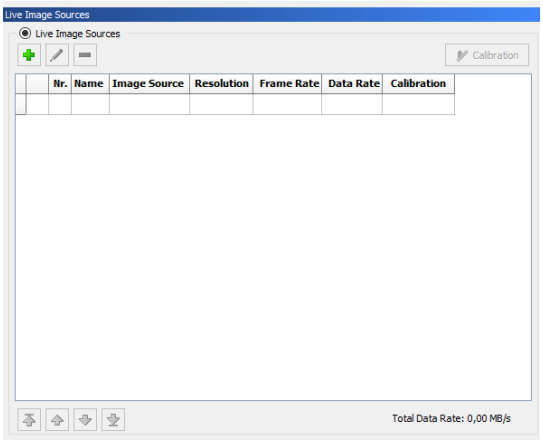
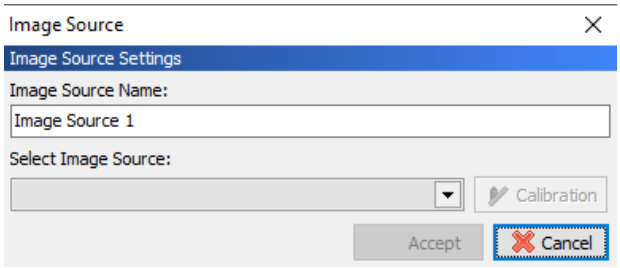
Live image source

Live image sources must be chosen and configured for any online tracking experiment.



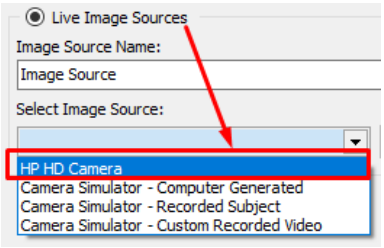
Live image selection



1. Click on  to select the option **Live image sources.**
2. Change the name of the image source if needed by using the **Image Source Name** text box.
3. Select the type of the source in the **Select Image Source** drop-down-list. The Image source list contains one element for each available (and supported) image acquisition device. Please note that corresponding drivers and related software must be installed for it to be recognized.
4. Click on  to edit the selected image source and on  to delete it.

The available elements are:

- A camera connected to the computer
  The camera should be connected to the computer and selected from the **Select Image Source** dropdown list:



  Depending on the type of digital camera selected, a different setting panel will be shown (see next section).

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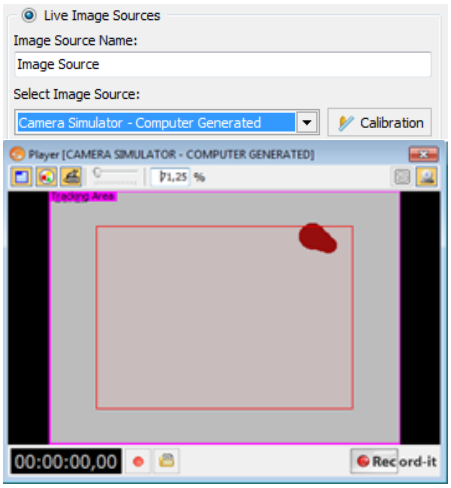
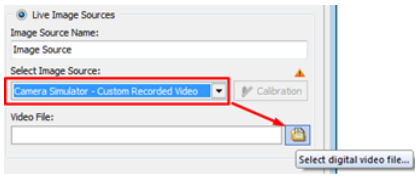
- A simulated live image
  A simulated live image is a live image that is not feed by a direct on-line camera but by digitally built or already recorded video file played in closed loop (see chapter 3.8 - SIMULATED LIVE IMAGE SOURCE). Options available are:

Computer generated

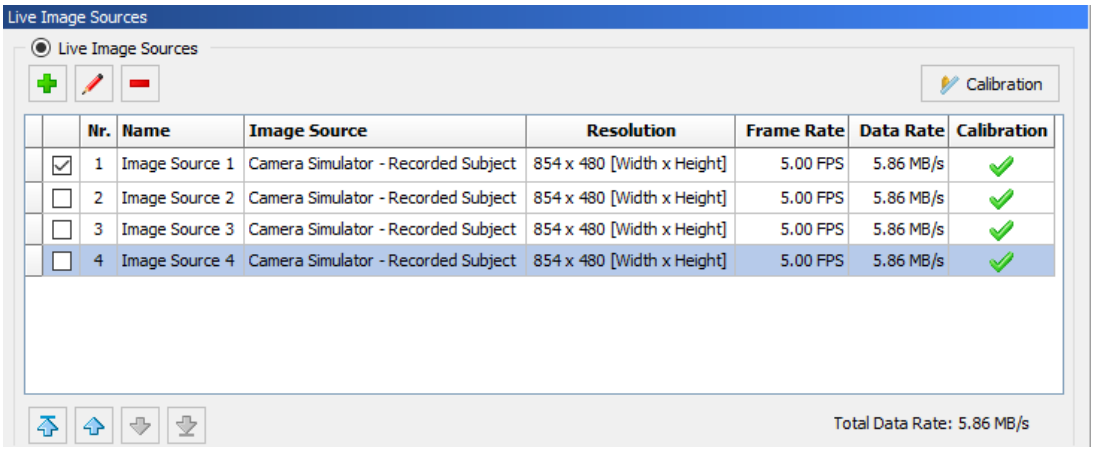


Recorded Subject

Custom Recorded video



After adding one or several image sources, the following panel will resume the information about each of them:



	Nr.	Name	Image Source	Resolution	Frame Rate	Data Rate	Calibration
☑	1	Image Source 1	Camera Simulator - Recorded Subject	854 x 480 [Width x Height]	5.00 FPS	5.86 MB/s	✓
☐	2	Image Source 2	Camera Simulator - Recorded Subject	854 x 480 [Width x Height]	5.00 FPS	5.86 MB/s	✓
☐	3	Image Source 3	Camera Simulator - Recorded Subject	854 x 480 [Width x Height]	5.00 FPS	5.86 MB/s	✓
☐	4	Image Source 4	Camera Simulator - Recorded Subject	854 x 480 [Width x Height]	5.00 FPS	5.86 MB/s	✓

A ☑ checkbox allows to show or hide the image source, in case it is not needed in a specific moment. The buttons allow to modify the order of the list of the image sources (Top, Up, Down and Bottom respectively).

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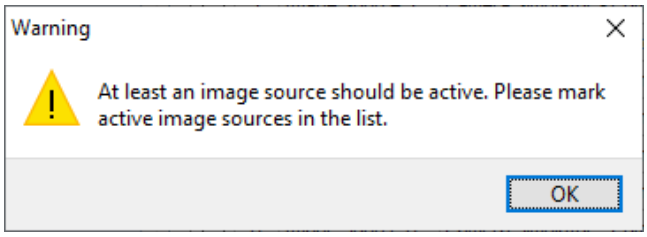
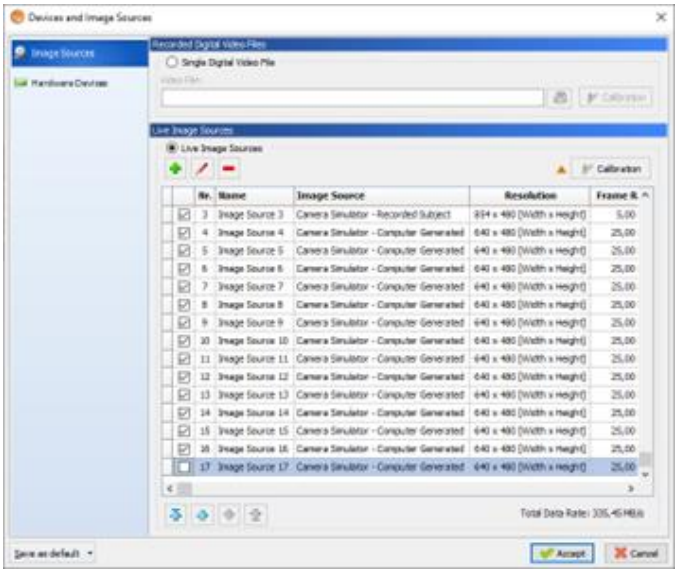
At least one image source must be activated to work with SMARTIO. If no image source is marked with the checkbox, the following message will be shown:



It is possible to add as many image sources as needed, but only 16 of them at the same time can be activated using the checkbox.



SMARTIO automatically enters in “Video Recorder” mode when more than 8 image sources are activated simultaneously. The tracking is only available for up to 8 image sources at the same time. In “Video Recorder” mode only recording and analyzing acquired trials will be possible (see chapter 18).

The **Data Rate** column indicates the amount of data required by each camera. In the bottom right angle, the line Total Data Rate reports the sum of the data rates of all the image sources. It has been validated a correct functioning of the system when the Total Data Rate is below 120 MB/s. In case its value is higher, a warning sign is shown:

1. Click on **Accept** to open simultaneously all the image sources.
2. Start a recording (see chapter 8.1 - LIVE IMAGE SOURCE PLAYER PANEL and chapter 16.2 - RECORDING SETTINGS)
3. Start the acquisition of data (see chapter 17 - DATA ACQUISITION).
4. Open the Task Manager and check that the CPU usage is below 50%. If it is higher, consider reducing the number of FPS, the frame size or the number of image sources simultaneously working.

The column **Calibration** reports whether the calibration of each image source has been correctly performed or not (chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration).

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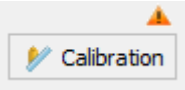
Live image source calibration



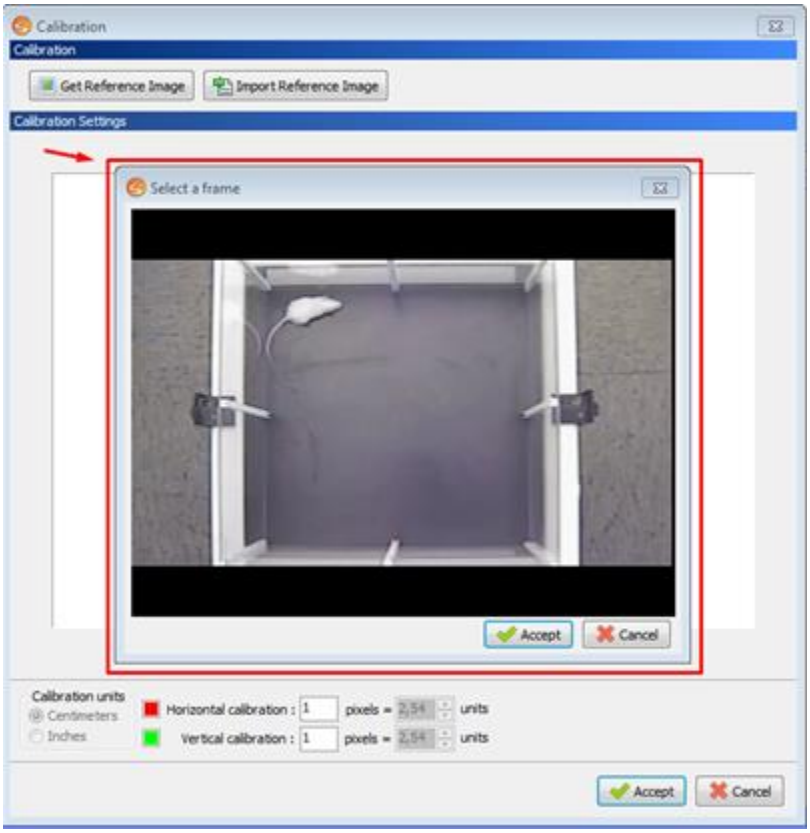
The calibration process allows SMARTIO to associate a fixed number of pixels to a real length dimension. This way, it will be able to calculate dimension-based variables (speed, distance, …) within the experimental area.

Digital images are formed by a group of pixels arranged in a rectangular matrix which dimensions depend on the image acquisition device (webcam, digital camera, etc.). The dimensions of that matrix are commonly called “resolution of the image”. For example, a typical webcam provides an image with a resolution of 640 x 480 pixels, that is, the pixels of each frame are arranged in a rectangular matrix which dimensions are 640 and 480 pixels respectively.

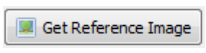
SMARTIO starts all its calculations (speed, status, etc.) by measuring distances in pixel units and, through the calibration process, it is transformed into real unit of distance (centimeters or inches).

In order to improve the precision of such calculations, use a higher resolution (and, if possible, an object with higher dimensions). However, increasing the resolution requires a greater computation to process each frame.

1. Click on the **Calibration** button available in the Live Image Source section.
   The software will display a current view of the live image and request the user to select a frame by clicking on the **Accept** button.



If no calibration has been performed before, the current frame of the image source is used as reference image to calibrate. If the calibration has been performed before, the previously used reference image is shown.



Use the **Get Reference Image** button to refresh the reference image from the currently opened image source.

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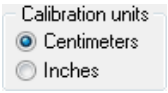
The user can also choose to set the calibration in an already saved reference image. In that case, the **Get Reference Image** button is used to import the image from an external file.

The reference image is shown with overlaid vertical and horizontal axes.

2. Choose an object of the scenery whose dimensions are known: it will be used as reference (for example: the area delimited by the distance between the base of the walls of the experimental area). Be sure to select an object on floor of the experimental area in order to obtain a more accurate calibration.
3. Drag the horizontal and vertical markers located at the borders of the calibration image to fit your reference object into the calibration lines.
4. In the “Calibration units” box, choose “Centimeters” or “Inches” as unit of length.



5. Introduce the horizontal and vertical dimensions of your reference object into the fields “**Horizontal calibration**” and “**Vertical calibration**”.

6. Click on the **Accept** button to apply the changes.

⚠	Calibration is stored into each trial during data acquisition to be used during the analysis process. This means that changing calibration will only affect the trials to be acquired and not the previously acquired trials. It is possible to change the calibration of registered tracks in case the calibration used during data acquisition was incorrect, however it is recommended, if possible, to discard and acquire the trial again as some detection processes (as TriWise) use the calibration to provide a more reliable tracking. More information about recalibrating trials once acquisition is finalized can be found in chapter 15.2 - Recalibrating an acquired trial.

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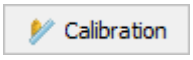
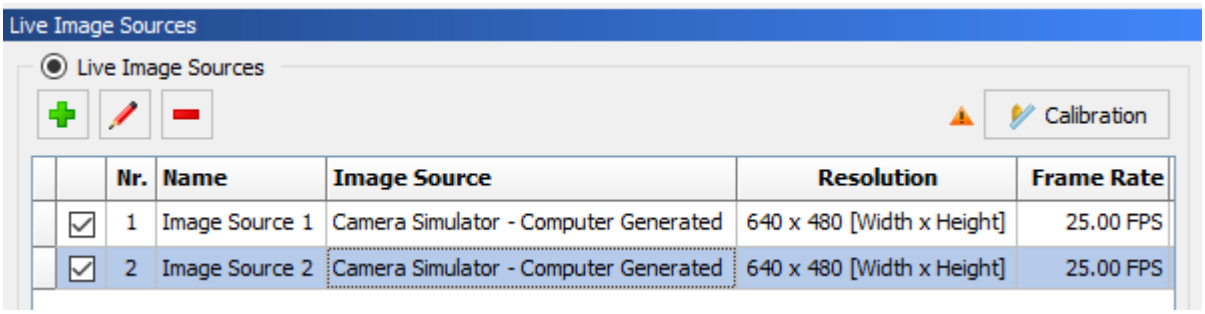
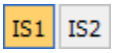
Multiple live image sources

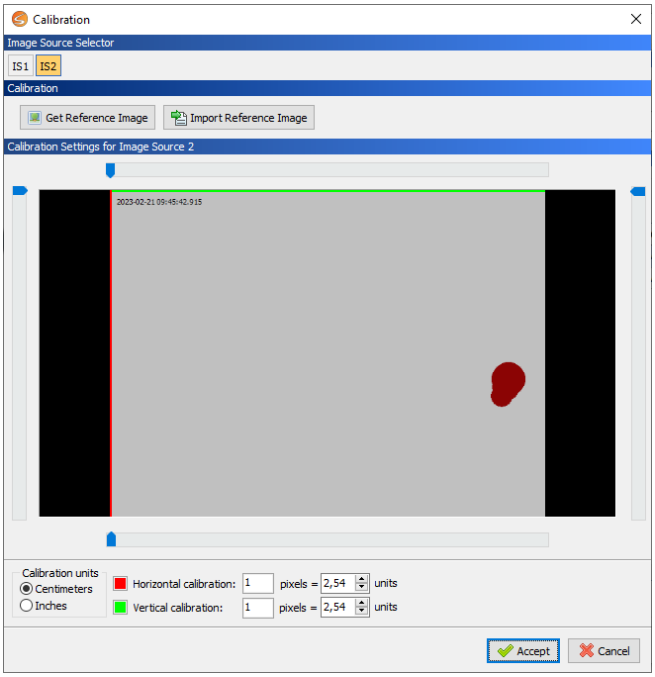
When several image sources are present, it is possible to calibrate them from the button 

in the Live Image Sources panel



From the following panel it will be possible to switch from one image source to the other to calibrate them one by one, using the 

buttons. The name of the image source is shown in the header.



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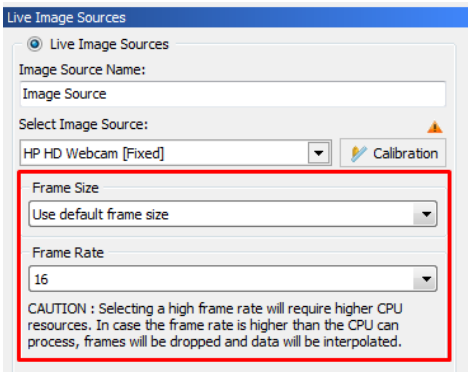
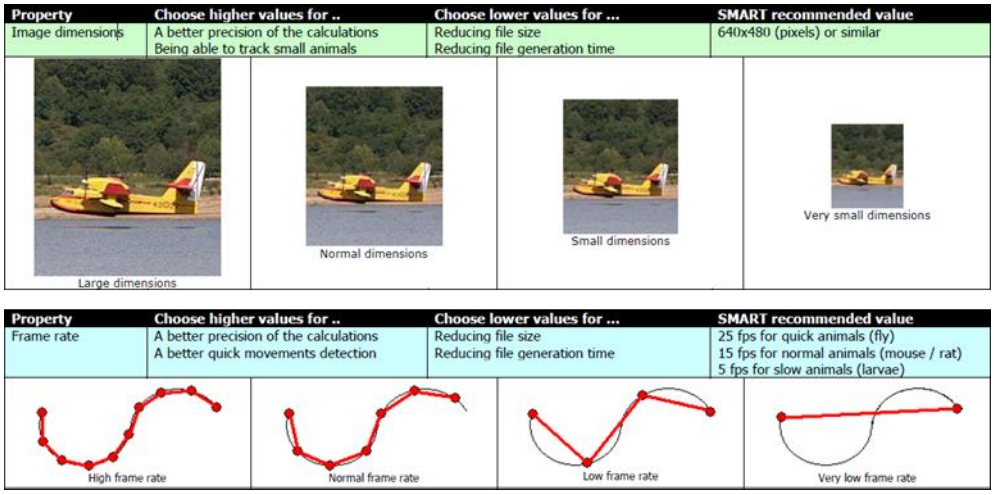
Live image source settings

If a life image source is selected (as a webcam or any other actual or simulated live image source), then some advanced settings will be available from the Live Image source section.

The advanced configuration panel will allow you to select the frame size and frame rate (in frames per second) to be provided by the selected image source device.



Depending on the device to be configured, different frame sizes and frame rates are available. Choosing the frame size and frame rate adequately will optimize the tracking process.



	Not all image sources provide the possibility to specify different frame sizes. In that case the panel will not show the frame size section. This is the case with simulated live image sources for instance.
	In many image source devices, choosing a bigger image size will result in a lower frame rate. Even if a higher frame rate is selected, the frame rate is always limited by the performance of the image source and the computer system on which it is running. This may cause the tracking and/or video recording to be performed with a lower frame rate than the one configured.

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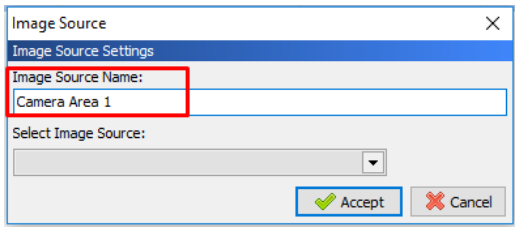
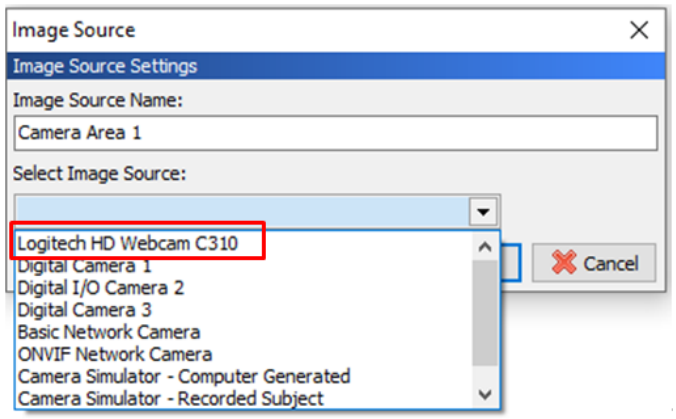
Webcam image source

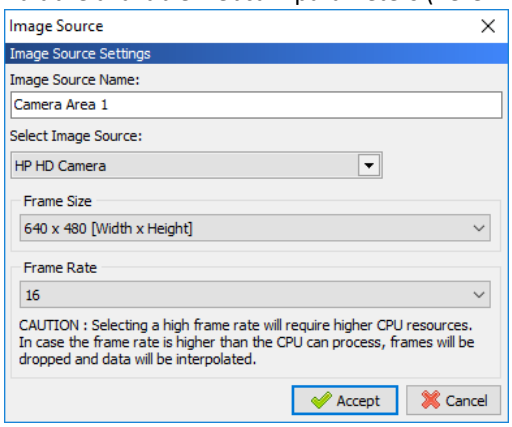
1. Click on the  button to open the **Image Source Setting** panel.
2. Edit the name of the camera in the **Image Source Name** text box (if needed).



3. Select the webcam from the list of image sources displayed in the **Select Image Source** dropdown menu.



Edit the available webcam parameters (here Frame Size and Frame Rate), if needed.



4. Click on the **Accept** button to exit.
5. The camera information is then displayed in the **Image Sources - Settings table**.

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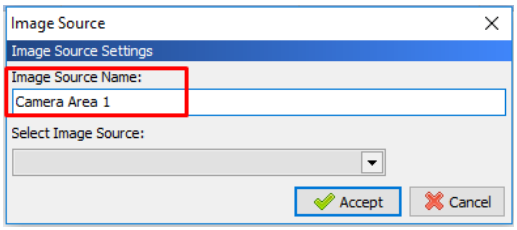
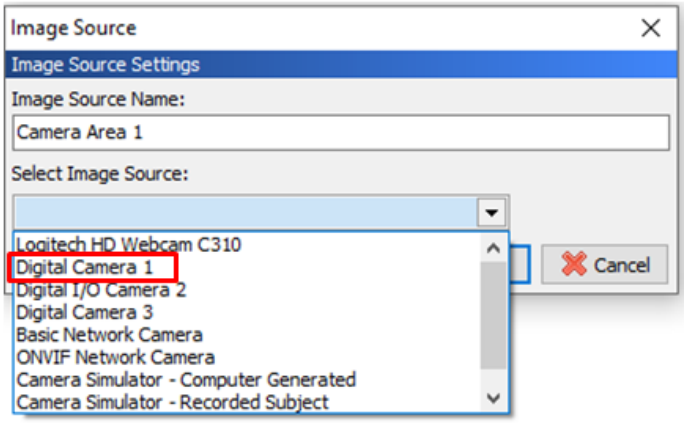
USB digital camera image source

1. Click on the  button to open the **Image Source Setting** panel.
2. Edit the name of the camera in the **Image Source Name** text box (if needed)

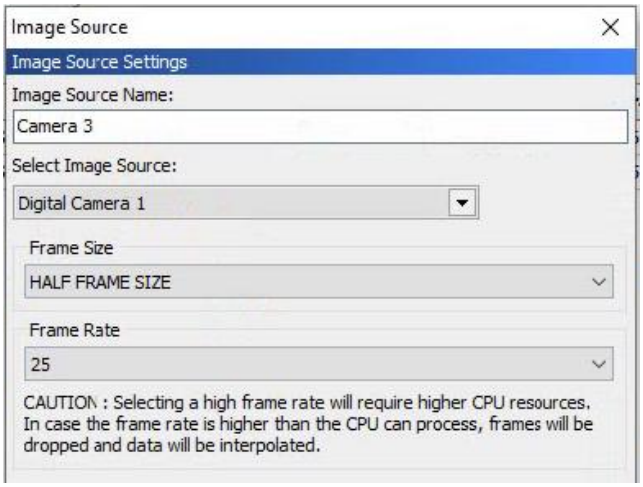


3. Select the **Digital Camera** image source from the list of image sources displayed in the **Select Image Source** dropdown menu.



Note: For the Basler USB 3.0 digital camera, the name of the camera has a different structure. For example: "GigE Vision pu280-544(40308285)"

4. Edit the available parameters (here Frame Size, Frame Rate, and other Parameters), if needed.



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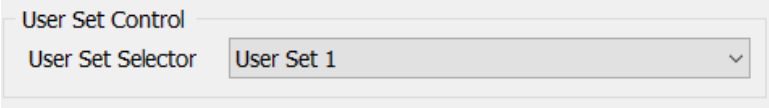
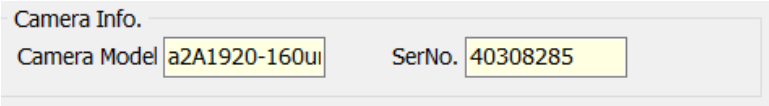
	It is important to set a frame rate optimal for the video recording process based on present system performance. Refer to chapter 3.1 - LIVE IMAGE SOURCES and Table 1, Table 2 and Table 3 to adjust the optimal frame rate based on the number of cameras selected. If the selected frame rate is greater than the value selected in uEye, some problems may occur with the display of the camera and video recording (performance issues, incoherent video file duration, …).
	TIS Digital Cameras have the binning disabled. Thus, changing the resolution from 1280x960 to 640x480 will not reduce the frame size but will just reduce the region of interest.

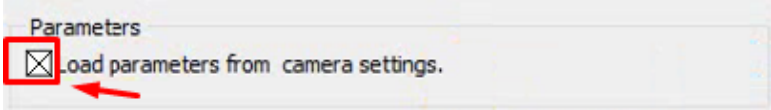
5. In the case of a Digital USB 3.0 camera set with Pylon Viewer (Basler, see chapter 3.3 - Configuring Basler USB digital cameras with Pylon Viewer) be sure to select the **User Set** defined in Pylon Viewer.



Also, in the bottom part of the panel, the info about the camera will be shown:



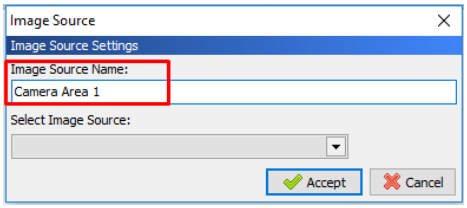
- In case of a Digital USB 2.0 or 3.0 camera set with IDS Camera Manager (see chapters 3.4 and 3.5) in the Parameters section, make sure that the Load parameters from camera settings option is checked.



6. Click on the **Accept** button to exit.
7. The camera information is then displayed in the **Image Sources - Settings table**.

Basic Network Camera image source

1. Click on the  button to open the **Image Source Setting** panel.
2. Edit the name of the camera in the **Image Source Name** text box (if needed).



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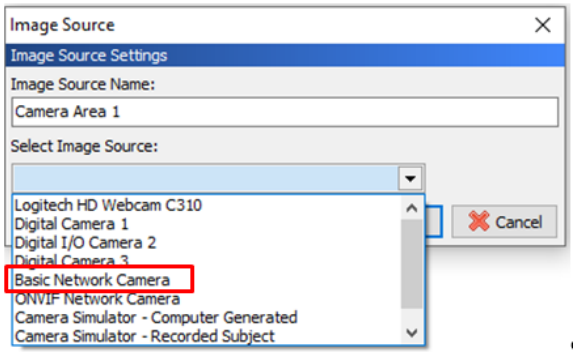
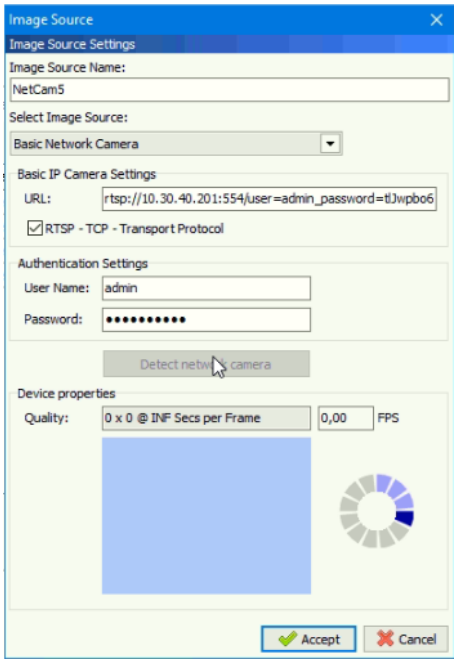
3. Select the **Basic Network Camera** option from the list of image sources displayed in the **Select Image Source** dropdown menu.



4. Enter the URL.

   - Different types of cameras have different URL formats, so you will need to find the RTSP URL that is correct for your camera stream. Contact the camera supplier/manufacturer for the correct RTSP URL address for your camera model.
   - Uncheck the "**RTSP – TCP – Transport Protocol**" option if the IP Camera does not allow RTSP-TCP-Transport.
   - Enter **User Name/Password** (if present) of the IP camera.



5. Click on the **Detect network camera** button to detect it.
   Once detected, the **Quality** text box of the **Device properties** will be filled, and the image of the camera should be displayed in the preview panel.

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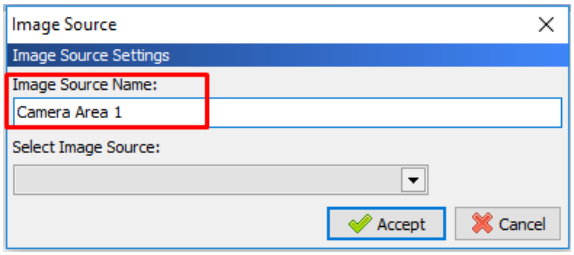
⚠	It is important to set a frame rate optimal for the video recording process based on present system performance. Refer to chapter 3.6 - NETWORK CAMERAS and Table 4 to adjust the optimal frame rate based on the number of cameras selected. If the selected frame rate is greater than the calculated optimal value, problems may occur with the recorded video (performance issues, incoherent video file duration…).

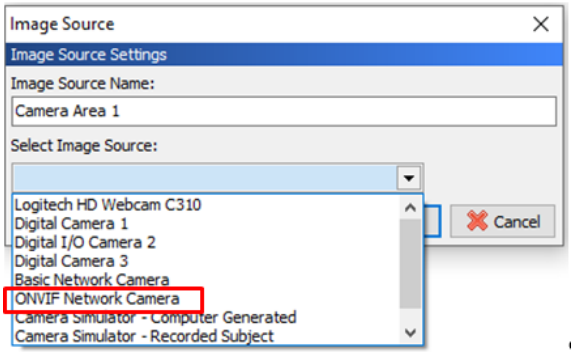
6. Click on the **Accept** button to exit.
7. The camera information is then displayed in the **Image Sources - Settings table**.

ONVIF Network Camera image source

1. Click on the  button to open the **Image Source Setting** panel.
2. Edit the name of the camera in the **Image Source Name** text box (if needed).



3. Select the **ONVIF Network Camera** option from the list of image sources displayed in the **Select Image Source** dropdown menu.



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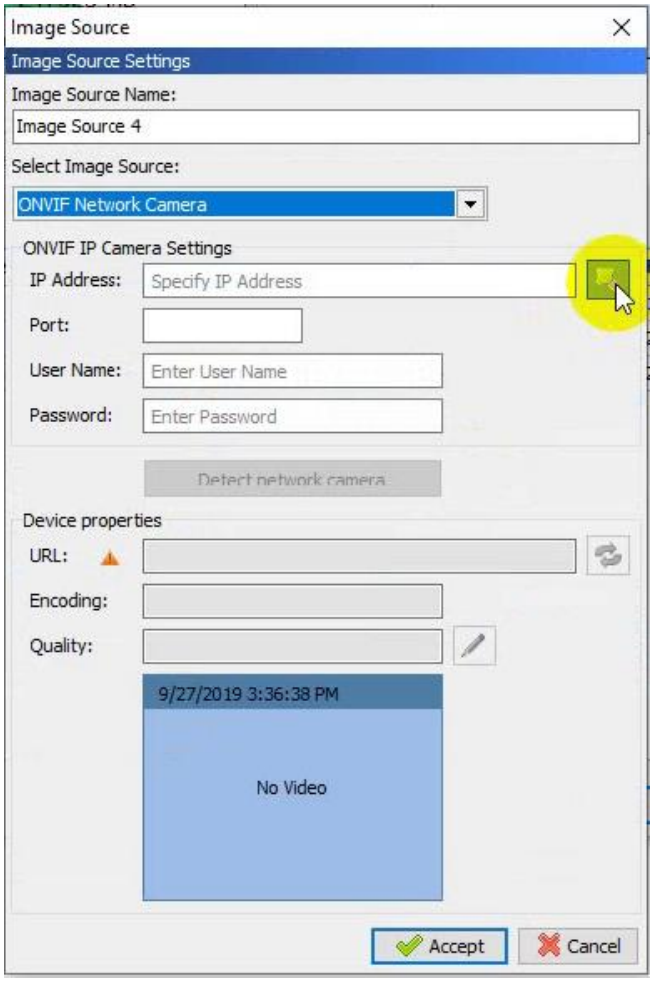
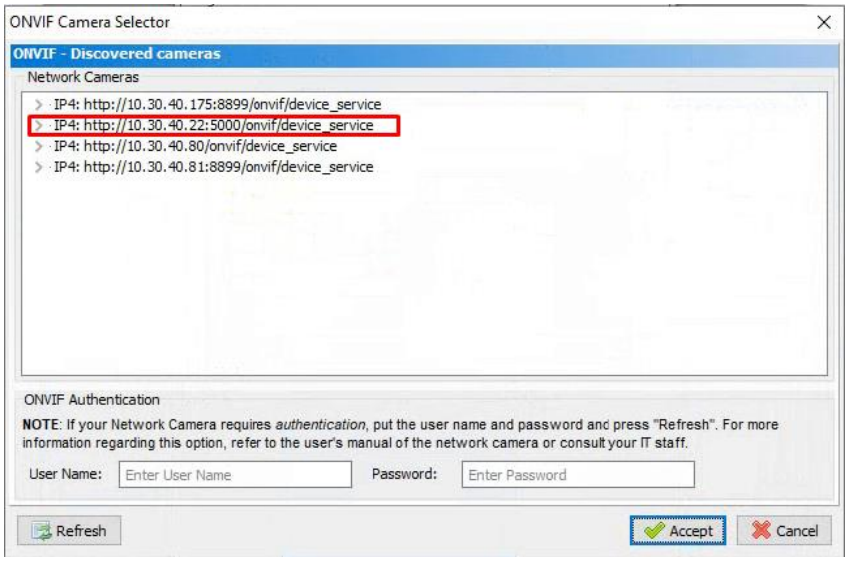
4. Click on the **Search** button of the **IP address** to have access to the available list of installed ONVIF cameras, select one of the cameras and then click on the **Accept** button.





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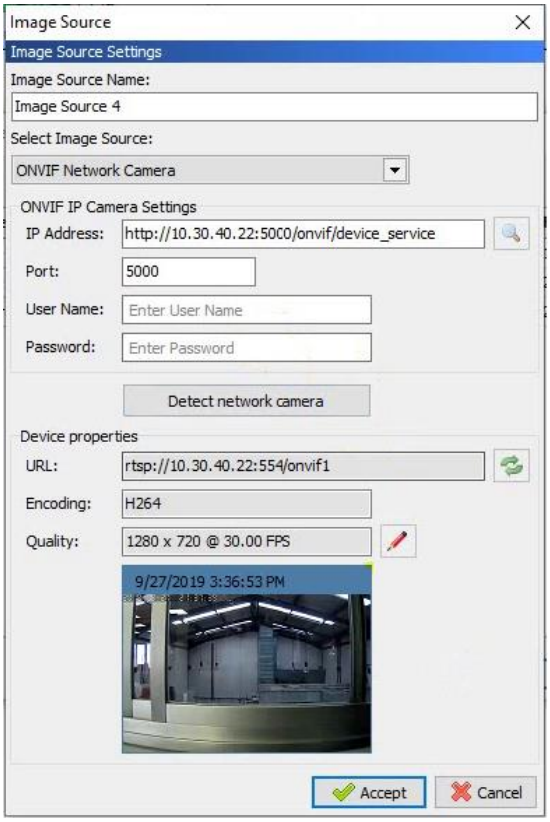
The corresponding **IP Address** and **Port** in the **Select Image Source** section as well as the devices properties will be filled with the corresponding information.



Click on the **Edit Quality** button  to select a different quality setting and then refresh the device properties by clicking on the **Refresh** button  .

	It is important to set a frame rate optimal for the video recording process based on present system performance. Refer to Table 4 to adjust the optimal frame rate based on the number of cameras selected. If the selected frame rate is greater than the calculated optimal value, problems may occur with the recorded video (performance issues, incoherent video file duration…).

5. Click on the **Accept** button to exit.
6. The camera information is then displayed in the **Image Sources - Settings table**.

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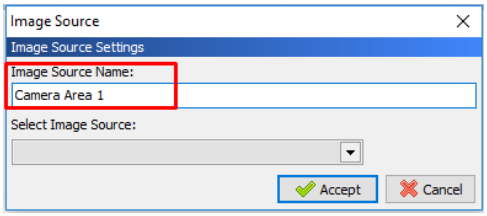
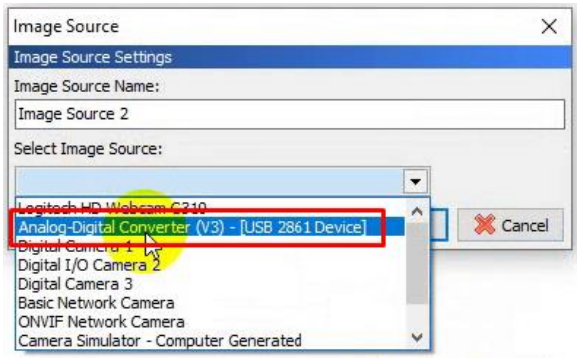
Analog camera image source

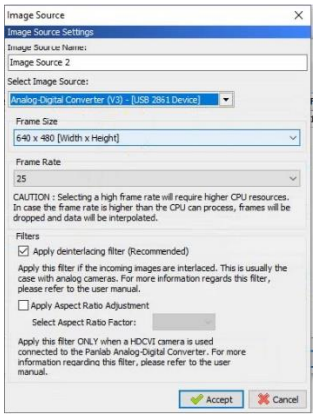
1. Click on the  button to open the **Image Source Setting** panel.
2. Edit the name of the camera in the **Image Source Name** text box (if needed).



3. Select the corresponding Analog-Digital Converter option from the list of image sources displayed in **the Select Image Source** dropdown menu.



4. Edit the available parameters (here Frame size, Frame Rate and Filters), if needed.



5. Apply the **Deinterlacing filter** if needed (see chapter 5.2 - Analog cameras interlaced video).
6. The **Aspect Ratio Adjustment** should be applied only when the CONVANAUSB is used to connect a HDCVI camera. With a HDCVI camera there is light distortion of the image, which stretches the image vertically. Review the document "User's Quick Guide_HDCVI Cameras" provided with the camera for additional information about this setting. These filters require the installation of FFDSHOW codec. The FFDSHOW installer is automatically launched when SMARTIO is installed, and no action is required by the user.
7. Click on the **Accept** button to exit.
8. The camera information is then displayed in the **Image Sources - Settings table**.

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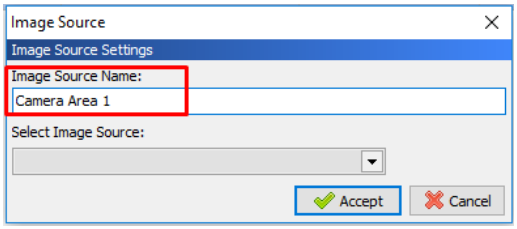
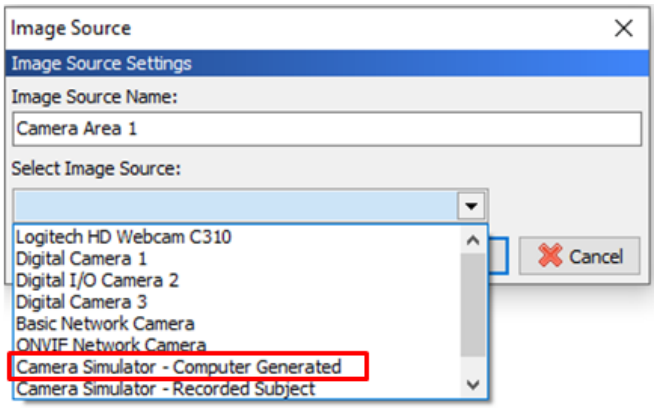
Camera Simulator – Computer Generated image source

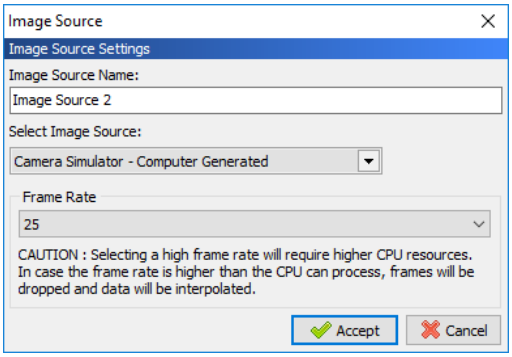
1. Click on the  button to open the **Image Source Setting** panel.
2. Edit the name of the camera in the **Image Source Name** text box (if needed).



3. Select the **Camera Simulator – Computer Generated** option from the list of image sources displayed in the **Select Image Source** dropdown menu.



4. Edit the available image source parameters (here Frame Rate), if needed.



5. Click on the **Accept** button to exit.
6. The camera information is then displayed in the **Image Sources - Settings table**.

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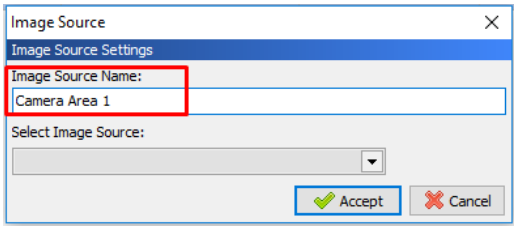
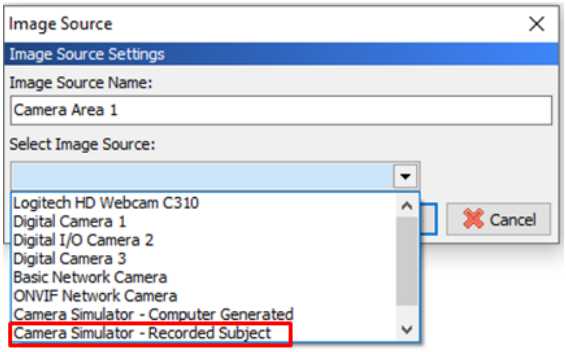
Camera Simulator – Recorded Subject image source

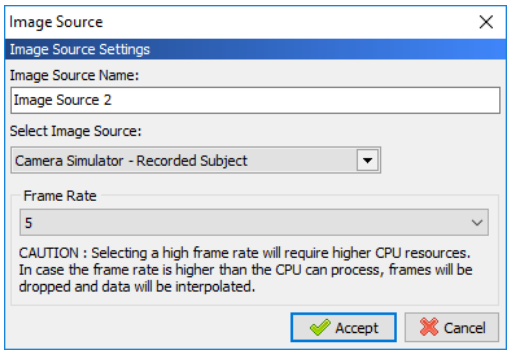
1. Click on the  button to open the **Image Source Setting** panel.
2. Edit the name of the camera in the **Image Source Name** text box (if needed).



3. Select the **Camera Simulator – Recorded Subject** from the list of image sources displayed in the **Select Image Source** dropdown menu.



4. Edit the available image source parameters (here Frame Rate), if needed.



5. Click on the **Accept** button to exit.
6. The camera information is then displayed in the **Image Sources - Settings table**.

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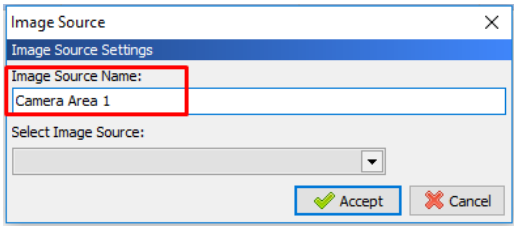
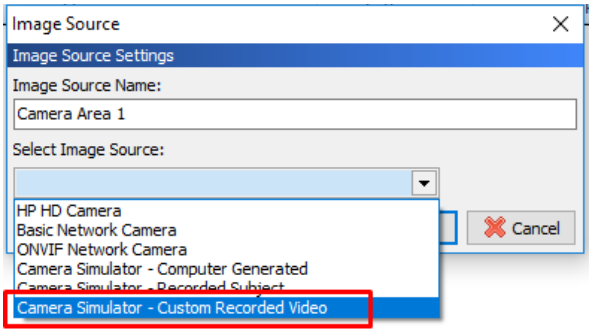
Camera Simulator – Custom Recorded Video image source

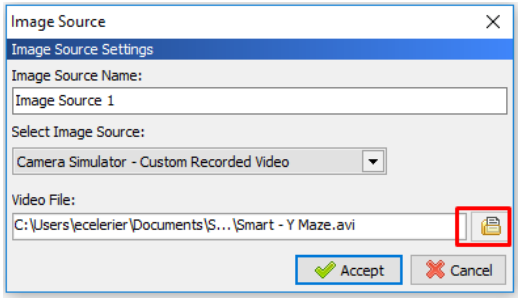
1. Click on the  button to open the **Image Source Setting** panel.
2. Edit the name of the camera in the **Image Source Name** text box (if needed).

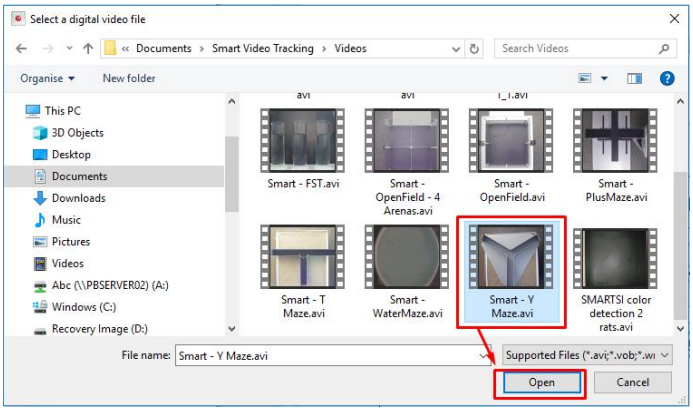


3. Select the Camera Simulator – Custom Recorded Video from the list of image sources displayed in the Select Image Source dropdown menu.



4. Select the Video File to be used as image source, Open it and then click on the Accept button.





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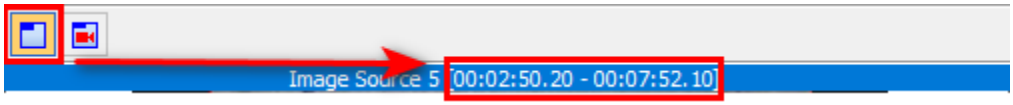
5. Click on the Accept button to exit.
6. The camera information is then displayed in the **Image Sources - Settings table**.

⚠	When using a Custom Recorded Video as image source, the video will be played in loop. Click on **Show/Hide Camera Name**, to show not only the name of the image source but also the playback position of the video 

Digital video source

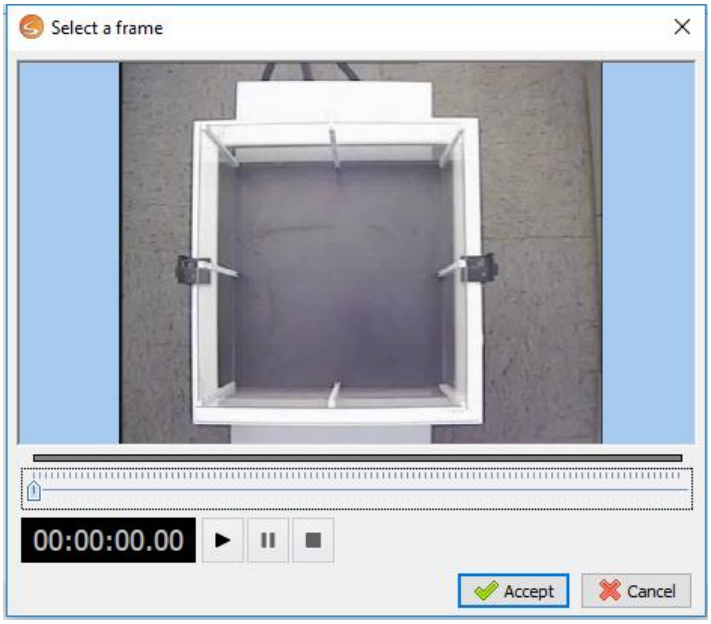
A digital video file recorded during an experiment can be opened by SMARTIO and processed again for behavioral data acquisition and analysis.

Video file selection

Select the **Single Digital Video File** option within the **Recorded Digital Video Files** section and select the video file name and location using the button associated to the field Video file.

Video file image calibration

The calibration of the image of a video file follows the same steps as the calibration of a live image (see chapter 7.1 - Live image source calibration) except for the selection of the frame to be used as reference image for the calibration. In the case of a digital video file, the user can choose the reference image by using the digital video control tools available below the image.



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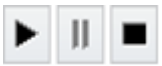
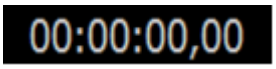
The digital video Control tools are the following:

	To search for a specific point of the video.
	To start, pause and stop the video reproduction.
	To visualize the current position within the video.

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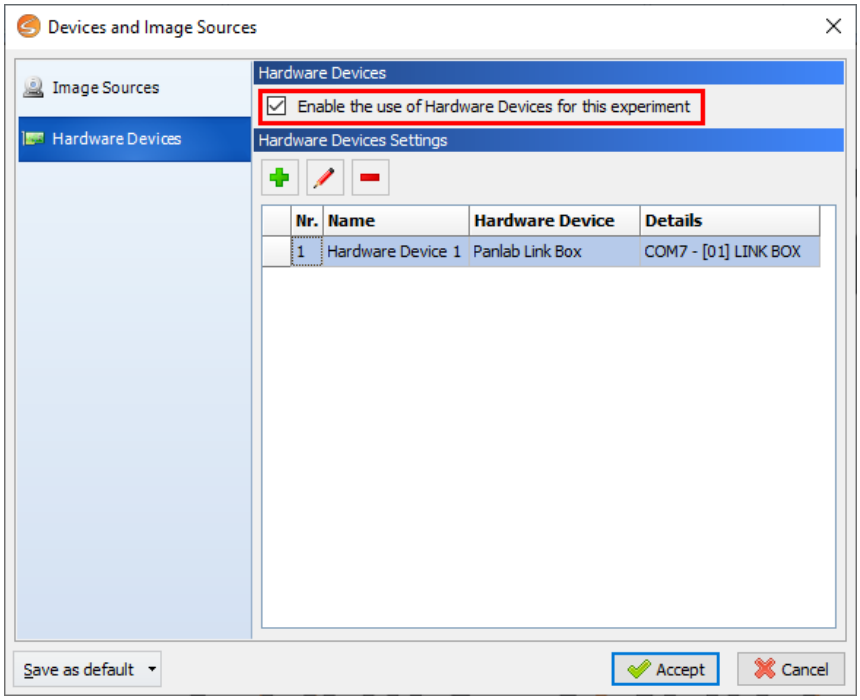
7.2. HARDWARE DEVICES

SMARTIO can interact with the Panlab Linkbox I/O interface (or any 3rd party stimulation) for triggering external laser optogenetic stimulator.

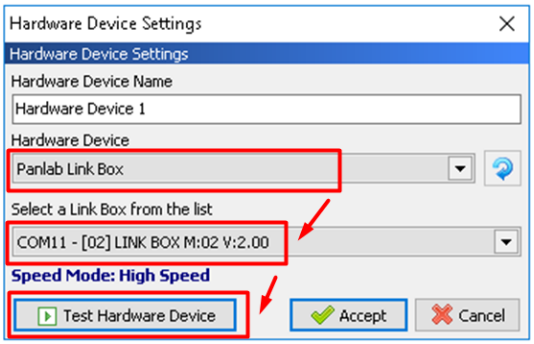
Set of the Panlab Linkbox I/O interface

In order to correctly set the Panlab Linkbox interface for its use to trigger an external laser optogenetic stimulator:

1. Select the **Hardware Devices** section and mark the option "Enable the use of Hardware Devices for this experiment".



2. Click on the  button to add the Linkbox interface, rename it if needed, select the Panlab Link Box option and click on the Test Hardware Device button to test the device.



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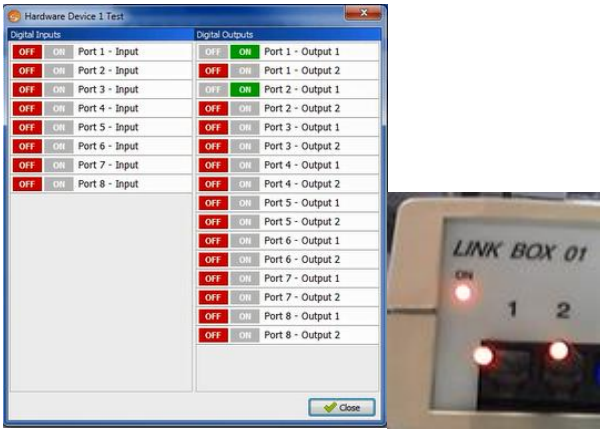
	If the connected Panlab Linkbox is the **Linkbox01HS/CONRS232USBHS adapter**, the Speed Mode used by the system to communicate with the external device would be the **High Speed mode**. If the connected Panlab Linkbox is the **Linkbox01/CONRS232USB adapter**, the Speed Mode use by the system to communicate with the external device would be the **Legacy mode**.

3. The Hardware Device Test panel will display the Input/Output port existing from the Linkbox interface.

	For the use of the Linkbox with an external optogenetic stimulator, only the first Outputs of the Port 1 and 2 are available.

4. Click on the **ON** button of the Port 1- Output 1 and Port 2 – Output 1 and check whether the external device is triggered. Click on the **OFF** button to deactivate these 2 output lines.



Once finished, click on the **Close** button.

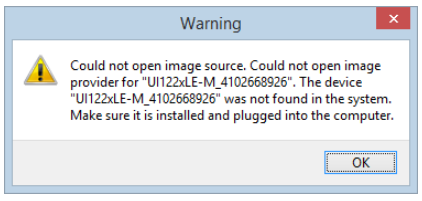
5. Click on **Accept** to the Hardware Device Settings panel.

7.3. EXIT THE DEVICES AND IMAGE SOURCES PANEL

Once the image source and hardware/software devices have been selected, set and tested, click on the **Accept** button to exit the devices and image sources panel.

The SMARTIO player will be opened displaying the image coming from the selected image source.

	When an experiment file is opened, SMARTIO automatically will try to connect the image source used. If this is not possible (because the device is not available yet), SMARTIO opens the experiment and a message similar to the following will pop up:



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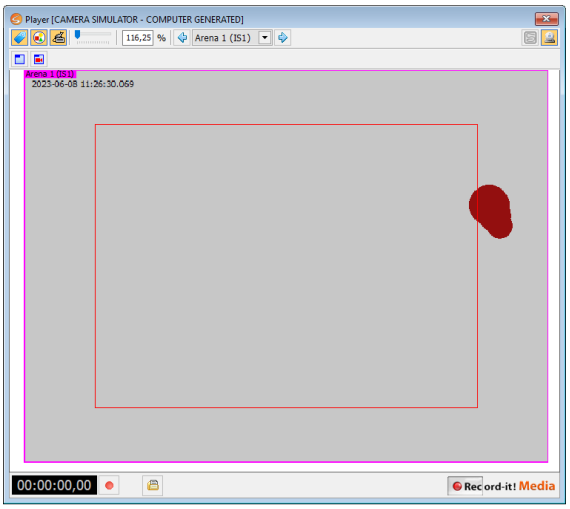
8. THE PLAYER PANEL

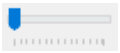
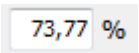
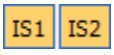
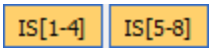
Once an image source is selected and correctly loaded, the Player panel is shown.

8.1. LIVE IMAGE SOURCE PLAYER PANEL



The **Player** panel shows the acquired image, zones and arenas of the selected zone definition. Please refer to chapter 11 - ZONES DEFINITION for more details about Zones Definition.

These buttons, located on the top bar of the panel, allow to:

	Shows or hides the tag of the arena.
	Shows or hides the zones within each arena.
	Shows or hides the tag of the subjects (Name and coordinates) during acquisition.
	Adjusts the opacity/transparency of the zones.
	Adjusts the zoom by entering the desired value or by directly resizing the player panel. This option is only available when a single image source is shown.
	Shows or hides the name of the image source.
	Shows or hides the camera status (see below).
	Shows one, four or all the image sources.
	Shows each image source individually (turns yellow when the image source is shown).
	Shows groups of four images sources (turns yellow when the image sources are shown).

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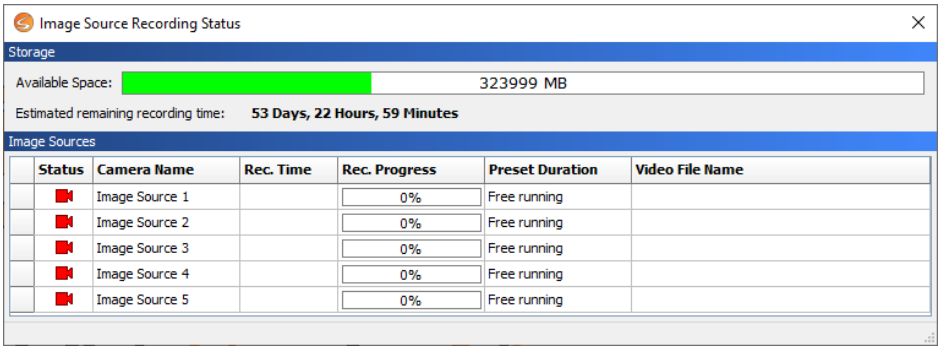
SMARTIO MA	SMARTIO-MA users are provided with a tool called “Arena Selector”. This tool is available in a variety of windows along the application and allows one to identify which arena is currently selected and also to select a different one by using the arrow buttons or the dropdown list. The Arena selector facilitates the task of configuring and managing each arena independently.

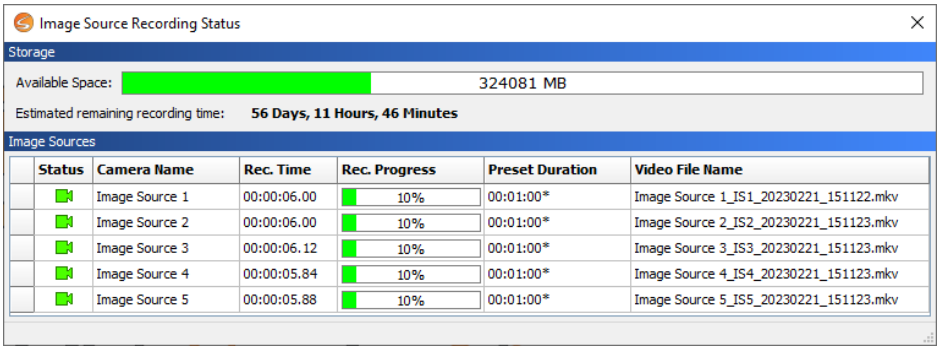
The button opens the Camera Status panel. This value is strictly related to the settings defined in Recording Settings (see chapter 16.2 - RECORDING SETTINGS).



In **Storage** there is a resume of the total space available on the local disk to store new videos. This is shown both as a graph bar with the total amount of MB left and as an estimation of the remaining recording time.

In the **Image Sources** section, it is shown a resume for each image source, showing:

- **Status:** a red icon if it is not recording, a green icon while a recording is ongoing.
- **Camera Name:** shows the name assigned to each image source.
- **Rec. Time:** elapsed time of recording.
- **Rec. Progress:** percentage progression of the recording (as defined in Recording Settings, see chapter 16.2 - RECORDING SETTINGS).
- **Preset Duration:** duration settings (as defined in Recording Settings, see chapter 16.2 - RECORDING SETTINGS
- **Video File Name:** name of the video (as defined in Recording Settings see chapter 16.2 - RECORDING SETTINGS).



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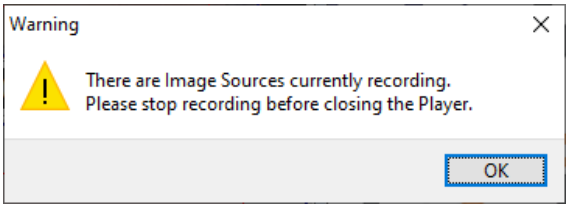
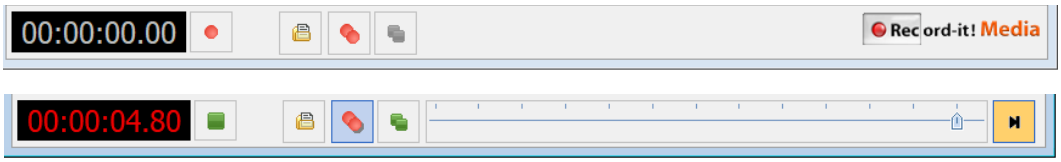
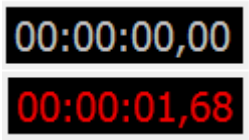
	When a recording is ongoing, it will not be possible to close the player panel unless the recording is stopped. The following message be shown: 

Live image source video recording options

When a live image source is selected, the **Player** panel provides an embedded module of RECORD-IT! Media for easily recording the image coming from the selected camera.



The RECORD-IT! Media toolbar is located at the bottom side of the Player panel and provides the following tools:

	To start recording the video.
	Recording Time Viewer. The label color changes from grey to red when during the recording.
	Time progression bar and cursor. This bar is shown during the recording provided with a cursor allowing the exploration of the recorded video during the recording process.
	Online progression button. When activated, the player shows the last current frame provided by the image source. When inactivated, the user can explore the video by using the cursor.
	To stop recording the video.
	Starts the recording in all image sources (when more than one is present).
	Stops the recording in all image sources (when more than one is present).
	To open the folder in which the video files are stored.

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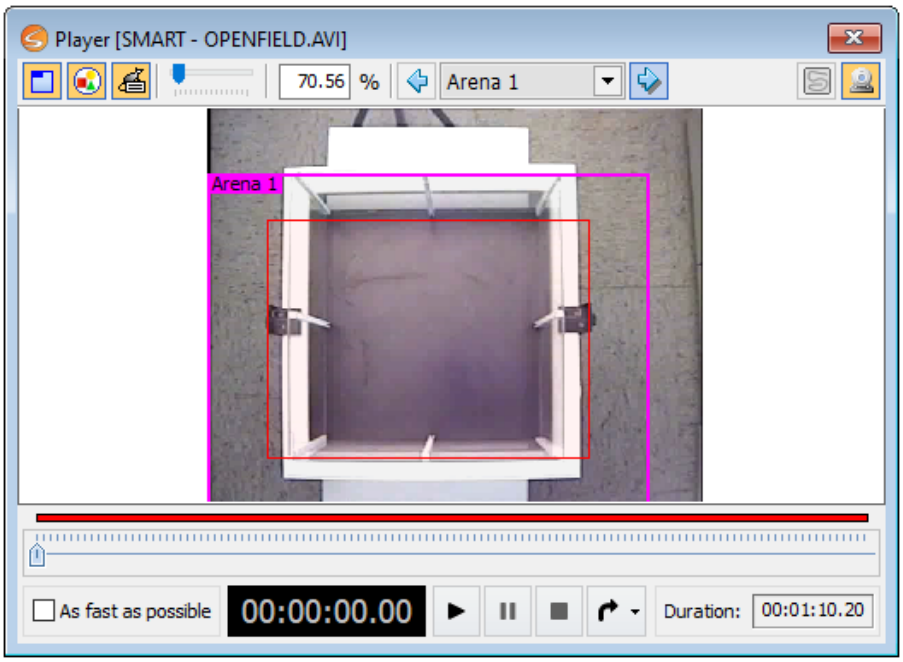
The video files recorded are automatically saved into the Video files folder configured in the Path Settings panel. The name of the digital video file recorded is composed by the name of the experiment file followed by the starting date and time in the standard format yyyymmdd_hhmmss.

Please refer to chapter 16.1 - PATH SETTINGS for more details on how to configure the default file paths of your experiment.

⚠	Recorded digital video files are compressed using the standard h.264 (XviD equivalent) codec so it must be installed previously on your computer. The h.264 codec is automatically installed with the SMARTIO software installation; see chapter 3.9 - CODECS AND SUPPORTED VIDEO FILES for more details.
	If the digital video file recorded will be used later for a data acquisition session, please remember the previous considerations regarding recording digital video files described in chapter 5.2 - Considerations about recording digital video files.

8.2. DIGITAL VIDEO FILE CONTROLS

If a digital video file was selected as image source, the Player panel provides an embedded module to facilitate the reproduction of the video (bottom section). The upper part of the panel is the same as the player panel described for live image sources (see chapter 8.1 - LIVE IMAGE SOURCE PLAYER PANEL).



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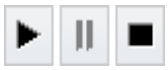
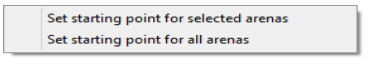
This bottom part of the display provides the following tools:



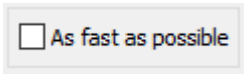
To start, pause and stop the video reproduction.

To go to a specific point of the video.

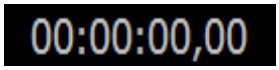


To set the starting point of the trial. This is the time of the video where the data acquisition will start. In case the SMARTIO-MA extension is available, a drop-down menu is available to apply the starting point only to the currently selected arenas or to all defined arenas.

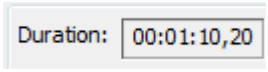
To visualize the time settings (latency, acquisition and delay time) of the current trial.



To select fast mode as video processing speed or unselect it to return to normal speed.



To visualize the current position within the video. Please note that this time can be different of that of the trial time shown in the Trial Time Control panel during data acquisition.



Duration of the video file

	If the subject is not detected when the trial is started, the time settings bars will not match with the trial execution. Please make sure to set the starting point so that the subject is properly detected when the trial starts.

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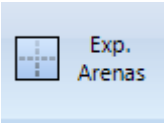
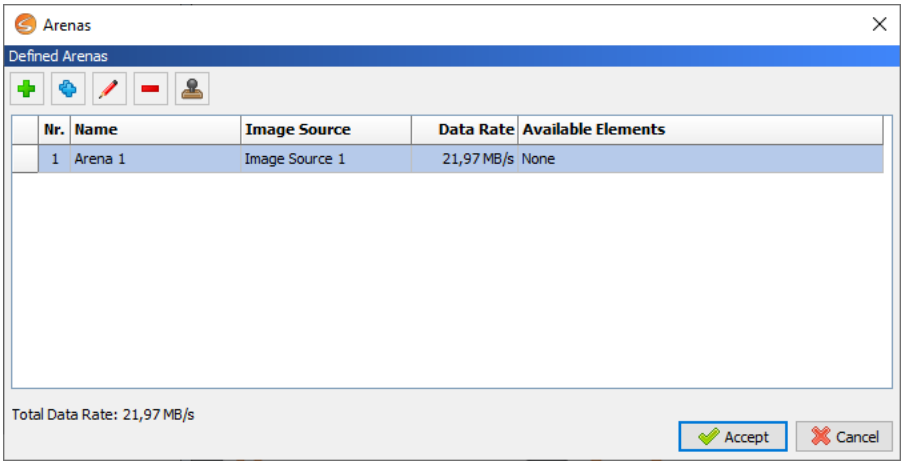
9. EXPERIMENTAL ARENA MANAGEMENT



The experimental arena is the working space in which the experiment will take place (maze, box etc.).

Click on the **Exp. Arena** button on the **Experimentation Assistant** bar to access to the Experimental Arena editor panel.

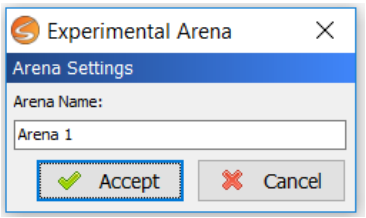
All SMARTIO experimental modules are provided with one arena and one zone by default.



SMARTIO MA	SMARTIO MA extension allows to work simultaneously with more than one arena. The rest of this chapter only applies to SMARTIO-MA users.

The "Arenas" window shows the arenas that are currently defined in the experiment. To add one more arena, click the button . To add multiple arenas at once, click the button , then type the number of new arenas you wish to add and click OK. To delete arenas, first select the arenas you wish to delete from the list and then click the button . Note that Arena 1 can never be deleted. Use the button to clone an arena and the button to edit the configuration of an arena.

After creating each arena, it is necessary to edit its configuration. Each arena must be associated with an image source and, if available, to a hardware device. If the arena is used for video-tracking only (no hardware device defined), the Experimental Arena panel only offers the option to change the name of the Arena.



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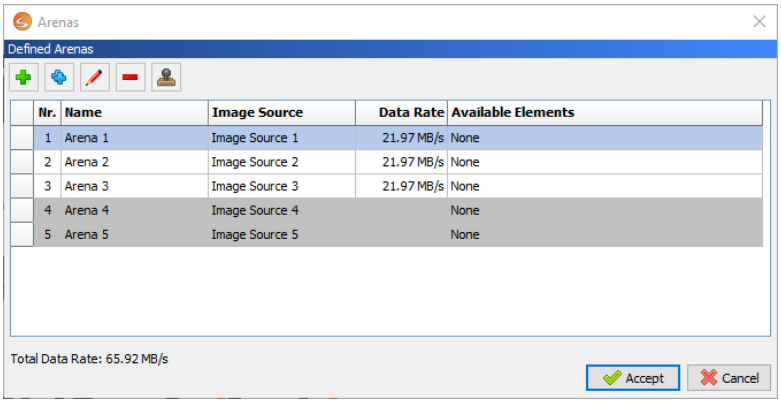
For each arena can be configured independently settings of:

- Hardware Devices
- Zones Definitions
- Detection Settings
- Time Settings
- Scheduler trials
- Speed Settings

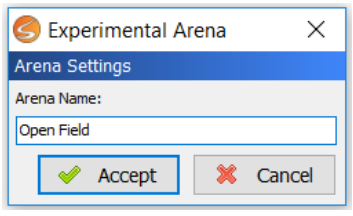
Deleting an arena deletes all related configurations, so this should be done with care. SMARTIO has an integrated security system, ensuring that any arena that has associated trials with data or zones defined cannot be deleted. To delete an arena which has zones or trials, you first must delete the zones and trials before deleting the arena. The Arenas shape and included zones are defined into the Arenas definition section of the Zone definition panel (see chapter 11 - ZONES DEFINITION).

When an image source containing an arena is deactivated in the Image Sources panel (see chapter 7.1 - Live image source), the corresponding arena is not deleted but appears in grey:



9.1. RENAME THE ARENA

Use the **Arena Name** text box to rename the experimental arena.



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9.2. ARENA ELEMENT DEFINITION

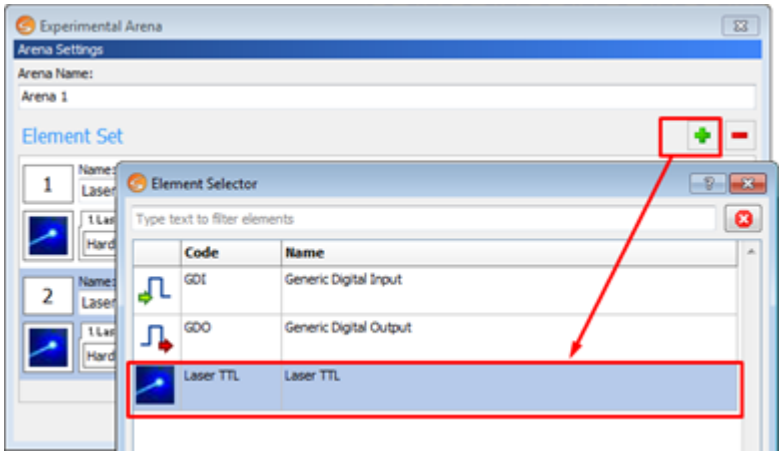
To add elements to the experimental arena, click on the button and chose the element from the available list.

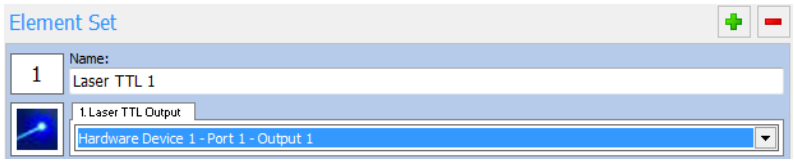
In the SMARTIO packages, the only element that can be set is laser optogenetic triggers (or any other 3rd party element connected to the Panlab Linkbox01).

Linkbox TTL ports selection for laser optogenetics

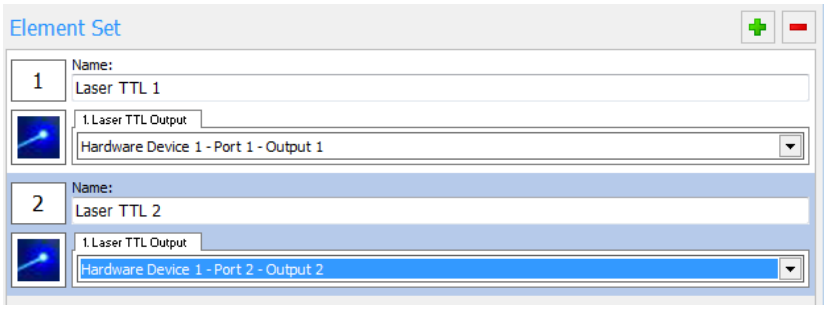
To select the ports of the Linkbox that will be used for the TTL triggering in the laser optogenetic stimulator follow the next steps:

1. Click on the button, select the Laser TTL element from the Element Selector and associate it with the Linkbox Port 1 – Output 1.





2. Click on the button again to select another Laser TTL element from the Element Selector and associate it with the Linkbox Port 2 – Output 1



3. Click on the **Accept** button.

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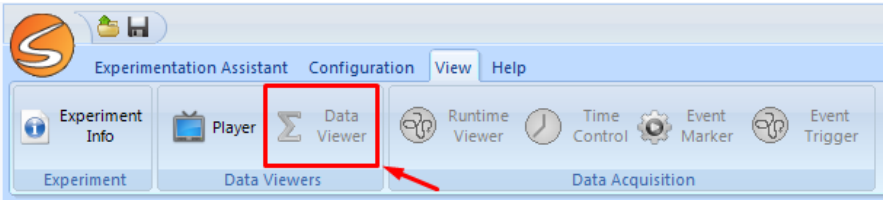
10. DATA VIEWER PREVIEW

A **Data Viewer - [Preview]** panel is displayed when at least one element has been selected in the experimental arena showing a timeline of the neural signal raw data or of activation/deactivation of the triggers.

Use the Data Viewer button of the View menu to show or hide the Data Viewer.



Press the  button to display or hide the channels in the Data view.

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11. ZONES DEFINITION

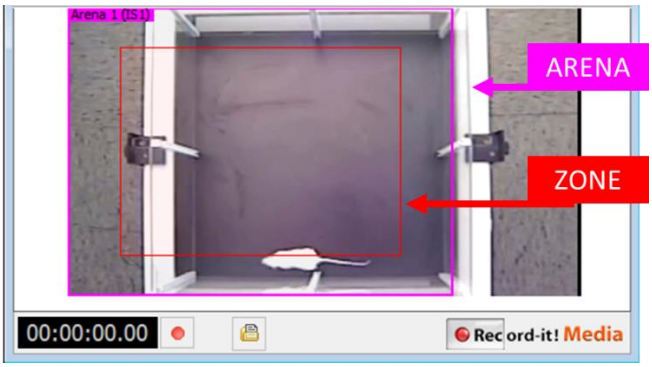
11.1. ARENA & ZONES CONCEPT

Zones definition section allows to determine the special regions that should be considered in the calculations of SMARTIO. Examples of such calculations are:

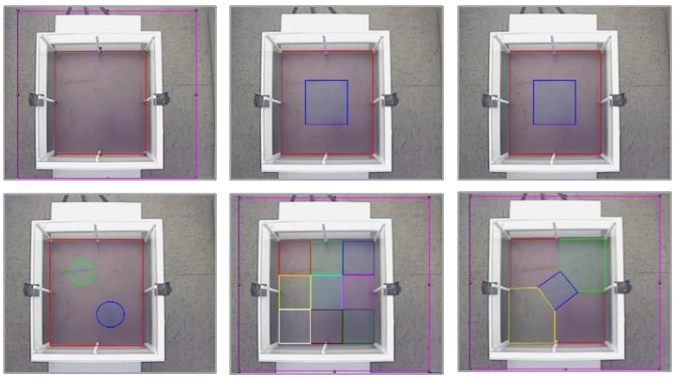
- Transitions count (number of transitions detected from one zone to a different zone).
- Permanence time and percentage (time or percentage of the whole time spent in each zone).
- Distance travelled (length of the path travelled by the subject in each zone).

Each zone definition is defined by an experiment workspace (arena) and zones.

The physical space in which the experimental trials are carried out is called an “arena”. Every experiment has at least 1 arena defined which is shown in the Player panel with a pink line (see image below) and the label “Tracking area” or “Arena 1”, depending on whether the SMARTIO-MA extension have been licensed or not. Each arena contains a zone or a group of zones that will be used for calculations (see red line in the image below).



A great number of different zones definitions can be defined and stored. The zone definitions can be then used indistinctly for data acquisition (tracking) and analysis.



SMARTIO MA	The tracking process can be carried out in several arenas at the same time, increasing the productivity of the experiments. Moreover, different arenas may have different zones definition even when acquiring data simultaneously from all of them.

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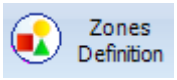
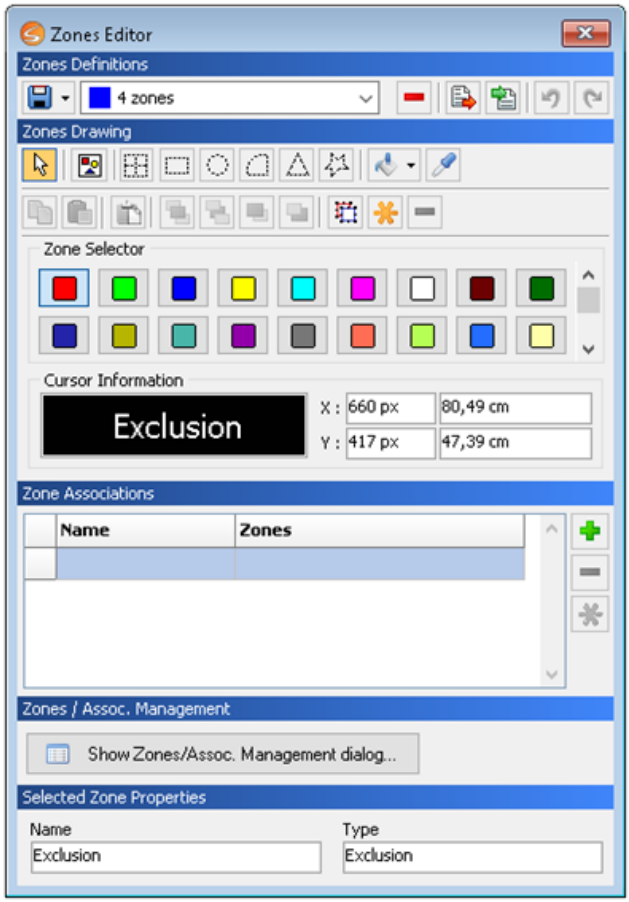
11.2. THE ZONES EDITOR TOOL



To open the zone editor tool, press the **Zones Definition** button in the **Experimentation Assistant** bar.

The SMARTIO-CS experimental module is provided with a flexible Zone Definition tool. The zones are freely editable. The user can decide:

- The number of arenas contained in each Zone definition (needs SMARTIO-MA if more than 1 arena).
- The number of zones contained in each arena.
- The shape of the zones.
- The name and properties of the zones.
- The associations between zones.



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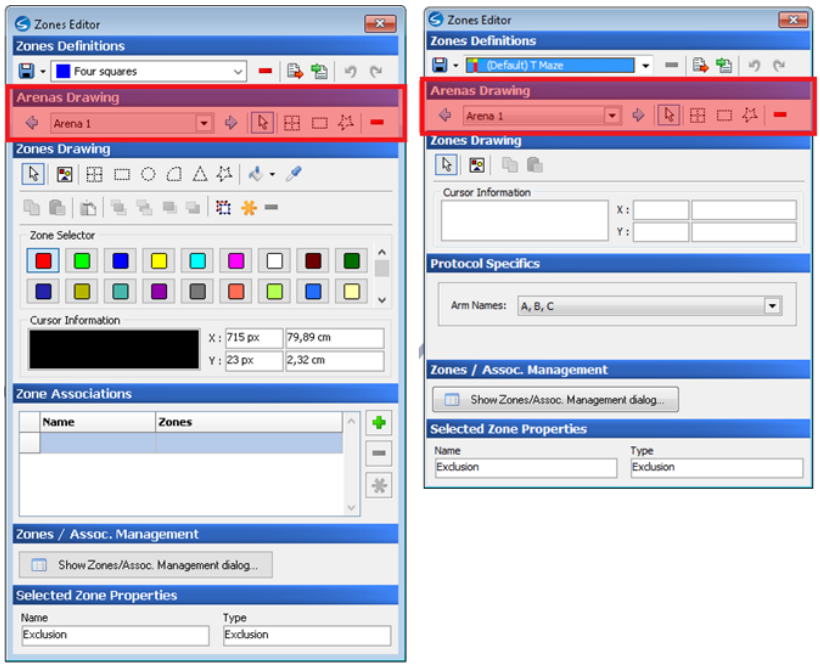
SMARTIO MA	SMARTIO-MA extension allow data acquisition/tracking simultaneously in several arenas. With this extension, the user can freely define more than one arena in which the available zones provided by the experimental module can be applied.
	

Drawing arenas

The **Zone Definition panel** presents in the **Arenas Drawing** section a set of tools to draw one or more arenas and adjust them to different kind of boxes, mazes and environments.

	Matrix with multiple rectangular arenas (only SMARTIO MA Users).
	Single rectangular arena.
	Single polygonal arena.

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SMARTIO MA	If several Arenas are drawn, please follow the next steps: 1. Select Arena 1 from the Arena selector. 2. Choose a drawing tool. 3. Draw the arena in the player. 4. Select Arena 2 from the Arena selector. 5. Choose a drawing tool. 6. Draw the arena in the player. 7. Repeat this process until all the Arenas have been drawn.

	Even if the Draw Arena tool allows the drawing of an unlimited number of arenas, the capacity for SMARTIO to carry out optimal data acquisition would depend on other factors such as the computer performance

	When drawing rectangular arenas using the button, be aware that you can only drag the mouse from left to right.

Once drawn, the arenas may be modified by resizing them using the markers or by displacing them by dragging the shape from an empty space within the Player panel. They can also be displaced through the keyboard arrows.

The shape of an arena may be deleted using the button of the Arenas Drawing section or by pressing the [DEL] key.

	Arenas cannot overlap. SMARTIO will prevent drawing an arena if it overlaps another one. In that case, the mouse pointer cannot be move into the zone of overlapping.

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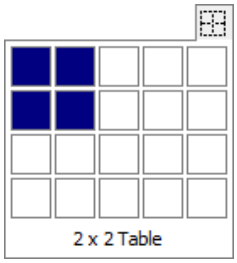
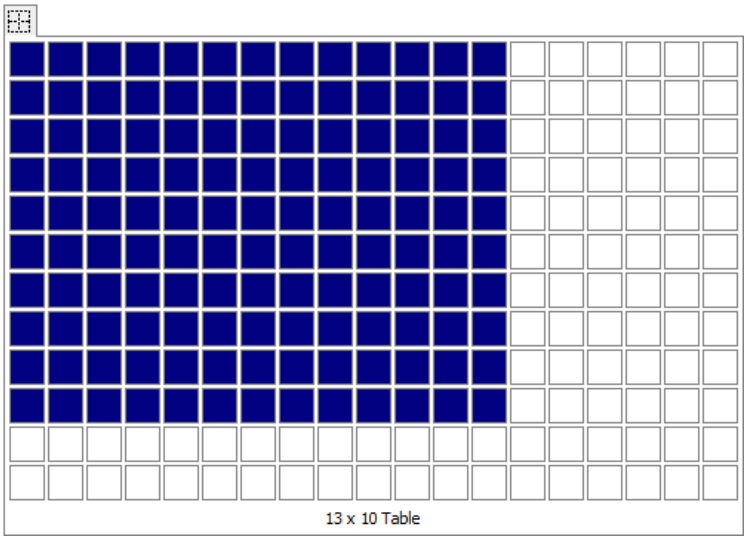
Matrix of rectangular arenas (only SMARTIO MA Extension)

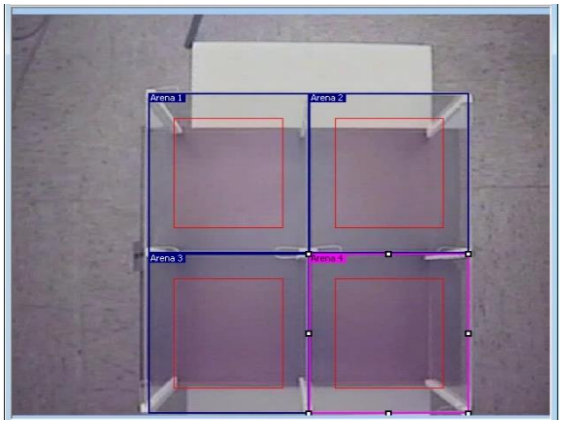
This tool allows drawing multiple rectangular arenas at once and arrange them in a matrix. To do so, follow these steps:

1. Click the tool and select the number of rows and columns of arenas desired. The selection can be extended to a higher number of arenas by displacing the cursor over the edges of the arena grid tool.





2. Draw a rectangle on the Player panel by dragging the mouse from top-left to bottom-right. A matrix will be shown. Release the mouse button and the arenas will be created with the selected default zone shown inside.



3. Modify each arena individually if needed.

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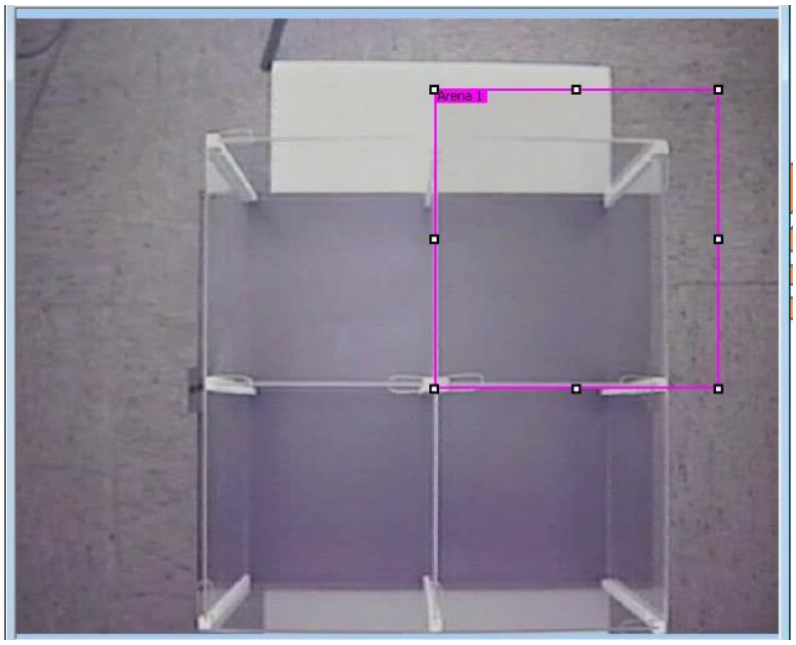
Single rectangular arena

This tool allows drawing a single rectangular arena.

To draw a single rectangular arena:

1. Select the arena to draw using the **Arena selector** tool. use the arrows at each side of the name of the arena or click on the black arrow to open a menu and select the arena.

2. Select the rectangle tool in the Arenas Drawing section.
3. Draw a rectangle on the Player panel by dragging with the mouse from top-left to bottom-right. A preview of the arena is shown.



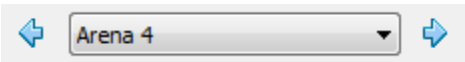
4. Release the mouse button and the arena will be created with the selected default zone shown inside.

Single polygonal arena

This tool allows drawing a single polygonal arena. Polygonal arenas can be drawn freely by connecting segments to fit the special shape - a box, maze or apparatus in general.

To do so, follow these steps:

1. Select the arena to draw using the **Arena selector** tool. Use the arrows at each side of the name of the arena or click on the black arrow to open a menu and select the arena



2. Select the polygon tool.

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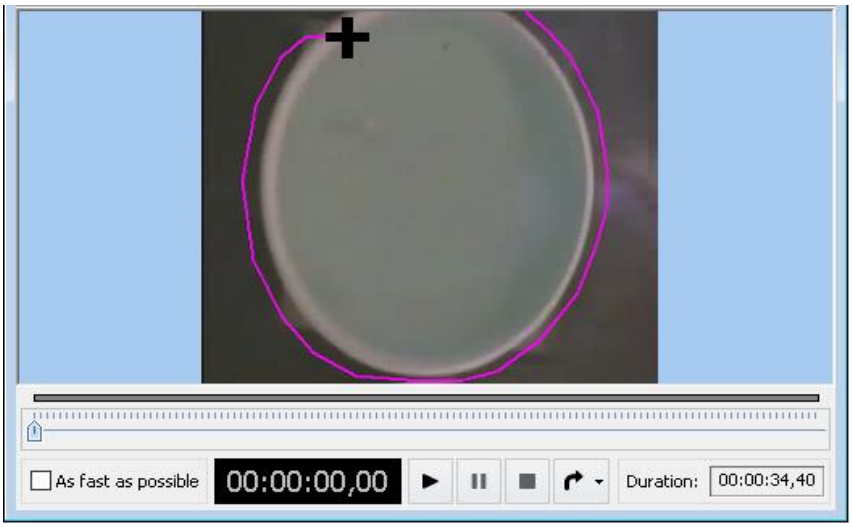
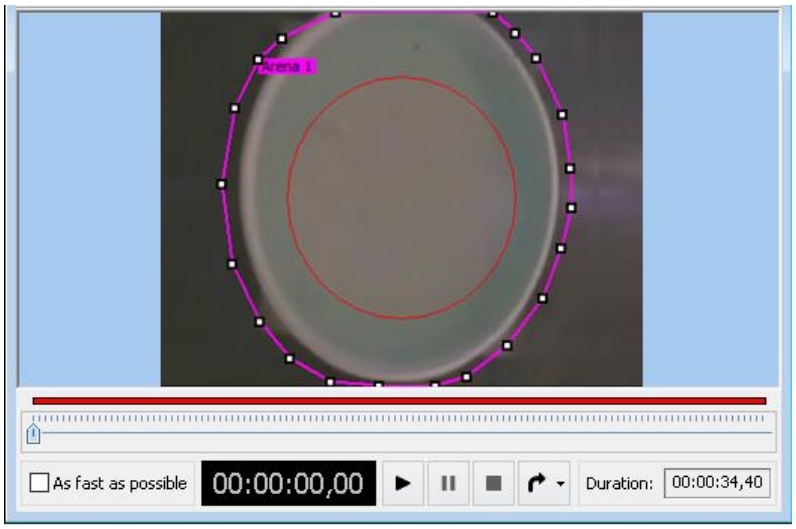
3. Draw the segments of the polygon where desired by clicking on the Player panel.



4. To close the polygon, click near the first segment. The cursor will change to a hand indicating that the arena will be closed upon clicking.



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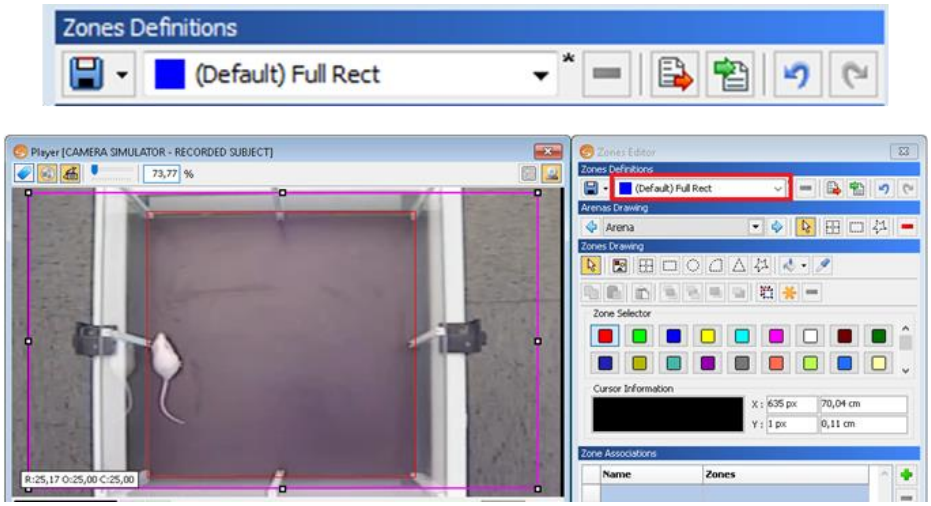
Zones definitions management

Several **Zones definitions** can be created within the same experimental file and can be chosen for data acquisition and analysis.

By-default zone definition

When creating a new experiment, the player is already associated with a **Zones Definition** selected by Default. In the classical SMARTIO-CS experimental module, a "Full Rect" zone definition is provided and selected by default. This zone definition contains 1 rectangular arena including 1 rectangular zone.

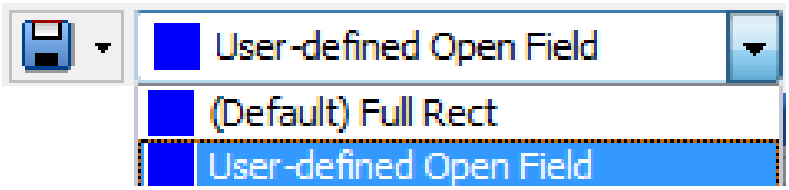


Saving new zones definitions

Changes done on a base (default) zones definition should be stored with a different name. To do this, follow the next steps:

1. Click on the Save button  located in the Zones Definitions section of the panel.
2. Enter the name of the new zones definition and click on the **Ok** button.

New zones definition is saved within the experiment file and automatically included in the list of zones definition.



To be valid, a zones definition should contain at least one Arena with a related Zone/Association.

Saved zones definitions may be deleted using the button in the **Zones Definitions** section. Default definitions cannot be deleted.

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Exporting and importing zones definitions

All the zone definitions within any experimental file can be exported and stored into external files. This operation is very useful to keep a backup of work done or to share zones definitions with a different experimental file.

To export the selected zones definition:

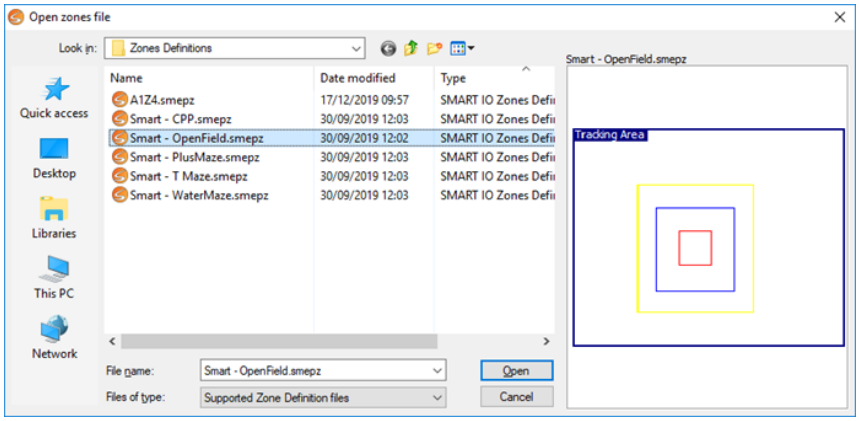
1. Click on the **Export zones definition** button located in the Zones Definitions section of the panel.
2. Enter the name of the exported zones definition file and click on the **Save** button.

Exported zones definition files are stored by default within the "Zones" folder configured and with the extension (.smepz). Please refer to the chapter 16.1 - PATH SETTINGS for more details on how to configure the default destination folders.

Exported zones definition files can be imported later into the same or different experimental file whenever both experimental files share the same experimental module.

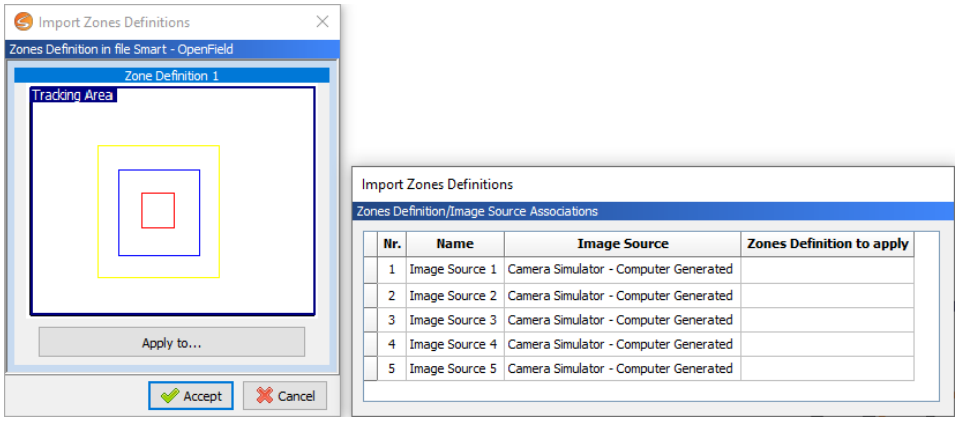
To import a zones definition file:

1. Click on the **Import zones definition** button located in the Zones Definitions section of the panel.
2. Select the zones definition file to import.



3. Click on the **Open** button to import the zones definition into your experimental file.

Imported zones definition is automatically applied to the experiment and is now available within the list of zones definitions. When more than one image source is available, clicking on the **Import Zone Definition**  button will open the following window:



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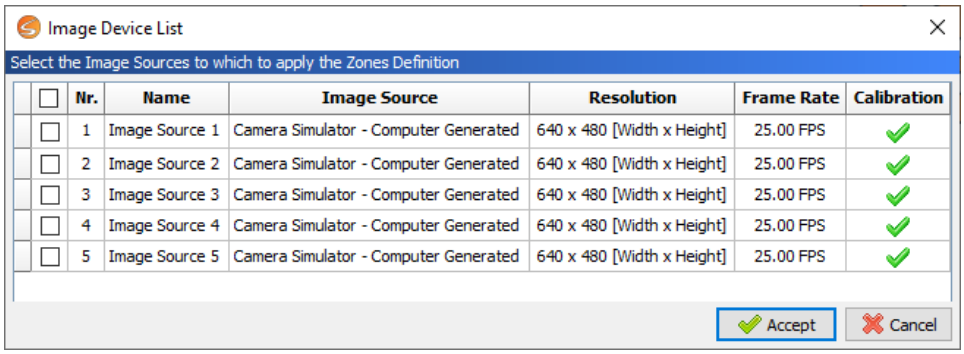
This box allows to select to which image source apply the zones definition selected. Clicking on **Apply to** will open the **Image Device List**



Click on each checkbox to assign the imported zone definition to a specific image source or click on the checkbox in the head bar to apply the zones definition to all the image sources.

The zone drawing section

SMARTIO-CS module enables users to freely draw, move, resize, rotate and, in general, adapt the shape of the zones to specific needs without the restrictions applied to the experimental modules.

Choose a zone color

Each zone is represented and identified by its colors in the **Zone Selector section**.

Each zone has a color assigned to it: red is for Zone 1, green for Zone 2 and so on. To define or modify a zone, the correspondent button in the **Zone Selector** section must be activated.

Draw zones

A few drawing functions allow to create a wide range of different geometric shapes. Select one on the Zones Drawing section and position the cursor over the Player panel to fix the tracing points, according to instructions given below. By default, the pointer tool is selected.

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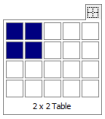
	**Matrix**: Use this tool to quickly draw a matrix of rectangular zones.
	**Rectangle**: Define a rectangular shape by two opposite vertices.
	**Ellipse-Circle**: Draw an elliptic shape by means of a rectangle. The sides of the rectangle will be parallel to the ellipse axis, and tangent to it, so the ellipse will be inscribed in the rectangle. If all sides are equal, then the rectangle becomes a square, and the ellipse is a circumference.
	**Pie/Sector**: This tool is particularly useful to define zones on a circular pool.
	**Triangle**: Define an equilateral triangle by its center and the length of one side.
	**Polygon**: Use this tool to draw irregular polygons. The polygon vertices are the tracing points to be fixed.

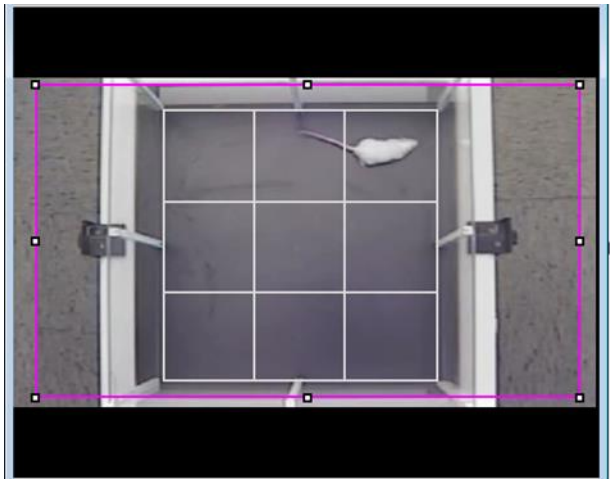
	When drawing rectangular zones using the  button, be aware that you can only drag the mouse from left to right.

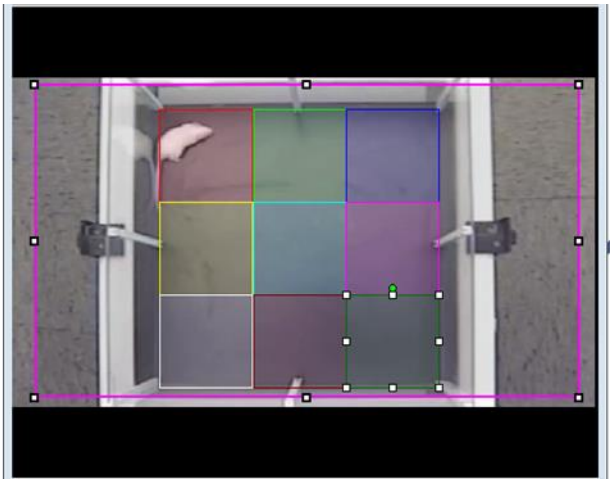
The process to draw zones using the above-mentioned tools is the following:

1. Select a zone number in the Zone Selector section
2. Select the zone drawing tool. In the specific case of the zone matrix, the number of rows and columns to be created must be selected. The selection can be extended to a higher number of zones by displacing the cursor over the edges if the zones grid tool.



3. Click on the left mouse button over the **Player** panel and, without releasing it, move the mouse pointer from left to right and from top to bottom.





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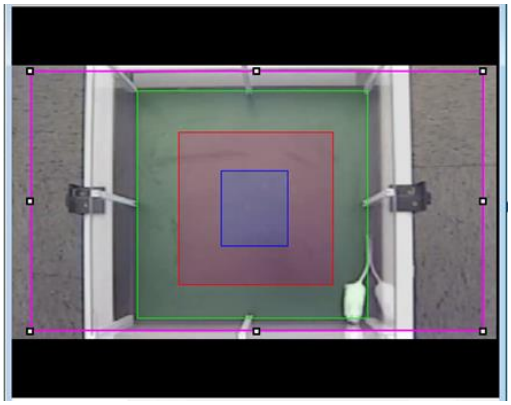
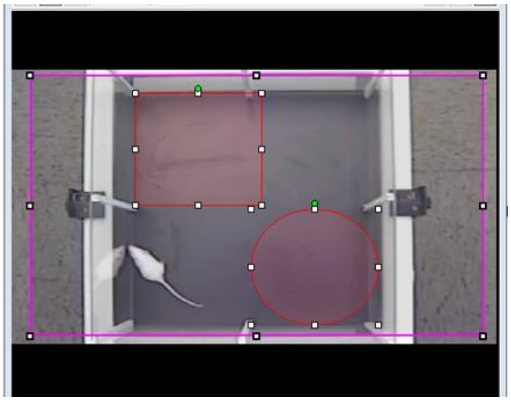
When a polygonal zone is being drawn, draw the segments of the polygon as desired by clicking on the **Player** panel. To close the polygon, click near the first segment. The cursor will change to a hand indicating that the arena will be closed upon clicking.

During this process, the zone shape is shown to facilitate adjusting the size. If the [ESC] key is pressed before releasing the left button of the mouse, then the zone creation is cancelled.

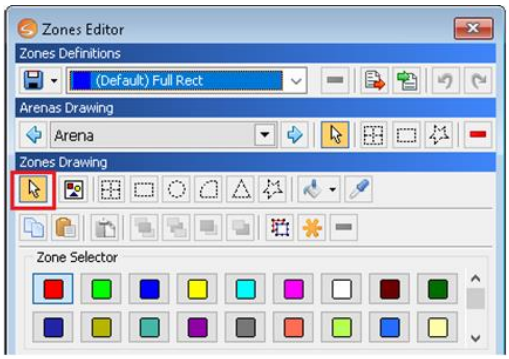
4. Repeat the operation until all the zones have been drawn. (Here, the example of 3 zones is drawn in an open field: center, border, and internal zone).



A single zone may be made up of two or more non-adjacent enclosures, each having its own boundary. These separate parts will be regarded as one in the analysis processes. The following is an example of two shapes that form the zone 1



5. Close the drawing process by selecting the pointer tool again.



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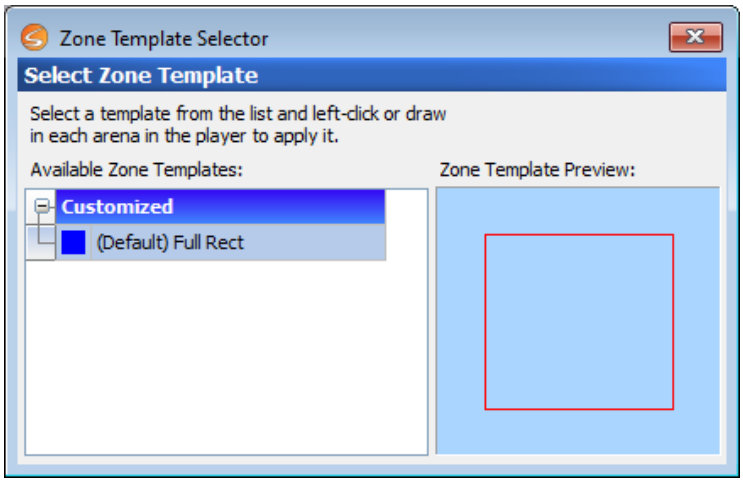
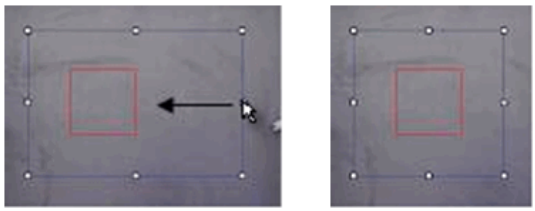
The button Zone templates  provides a template for a rectangular zone.



Adjusting the zones

Once a zone or template has been applied, it is possible to adjust it by moving or resizing each zone shape. To do this, follow the next steps:

1. Select a zone shape clicking inside it in the **Player** panel. White rectangular markers will appear surrounding the selected shape.
2. Click on one of the markers and drag to **resize** it. Please note that resizing operation can be applied only to a single zone at once.



3. Click inside the zone shape and drag to **displace** it. To facilitate the displacement of all the zones at once, click on the **Select all zones** button in the **Zones Drawing** section and displace the zones. The [ARROW] keys combined with the [CTRL] key facilitate a fine adjustment by doing smaller displacements of the zones.
4. **Rotate** a zone. Select the zones and drag the green circle located close to the first vertex of the shapes. All the zones will be rotated around the center of the rectangle in which the selected zones are inscribed.
5. Use the Undo and Redo buttons to undo and redo changes made in the zones.

Depending on the selected protocol, adjusting a particular shape could imply a general adjustment in order to maintain the coherence of the whole definition. Some specific adjustments can also be done by mean of the Protocol Specifics section of the panel.

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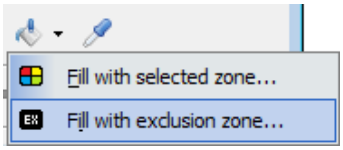
Additional tools

Pointer: Use the pointer tool to select zones or arenas. In combination with the [SHIFT] and [CTRL] keys, multiple zones and arenas can be selected.

Copy / Paste: Facilitates copying the zones from one arena to other. Select one arena, press the Copy button, select the other arena and press the Paste button to copy the zones. Create new arenas.

Copy / Paste Zone: Facilitates copying the zones inside the same arena. Select a zone in an arena, press the Copy/Paste Zone button and click on one or more positions in the same arena. Finish the paste process by selecting the pointer tool again.

Filling: This tool changes the color of a given region. Select the desired color, place the cursor on the region and click.



The Filling button provides a dropdown menu with the following options:

- Fill with the selected zone: to fill the area with the selected color.
- Fill with exclusion zone: to convert a zone to “exclusion zone” (see chapter 11.2 - The exclusion zone for a definition of an exclusion zone).

Color / Zone Selection: Use this tool to select one existing color and zone. Place the cursor over the desired zone in the Player panel and click to select color and area.

Zone layers: The zones mutually exclude each other so that a given point of the image always belongs to just one zone. As previously noted, if there are no zones defined, every point in the image belongs to the exclusion zone.

These tools make the selected zones (enclosures) to be moved onto the upper / lower layers, when configuring a multiple layer zones set-up.

Select all zones: This button selects all the zones in the arena/working area to facilitate resizing, displacing or rotating all of them at the same time.

Clear all zones: Deletes all the zones in the selected arenas/working area.

Delete selected zones: Deletes the selected zones in the selected arenas/working area.

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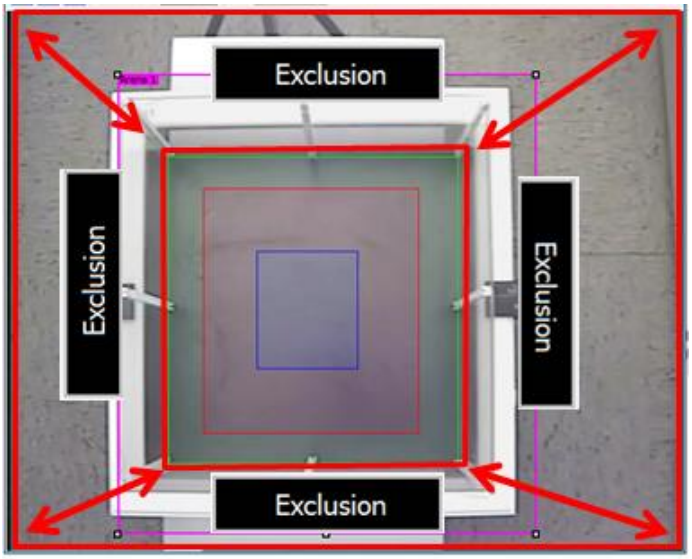
The exclusion zone

The “exclusion zone” is a special zone conceived as the area where no subject will ever be. SMARTIO will not process this part of the image to save resources and prevent interferences. The exclusion zone is a user-defined zone from which no type of data is obtained. If no zone has been defined, the exclusion zone takes the whole surface so that SMARTIO will not “see” anything. It is the experimenter who, by drawing zones on the image, tells SMARTIO what parts of the image are to be processed. To obtain practical data from an experiment at least one zone other than the exclusion zone must be defined.

In a general manner, any spaces out of the defined zones are automatically considered as Exclusion zone.



Using the keyboard to edit zones efficiently

Although the mouse is the most common device to interact with the software, it is not always the most efficient. The keyboard provides a much faster way to carry out many of the operations regarding the edition of zones.

SMARTIO Zones Editor is designed to accept the use of the keyboard to accelerate the setup.

The most useful keys and combinations are:

Key / Combination	Effect
[CTRL] + Mouse click	Selection of multiple independent zones.
[SHIFT] + Mouse click	Selection of multiple zones or arenas in sequence.
[CTRL] + Resize	Resizes the selected zone or arena keeping its aspect ratio. Only applies to rectangular and elliptical zones.
[DEL]	Removes the selected zones or arenas.
[ESC]	Cancels the current edition task (creation, resizing, displacement or rotation).
[CTRL] + Mouse click	Selection of multiple independent zones.

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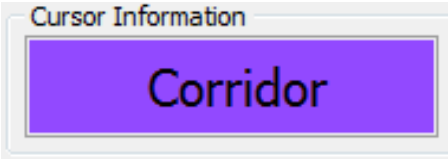
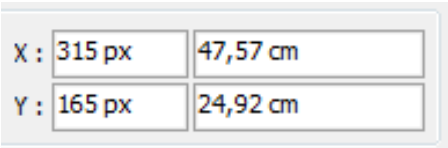
Cursor information

When the mouse is moved over the **Player** panel, SMARTIO automatically updates the Cursor Information section of the **Zones Editor** panel showing the following information:

- The name and color of the zone where cursor is located.



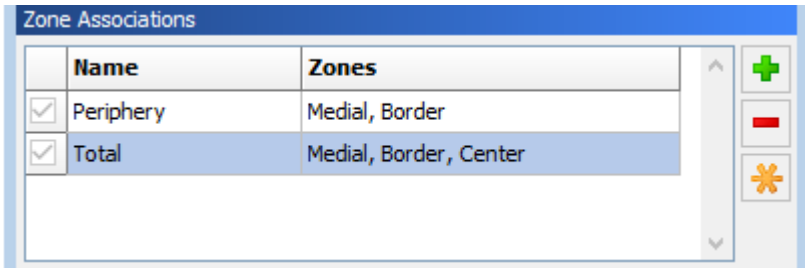
- The X and Y coordinates (in pixels and in the calibration units, see chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration) in which the mouse is located.



Create an association of zones

SMARTIO-CS module enables to freely define associations between the zones. This option allows combining different zones and then obtaining a new set of combined zones. As an example, the **Border** and **Medial** zones of an open field can be combined to create a new zone association defining the **Periphery** of the experimental area in a paradigm aiming to assess anxiety in rodents. All the zones can also be combined (Border + Medial + Center) to define a new zone referring to the **Total** experimental area.

1. Select the zones you wish to associate by clicking on the zones with the left button of the mouse in combination with the [CTRL] / [SHIFT] keys. Please note that to create an association, at least two zones must be selected. Exclusion zone cannot take part in an association.
2. Press the button located at the right side of the table in the **Zone Associations** section.
3. Enter a unique name for the new association and click on Ok.
4. Repeat the operation for all the associations requested.



5. Use the button to delete the association selected in the table.
6. Use the button to delete all the associations in the table.

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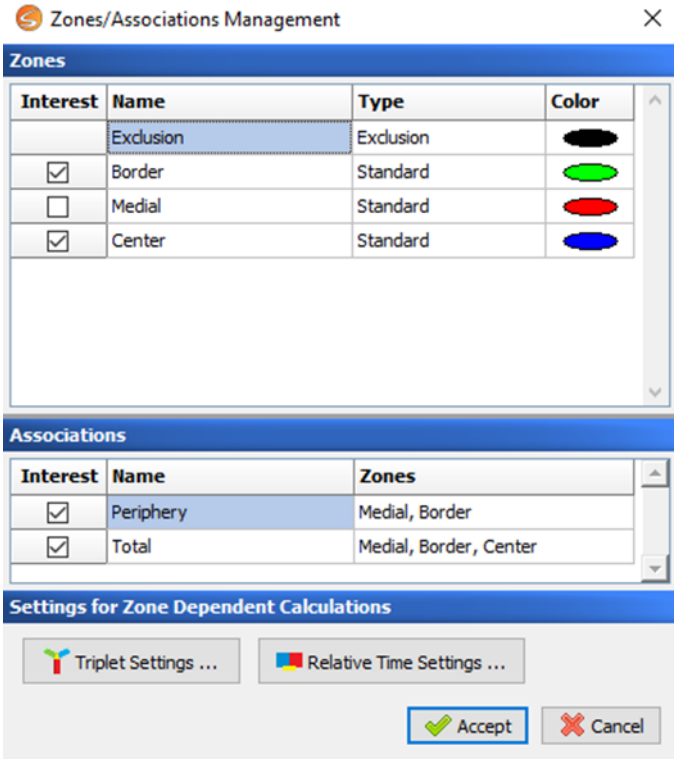
Some useful tips related to zone associations:

- Zones templates provided with experimental modules already include the standard associations, so it is not necessary to create them again.
- Double-click an association row within the Zone Associations section to highlight the corresponding zones in the Player panel.

If a zone included in an association is deleted, it will be removed from the association as well.

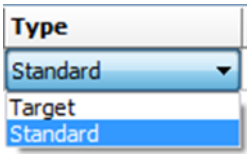
Zones and associations management

The **Show Zones/Assoc. Management** dialog button located in the Zones/Assoc. Management section provides a tool to edit the zones and associations properties.



By default, zones are named as Zone 1, 2, 3, … However, SMARTIO-CS users can freely edit the properties of the zones such as their names (by a more meaningful name related to the current experiment) and types, although not the color. To do so, click in the corresponding cells in the table and change the property. None of the properties of the Exclusion Zone can be changed.

The zones are categorized in two different types:



- Standard: no special features. All zones are standard by default.
- Target: will allow configuration of a special timing condition stopping the tracking process when the subject is detected in the Target zone (commonly used in water maze experiments in which it is required to stop the tracking process when the animal found the platform. In this case, the Platform zone is set as a Target zone). More than one zone can be defined as Target.

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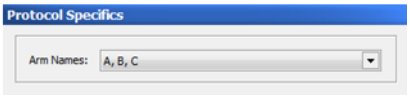
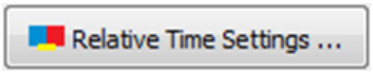
The Interest checkbox allows you to select which zones will be considered for data visualization in the runtime panel. Regarding zone associations, both the Interest checkbox and the name can be also edited in the corresponding table.

SMARTIO also provides two additional options to configure the evaluation process of zone dependent calculations:

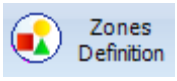
- **Triplet Settings**: allows selecting which zones in the definition will be considered in the evaluation of the alternation triplet calculation used in T/Y mazes. Please refer to <u>chapter 21.3 - Alternation Triplet</u> for more information about this calculation.



- **Relative Time Settings**: allows selecting the zone which time will be proportionally distributed between all the other zones contained in the current zone definition. This parameter is required to evaluate the relative time in the zones of a CPP test. Please refer to the <u>chapter 21.3 - Relative Time in Zone (%)</u> for more information about this calculation.



To Exit from Zones definition editor panel, click again on the Zone Definition menu button-



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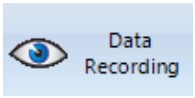
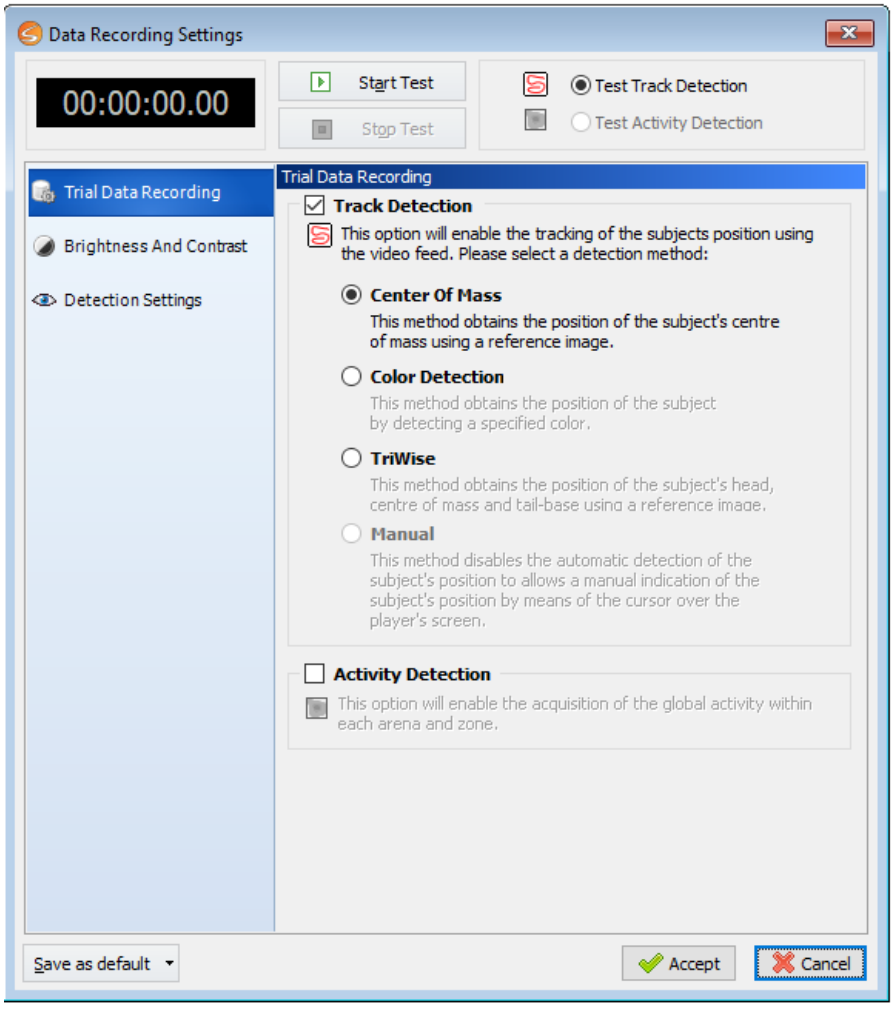
12. DATA RECORDING & DETECTION SETTINGS

Detection adjustments are needed for a precise detection of the position of the animal in the image. The tracking process requires a clear and well-contrasted image.



Click on the **Data recording** button in the **Experimentation Assistant** bar to adjust those parameters and test the tracking before starting the data acquisition sessions.



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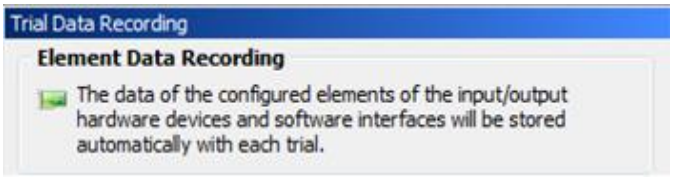
12.1. TRIAL DATA RECORDING

Element data recording

This Information will only be displayed if a Hardware device has been set. As indicated in the software, the data of the configured elements of the input/output hardware devices and software interfaces will be stored automatically with each trial.



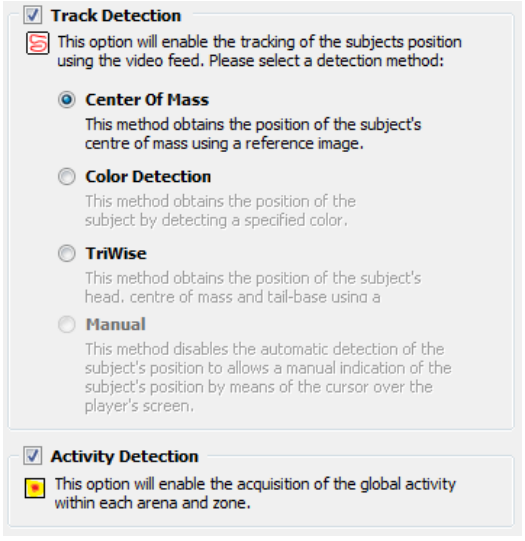
⚠	This option will not work when using one of the Camera simulated image sources provided by the application.

Track detection

Four automatic detection modes are available in SMARTIO:

- Centre-of-mass tracking detection mode
- TriWise, 3-points tracking detection (head, center, tail)
- Color tracking detection mode
- Global Activity detection mode

Each detection mode is selected into the Trial Data Recording section of the Data recording Settings panel. Moreover, a Manual mode is also available when using a video as image source.



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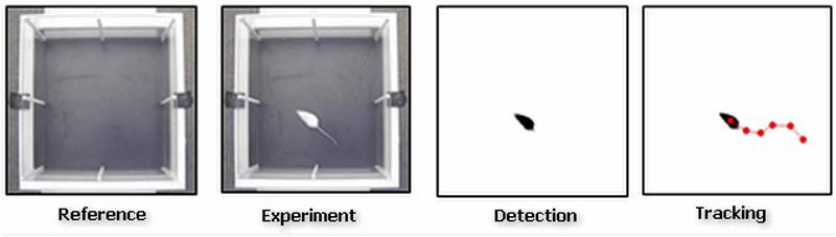
Center of mass tracking mode

In the tracking mode, an image of the experimental area without an animal is used as reference and compared with each frame containing the animal from the image source during the experiment. The difference between both images (Reference and Experiment images) is detected by the system and converted into pixels. The position of the animal is then calculated for each image as the mean of all the pixels detected by the system (resulting in a black blot in the Animal Detection image). A track is formed by consecutive detection of the animal position along the time.

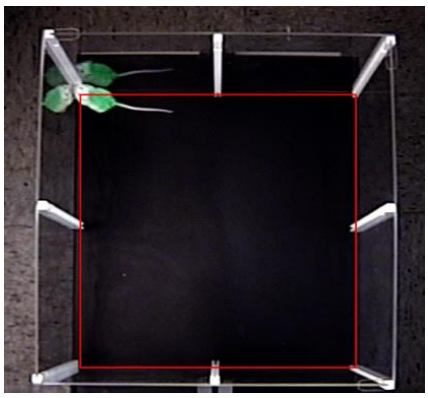


SMARTIO uses the "Static background" technique to convert the real image of the subject into a black pixel blot: the detection image is given by the difference between the current experiment image and the background image taken as reference.

Recommended use for center of mass tracking detection: Tracking the center of mass is particularly recommended for the analysis of animal displacement and position allowing the calculation of a wide variety of related parameters (speed, distance, resting/slow/fast displacements, parallelism, directionality, turning angle, entries into user-defined zones, permanence time into user-defined zones, etc.…). Some examples of standard applications: Morris water maze, elevated-plus maze, open-field, radial maze, locomotor response to novelty, etc.…

Color tracking mode

SMARTIO provides an additional advanced detection mode for color tracking. In this mode, any color can be considered as a mark that can be tracked by SMARTIO (black, grey scale and white colors included).



In this mode, there are no comparisons of the image of the experiment with a previous image of reference.

The technique consists in indicating to the system which color must be tracked using the color-selector tools provided by SMARTIO (see chapter 12.3 - Color mode detection settings). SMARTIO will register a color pattern of all the color of the pixels contained in the area selected by the user.

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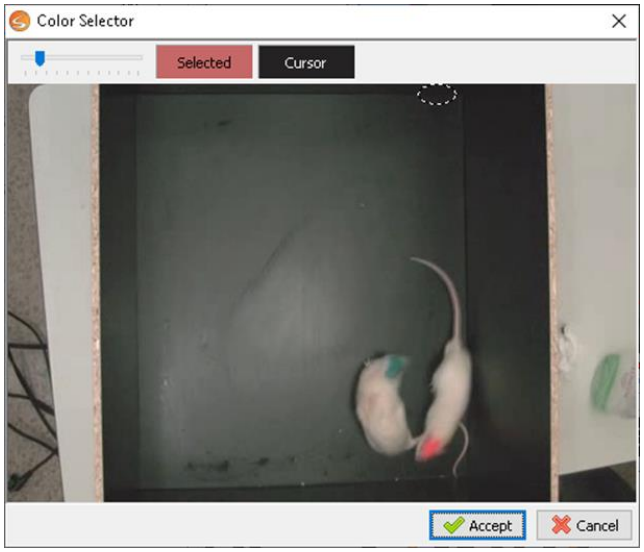
During the tracking process, the dots of pixels showing the selected color pattern will be recognized by the system. The position of the detected color dot is then calculated for each image as the mean of all the pixels detected by the system. The track is formed by consecutive detection of the color dot position along the time.

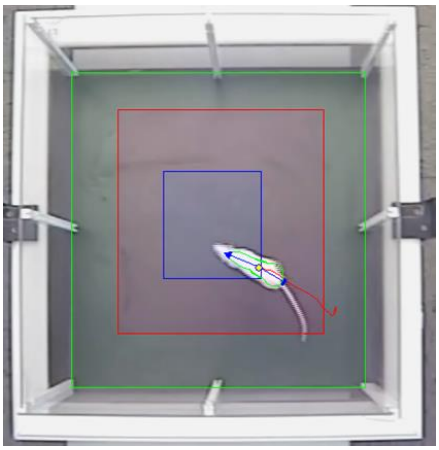


Recommended use for color tracking detection: This mode is particularly useful when it is important to track the position of a specific part of the body of the animal such as the head, center and/or base-tail-plus (object recognition test, social interaction, etc.…) .

TriWise tracking mode (only SMARTIO TW Extension)

Standard video-tracking systems use the detection of the center of mass to study the behavior of experimental subjects in a wide variety of different tests. Nevertheless, this method may not be considered precise enough or sensitive in some applications with very specific needs.

The TriWise technology is an innovation of the Panlab Harvard Apparatus Research and Development team enabling the SMARTIO video-tracking system to extract an advanced model of the animal motion based not only on its center of mass but also on its head and tail position.



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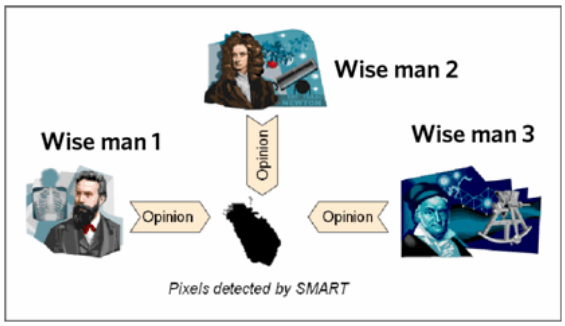
The TriWise technology is based on artificial intelligence techniques applied to obtain always the most natural, realistic, and reliable solution.

A range of different criteria are considered to determine the position of the head and tail-base from the video sequence. Each criterion is evaluated independently by a specialized “wise man” who gives his opinion about the present and past data. This answer is always accompanied by a reliability level. All the opinions and reliability levels are considered and weighted to obtain the final decision.

This technology enables SMARTIO to automatically identify and register complex animal behaviors like exploration, rotations, rearing, and head-head contacts between identified animals using only the image coming from the camera or digital video file, without requiring additional external electronic devices or animal painting.

Furthermore, zone entries criteria become more realistic as more morphological information is considered during the evaluation of the zone transitions.

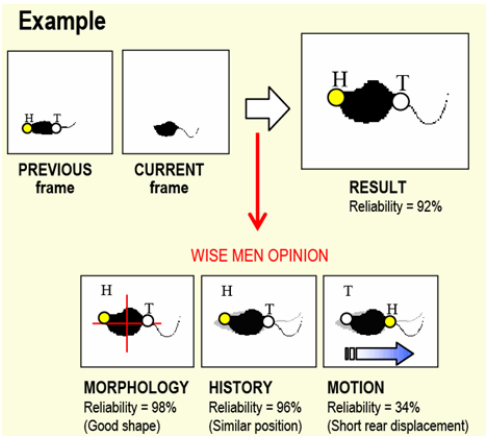


To determine the position of the center, the head and the tail base of the animal, the following criteria are evaluated:

Morphology: studies the animal’s shape and mass distribution.

History: keeps record of the past head and tail base positions.

Motion: its answer is based on the animal displacement direction.



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TriWise uses these three criteria to determine the position of the head, center and tail. The weight of each criterion represents its importance. If, for example, the weight of movement is higher than the other, TriWise will consider the movement more important than morphology and the historic stability and will determine the position of the three points based mainly on the movement of the subject.

Recommended use for TriWise detection: This tracking mode may be very useful when defining precisely the criteria for considering an entry into a zone (elevated-plus maze, etc.…) is needed. Also, it is recommended for registering complex behaviors such as rearing and rotations (open-field test, exploration tests, apomorphine test in unilateral lesions, etc.…). Using this technology not only avoids having to mark the animal but also allows a more detailed evaluation of some specific behavioral items such as animal exploration (hole-board test, object recognition test, novel object test), location choice (elevated-plus maze, T/Y maze, open-field), rearing (open-field, exploration studies), and rotations (studies using unilateral lesion of dopaminergic systems, cycling behavior).

Global Activity detection (only SMARTIO GA Extension)

Global Activity measurement is the task of detecting animal movements even without animal's displacement. The activity is evaluated by counting the pixel change rate between two consecutive images. Unlike the tracking mode, all the pixels detected are taken into account allowing a fine evaluation in the change of the animal shape.

Recommended use for Global Activity detection: This detection is particularly recommended for the analysis of animal movements (with or without any displacement) allowing the calculation of a wide variety of related parameters (amount of activity, resting/slow/fast movements, immobility episodes). Some standard applications are animal(s) global activity, forced swimming, fear conditioning (freezing) and tail-suspension, activity of a group of animals in a social interaction study.

SMARTIO GA	Global Activity detection can be used in parallel with any of the tracking detection modes described above.

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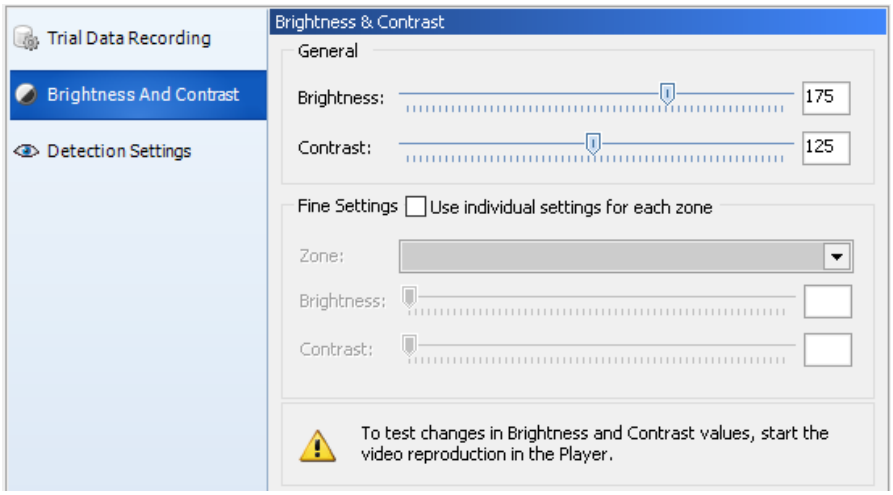
12.2. BRIGHTNESS AND CONTRAST

To improve the detection, SMARTIO includes a fine adjustment of general brightness and contrast parameters in the section **Brightness & Contrast** of the **Data Recording Settings** panel.

These settings can be adjusted for the whole image or for one specific zones.



SMARTIO MA	An "Arena selector" control within the **Brightness & Contrast** section allows to adjust the detection parameters for each arena independently.  Select the arena to configure (with the selector or by clicking directly at the Player panel) and adjust the parameters freely. The image will be automatically updated to reflect the new settings during the acquisition test. In order to apply the new settings to all the arenas simultaneously, check the box **Apply to all arenas**.

SMARTIO has default values for each of the parameters that should be chosen for standard light conditions. However, these parameters can be modified for optimal results.

- **Brightness:** The range varies from 0 to 255. A zero value will give a very dark image while 255 would display a very white image.
- **Contrast:** Modifying this value varies image contrast. The values may be between 0 and 255 also.

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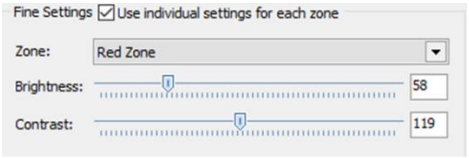
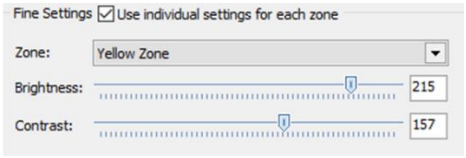
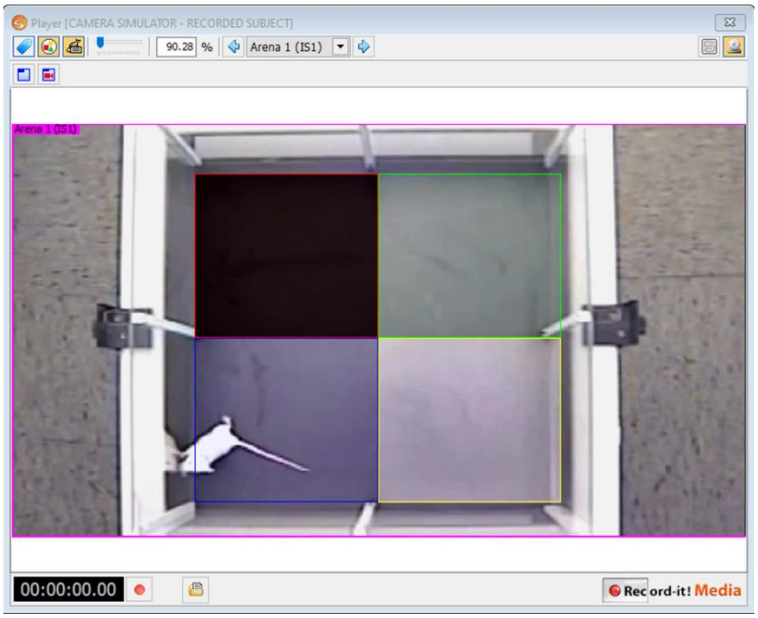
Lighting conditions for specific zones

Particular lighting conditions can be established for these specific zones using the setting “Use individual settings for each zone”. This function may be very useful to correctly detect animals also in zones with too much illumination or shadows.







1. Check the **Use individual settings for each zone box**.
2. Select the zone to which the special adjustments must be made.
3. Tune the brightness and contrast parameters.
4. Repeat steps 2 and 3 for each zone in which special adjustments are required.

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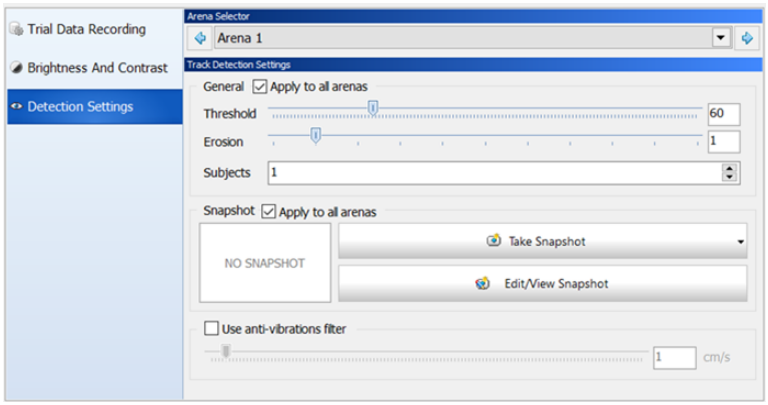
12.3. DETECTION SETTINGS

The detection settings are very important for ensuring reliable detection of the animal during the experiment.

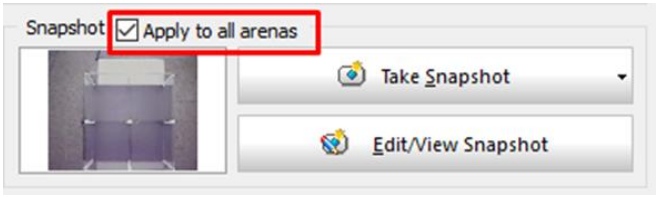
Select the **Detection Settings** tab of the **Data Recording Settings** panel.



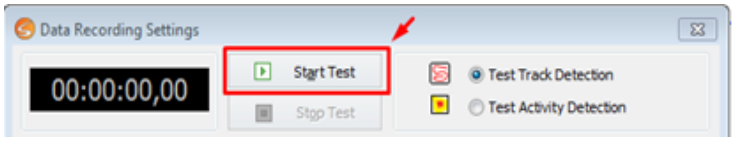
Center of mass mode detection settings

To check the detection of the animal using the center of mass mode:

1. Remove any subject from the experimental area for taking the snapshot that will be used as image of reference. The only difference between the experiment image (with animal) and the reference image (without animal), is the presence or absence of the experimental subject: lighting and background conditions must be the same. If a video digital file is used, move the cursor of the Digital Video tool section of the player until a frame without animal is found.
2. Click on the **Take Snapshot** button to record the current image (frame) as the image of reference (snapshot). If the video does not contain any image of reference without animal, please refer to chapter 12.3 - Snapshot editor for more details on how to edit a snapshot.

SMARTIO MA	 Apply to all arenas option is checked by default. Uncheck this option for taking and using a different Snapshot individually for each arena.

3. Put a pilot subject (or object with similar color) into each arena to test the detection process. If your image source is a digital video file, use the **Digital Video Control** tools to set a new starting point for the tracking detection test.
4. Click on the **Start Test** button to verify if the detection process can identify the subject correctly.



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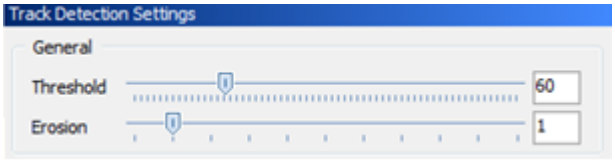
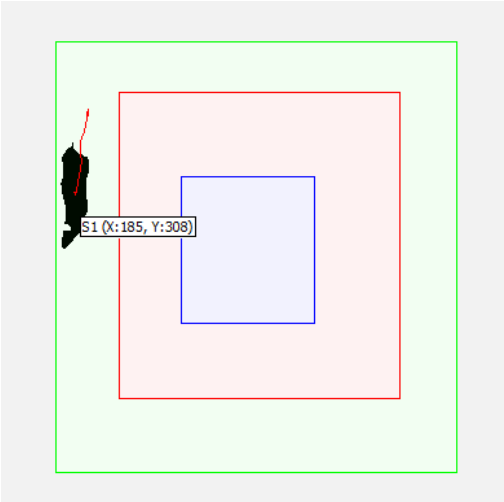
The Calibration process (see chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration) must be done before starting the Test. The difference between the current image and the image of reference is converted in pixel (black dots).
Adjust the **Threshold** and **Erosion** to obtain an optimal detection.



Detection is considered as optimal when the black dots perfectly reproduce the shape of the animal. In that case, the red tracking line should closely follow all the animal displacements and a white label with the animal number and coordinates is shown.



The **Threshold** level is the confidence to differentiate whether changes in the image arise from real movements and not by reflections, shadows or small bits of not-under-study objects: the lower it is, the more these are considered part of the subject. Increasing the Threshold value progressively removes the background noise (reflection, shadow, small displacement of the camera or the scenery) until only the animal is detected.
The **Erosion** level is the confidence to ignore the boundary outline of the shape, which can produce artifacts: the lower it is, the more these boundaries are considered in the detection. At all times, SMARTIO calculates the tracking points of the subjects (assuming a homogenous surface weight distribution). Concentric zones are defined from the center of mass out to the exterior perimeter. The external zone (outermost rings) would be likely to produce artifacts, as this is where shadows and reflections are most difficult to discriminate. To avoid this problem, the Erosion control enables these external rings to be eliminated. To find the optimum setting, start with a value of 1 and gradually increase until the image displayed shows only the shape of the subject. The Erosion is also used to eliminate the animal tail detection

We first recommend the user to set the Threshold and Erosion to the zero value. A lot of background noise is detected during the test.

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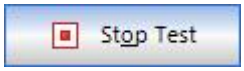
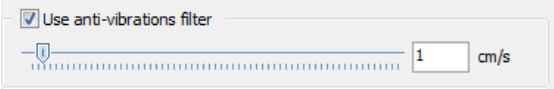
	It is strongly recommended to ensure that a stable volume of pixels of the animal is detected in all the sectors of the experimental area. If the detection is still difficult to obtain, change the image brightness and contrast (see chapter 12.2 - BRIGHTNESS AND CONTRAST) or optimize the experimental detections (see chapter 12.3 - DETECTION SETTINGS).

5. When the tracking path is correctly detected, click the **Stop Test** button.



If the detection is still difficult to obtain, ensure that the targeted colors are only existing on the animals, and not in any other area of the experimental area. Additionally, change the image brightness and contrast (see chapter - 12.2 - BRIGHTNESS AND CONTRAST) or optimize the experimental detection (see chapter 5 - VIDEO-TRACKING PREVIOUS CONSIDERATIONS).

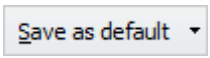
6. Set the **anti-vibrations filter**, if required (see chapter 20.4 - Filtering and smoothing techniques in SMARTIO).



Due to the own nature of the basic video-tracking systems, image noise could produce little variations of the center of mass that could imply a considerable error in the total distance travelled. To minimize such errors, check the box Use anti-vibrations filter and select a filtering value.
The filtering value depends on the level of noise detected in the image. To determine it, put a static object into the arena and try to track it starting with the filter set to zero. If the distance increases, this means that the noise level is high introducing a vibration of the tracking point. Increase the filter value until distance is only increased when the object is moved.

	Setting the noise filter too high can cause the system to ignore a real movement of the subject between frames, resulting in a loss of accuracy in tracking detection. The selection of an optimal anti-vibration filter is not critical during the acquisition of the data, since this parameter can be modified also during the analysis process.

7. Click on **Save as** default if f these adjustments are going to be used for every new experimental file



8. Click on the **Accept** button to save the new detection settings.

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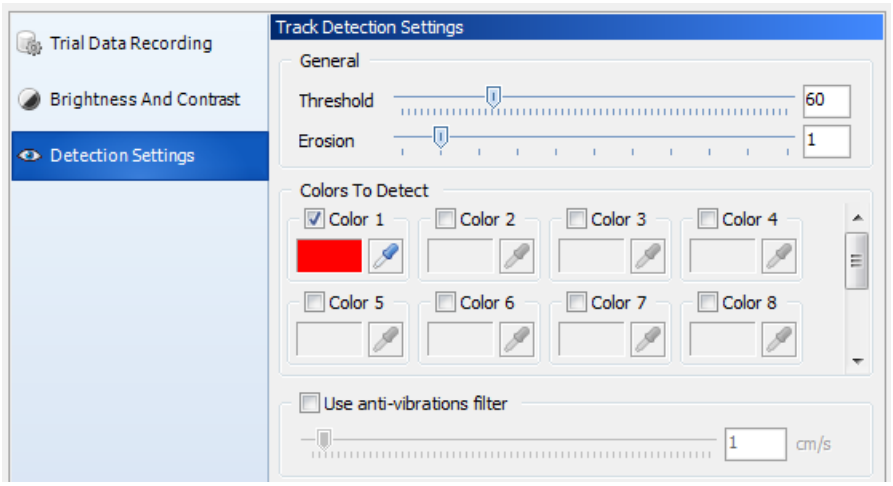
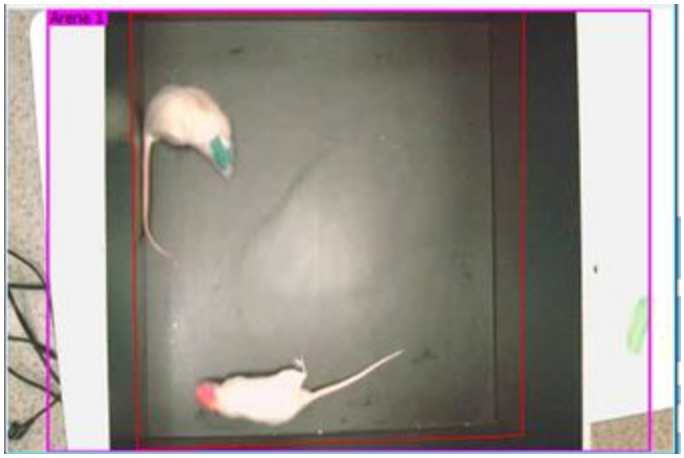
Color mode detection settings

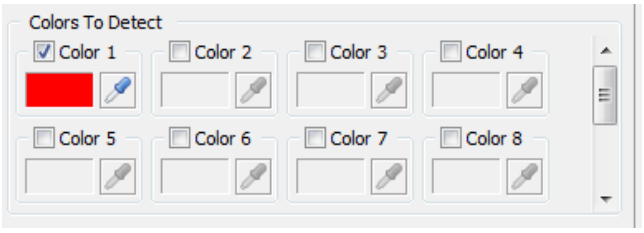
The following panel allows to set and check the detection of the animal using the color detection mode.



1. Put one pilot subject marked with one color (or an object with similar color) into the arena. If your image source is a digital video file, use the **Digital Video Control** tool section of the player to set a new starting point for the tracking detection test in which the animal is seen.



2. In the **Colors to Detect** options, check **Color 1**



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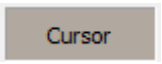
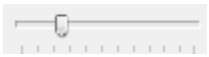
3. Use the  button to select the color to detect.

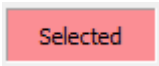
4. Move the cursor on the mark of the animal. The color detected by the cursor is the average color of all the pixels included in the surface of the cursor and is shown in the **Cursor button**.



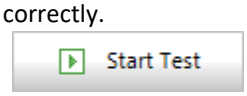


Use the scroll (or scroll the roll of the mouse) tool for reducing the surface of the cursor until the Cursor button shows the color to detect.

5. Click on the cursor mouse to accept the selection. The selected color will be then shown in the **Selected button**.



6. Click on the **Accept** button when the color selection is finished.
7. Click on the **Start Test** button to check whether the color detection process can identify the subject or the subjects correctly.



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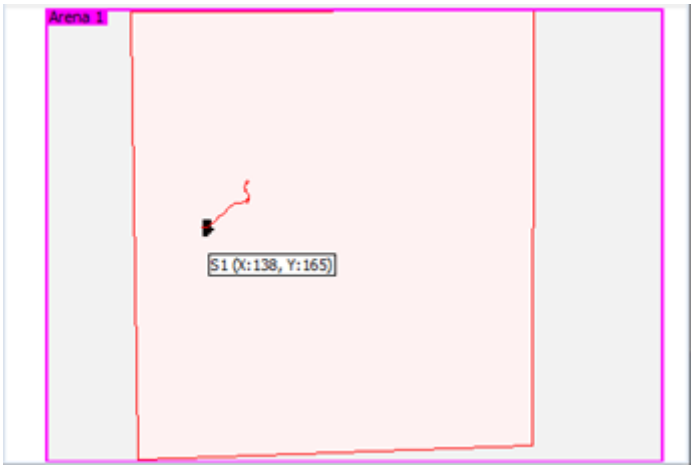
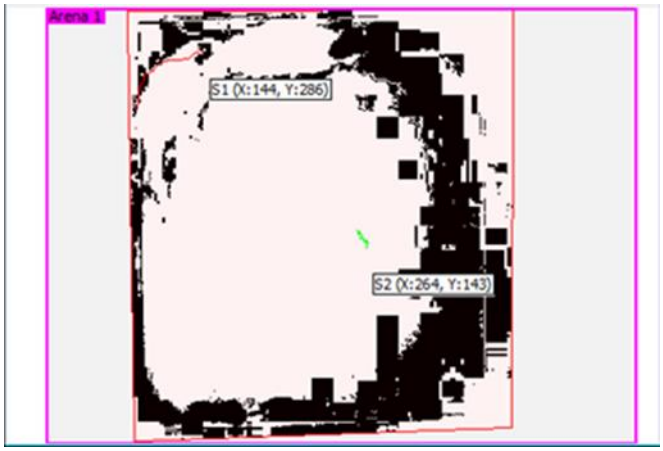
The Calibration process (see chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration) must be done before starting the Test. The difference between the current image and the image of reference is converted in pixel (black dots). Detection is considered optimal when the only black dot shown in the player is the targeted dot of color. In that case, the red tracking line should closely follow all the color dots displacements and a white label with the animal number and coordinates is shown.



8. If such detection is not obtained, adjust the **Threshold** and **Erosion** parameter for optimizing the detection process (see chapter 12.3 - Center of mass mode detection settings).
   We first recommend the user to set the Erosion to the zero value. Depending on the Threshold value set a lot of background noise is detected…or nothing is detected!



- Threshold
  In the color detection mode, the threshold is an automatically calculated index mixing Color Hue and Saturation parameters.
  The **Hue** is an angle dimensioned parameter (between 0 and 360º) related to tonality. The entered value is an interval of tolerance for color distinction. Normally, it ranges from 10 to 20º. A very narrow tolerance would make the system consider as different colors two points of the same-colored surface, while a very wide one would cause two different but similar colors to be regarded as one.

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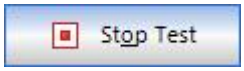
The figure on the left shows an example of what could happen when the Hue Threshold value is higher than it should be: SMARTIO mixes the colors of the subjects, and so the positions of the subjects are incorrectly calculated. On the contrary, the figure on the right is an example that shows the result of a correct configuration of the Hue Threshold control (note that the shape of the pixels detected is clearly painted with a single color).

The **Saturation** is the saturation tolerance range for a subject color (it works the same as explained for hue). It is a color/brightness ratio, in percentage (0% = black; 100% = pure color). A tolerance interval is given here to exclude wrongly detected changes due to glints. Users should be advised that, internally, SMARTIO rejects all those pixels which have a saturation lower than 25% (they can be considered grey tonalities and not color tonalities).

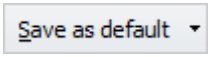
- Erosion
  When using color detection, the Erosion parameter is commonly set to the zero value because the SMARTIO will focus the detection to the targeted colors and ignore any of the other colors displayed in the experimental area.

If the detection is still difficult to obtain, ensure that the targeted colors are only existing on the animals, and not in any other area of the experimental area. Additionally, change the image brightness and contrast (see chapter - 12.2 - BRIGHTNESS AND CONTRAST) or optimize the experimental detection (see chapter 5 - VIDEO-TRACKING PREVIOUS CONSIDERATIONS).

9. When the tracking path is correctly detected, click the **Stop Test** button.



10. Click on **Save as** default if these adjustments are going to be used for every new experimental file.



11. Click on the **Accept** button to save the new detection settings.

TriWise mode detection settings

The following points are useful recommendations for setting the experimental conditions for optimal subject detection when the TriWise detection mode is selected:

- Maximize the image contrast
  With all video-tracking systems, the better the image quality and contrast, the better the performance obtained. This point is critical when using the TriWise technology. Optimum reliability is obtained with black subjects on a white background or white subjects on a black background. High reliability cannot be ensured in low contrast conditions. Adjusting the camera lens and using the SMARTIO lighting configuration will help produce the sharpest and most contrasted image possible. Always avoid the undesirable artefacts (shadows, reflections, etc.). Always use indirect lighting for homogeneous lighting distribution in the experimental area.

- Avoid “obstacles” in the experimental area
  We recommend working in experimental conditions in which the shape (or a part of the body) of the animal will not be hidden by “obstacle(s)” placed in the experimental area or related to the structure of the maze used.

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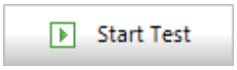
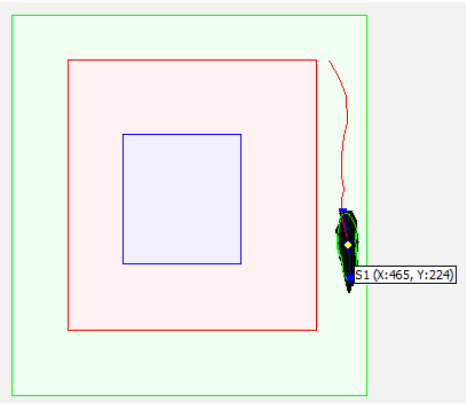
To set and check the detection the next steps must be followed:

1. Remove any subject from the experimental area for taking the snapshot that will be used as image of reference. The only difference between the experiment image (with animal) and the reference image (without animal), is the presence or absence of the experimental subject (the lighting and background conditions should be the same). If a video digital file is used, move the cursor of the Digital Video Control tool section of the player until a frame is found without animal.
2. Press the Take Snapshot button to record the current image (frame) as the image of reference (snapshot). If the video does not contain any image of reference without animal, please refer to chapter 12.3 - Snapshot editor for more details on how to edit a snapshot.

3. Put a pilot subject (or an object with similar color) into each arena to test the detection process. If your image source is a digital video file, use the Digital Video Control tool section of the player again to set a new starting point for the tracking detection test.
4. Press the **Start Test** button to verify if the detection process can identify the subject correctly. The Calibration process (see chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration) must be done before starting the Test. The difference between the current image and the image of reference is converted in pixel (black dots). Detection is considered optimal when the only black dot shown in the player is the animal. In that case, the red tracking line should closely follow all the animal displacements, a white label with the animal number and coordinates is shown, and a blue arrow showing animal head (triangular extremity), center (central circle) and base-tail (short line extremity) placements.





If such detection is not obtained, adjust the Threshold and Erosion parameter for optimizing the detection process.

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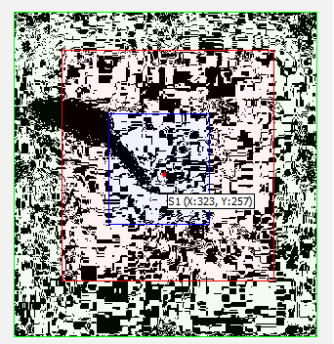
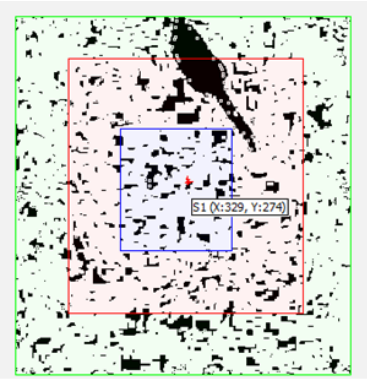
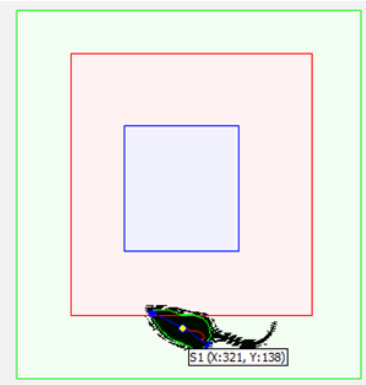
5. Adjust **Threshold** and **Erosion** parameters to get a sharper and noise-free test image. To differentiate changes in the image due to real movements from those produced by reflections, shadows, or small bits of not-under-study objects, a tolerance Threshold is defined. On the other hand, the boundary outline of the shape is likely to produce artefacts, but it may be disregarded with this Erosion control. We first recommend the user to set the Threshold and Erosion to the zero value. A lot of background noise is detected during the test shown below.



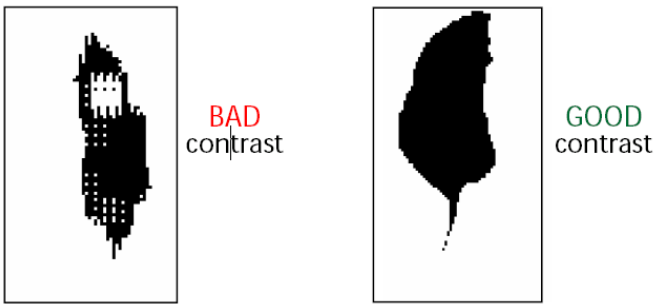
- Threshold

  Increasing the Threshold value removes progressively the background noise (reflection, shadow, small displacement of the camera or the scenery) until only the animal is detected.





Although they are great tools to optimize the tracking process, both tend to distort the animal shape that makes the automatic visual recognition difficult. Try to keep the animal shape as natural as possible, avoiding blurred edges. The shape of the animal must be as sharp as possible.



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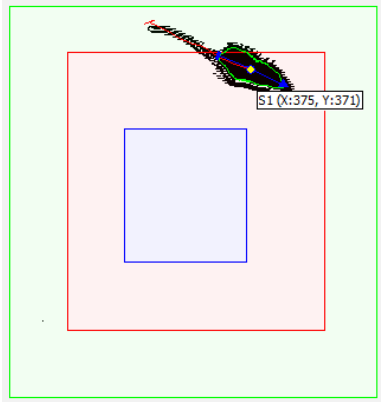
- Erosion

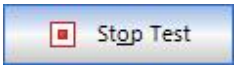
  At all times, SMARTIO calculates the tracking points of the subjects (assuming a homogenous surface weight distribution). Concentric zones are defined from the center of mass out to the exterior perimeter. The external zone (outermost rings) would be likely to produce artifacts, as this is where shadows and reflections are most difficult to discriminate. To avoid this problem, the Erosion control enables these external rings to be eliminated. To find the optimum setting, start with a value of 1 and gradually increase until the image displayed shows only the shape of the subject.



  An erosion value set to zero (or very close to zero) is highly recommended. If some small pixel dots can be observed in the Test player when the Erosion is set to the minimum value, it is suggested to use the additional internal filter included in the TriWise option to ignore them.

If the detection is still difficult to obtain, ensure that the targeted colors are only existing on the animals, and not in any other area of the experimental area. Additionally, change the image brightness and contrast (see chapter - 12.2 - BRIGHTNESS AND CONTRAST) or optimize the experimental detection (see chapter 5 - VIDEO-TRACKING PREVIOUS CONSIDERATIONS).

6. If the tracking path is correctly detected, click on the **Stop Test** button.



7. Set the anti-vibration filter, if required (see chapter 20.4 - Filtering and smoothing techniques in SMARTIO)

Due to the own nature of the basic video-tracking systems, image noise could produce little variations of the center of mass that could imply a considerable error in the total distance travelled. To minimize such errors, check the box Use anti-vibrations filter and select a filtering value.

The filtering value depends on the level of noise detected in the image. To determine it, put a static object into the arena and try to track it starting with the filter set to zero. If the distance increases, this means that the noise level is high introducing a vibration of the tracking point. Increase the filter value until distance is only increased when the animal moves.

	Please note that setting the noise filter too high can cause the system to ignore a real movement of the subject between frames, resulting in speed values that are not accurate.

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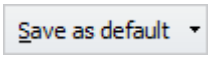
The selection of an optimal anti-vibration filter is not critical during the acquisition of the data, this filter can be changed again during the analysis process for getting a new set of data.

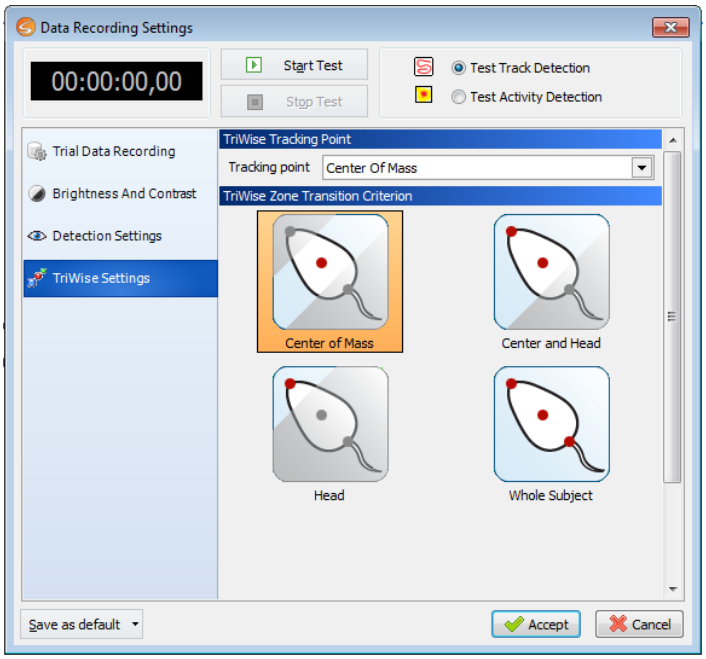
8. Click on **Save as** default if f these adjustments are going to be used for every new experimental file



9. Click on the **Accept** button to save the new detection settings.

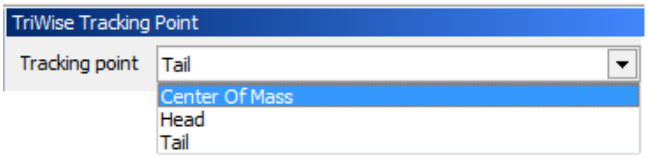
TriWise mode additional settings

SMARTIO users are provided with an additional **TriWise Settings** panel to configure the capabilities of the extension.



The **TriWise Settings** section helps the user to adjust the following TriWise tracking detection parameters.

- **Tracking Point**: allows choosing the tracking point (head, center or tail), and therefore which point SMARTIO will use to calculate parameters such as distance covered and speed during the acquisition process. The coordinates of the 3 points will be registered internally, so the Tracking point can be changed afterward during the Analysis process for generating a new set of data.



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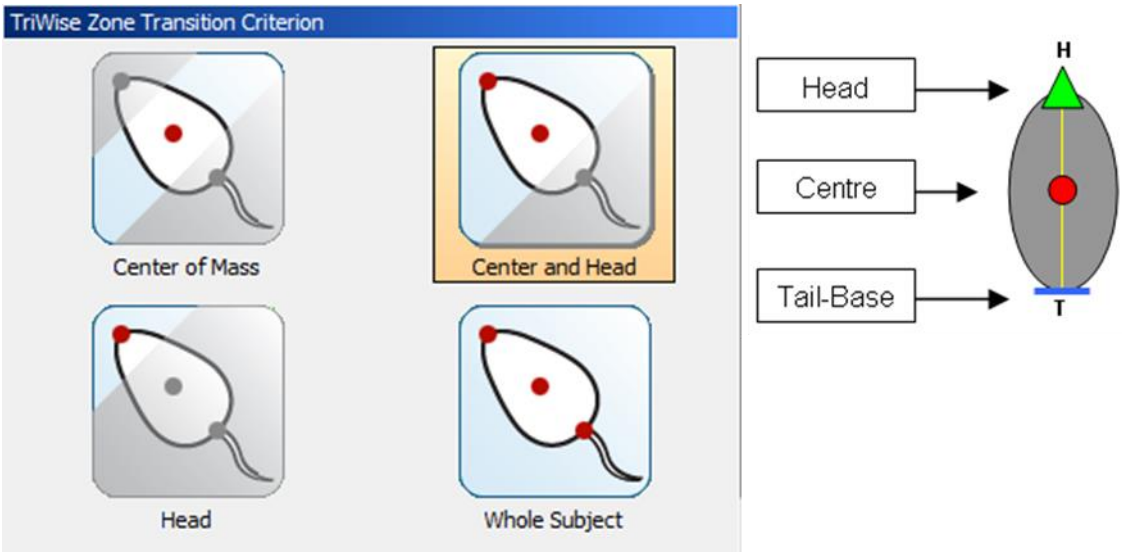
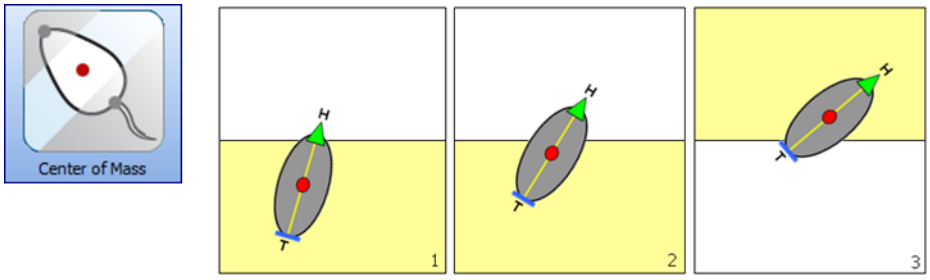
- **Zone transition criterion**: knowing the position of the head, center and tail of the animal, it is possible to define advanced zone transitions criteria.

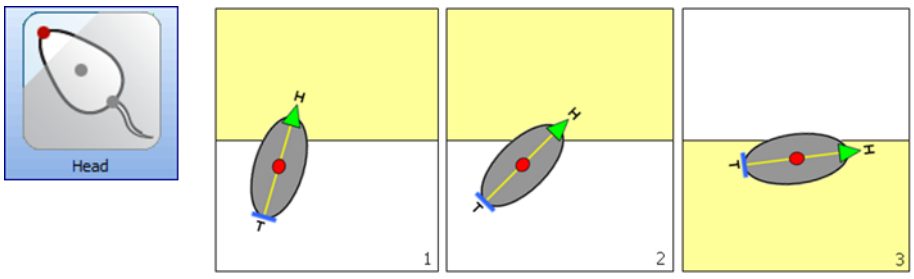


This configuration will be used during the acquisition process for determining when a zone transition occurs. This configuration can be changed afterward during the Analysis process for generating a new set of data. TriWise provides the following options to decide when a subject moves from one zone into another:

- Center of Mass: The animal is considered in the zone when the center point is detected within that zone. For this determination, only the center point is used in the evaluation.



- Head: The animal is considered in the zone when the head point is detected within that zone. For this determination, only the head point is used in the evaluation.



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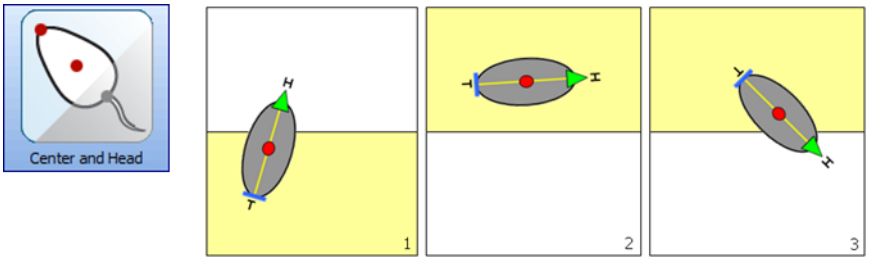
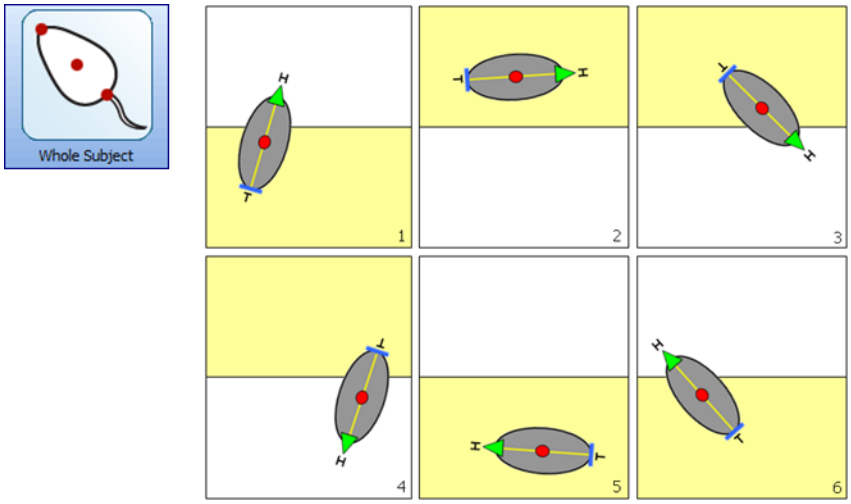
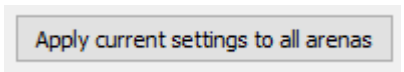
- Center and Head: The animal is considered in the zone when the center AND head points are detected within that zone. For this determination, only the center and head point are used in the evaluation. If the center and the head are in different zones, no transition is considered to occur. In the first sample acquired, if center and head are in different zones, it is considered that the subject is in the zone where the center point is.



- Whole Subject: The animal is considered in the zone when all three points are detected within that zone. For this determination, all three points are used in the evaluation. If the center, head and tail-base are in different zones, no transition is considered to have occurred. If the points are in different zones, SMARTIO will consider the subject to be in the zone where the center point is observed.



SMARTIO MA	The TriWise Tracking Point and TriWise Zone Transition Criterion can be applied to all arenas by pressing the “Apply current settings to all arenas” button. 

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Manual detection

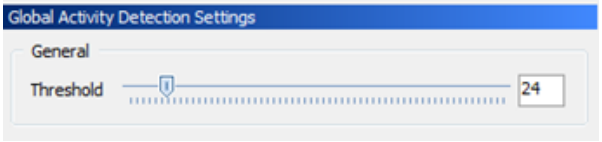
In the manual tracking mode, the position of the subject is not detected automatically and must be indicated by the user using the mouse-cursor over the image shown in the player. It is a useful tool for experiments in difficult conditions where automatic track detection is not possible or not reliable. The manual tracking mode works in the same way as the center of mass tracking detection mode, with the difference that the position of the subject is not detected automatically but indicated by the user. All other functionalities remain the same as in the center of mass tracking detection mode with some limitations described further ahead in this chapter.

- Detection test: As the position of the subject is not detected automatically in the manual tracking mode, no detection test is necessary.
- Anti-vibration filter: Please refer to chapter 20.4 - Filtering and smoothing techniques in SMARTIO.
- Limitations of the manual tracking mode: As there is only one cursor available to indicate the position of only one subject at a time, the following restrictions apply when using manual tracking mode:
    - When using the manual tracking mode, it is not possible to record more than one track in one trial. Each trial to be acquired can contain only one subject. Social interaction specific data cannot be obtained using this mode.
    - Although multiple arenas may be defined, when using the manual tracking mode only the data acquisition of one arena can be executed at a time. It is not possible to acquire data of multiple arenas simultaneously using this method.
    - Given the nature of this tracking mode, it only will be available with digital video files as image sources.

As the automatic detection of global activity is independent of the automatic detection of the subject's position, SMARTIO can acquire simultaneously global activity data when using the manual tracking mode.

Global activity detection settings

When the **Activity Detection** mode is selected, the following settings panel will be available from the **Detection Settings** section.



The following steps must be followed to check the detection:

1. Put a pilot subject into each arena to test the detection process. No snapshot must be taken to detect activity. If your image source is a digital video file, use the Digital Video Control panel to set a starting point for the video.

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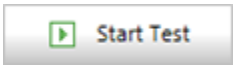
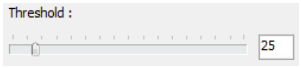
2. Click on the **Start Test** button to check whether the detection process can identify the subject correctly. The Calibration process (see chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration) must be done before starting the Test. The image will turn to test mode, allowing real-time visualization of adjustment effects.



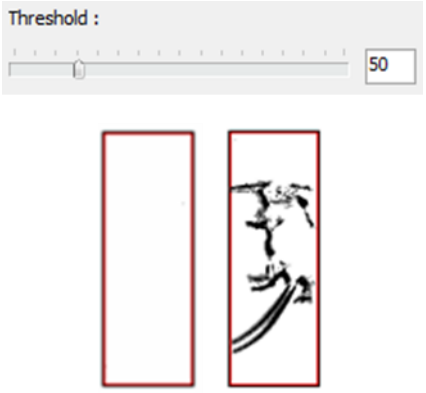
3. The difference between two consecutive frames (that is, what is being detected and processed by SMARTIO), will be numerically materialized in pixel format (black dots).
4. Adjust the **Activity Threshold**



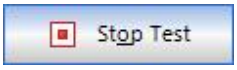
For calculation purposes, activity is measured as surface change rate. SMARTIO evaluates pixel changes rate and converts it to a surface change rate using the calibration values. Since a perfectly static image is not possible (interferences always cause a few pixels to change) a threshold must be defined. See the Activity Threshold box at the bottom of the screen. A threshold equal to zero will make the system consider all changes in the image, while higher values would only consider significant changes. The difference between two subsequent frames (that is, what is being detected and processed by SMARTIO), will be numerically materialized in pixel format. The identical areas between the two images will be displayed as white. The optimal threshold setting makes the test image white; illustrating no displacement is produced and black spotted at the slightest movement.
Here is an example for setting threshold for a forced-swimming test.

Threshold too low

Optimal threshold



5. Once the activity parameters have been set, return to the normal camera image by pressing the Stop Test button.
6. If the tracking path is correctly detected, click on the **Stop Test** button.



7. Click on **Save as** default if these adjustments are going to be used for every new experimental file

8. Click on the **Accept** button to save the new detection settings.

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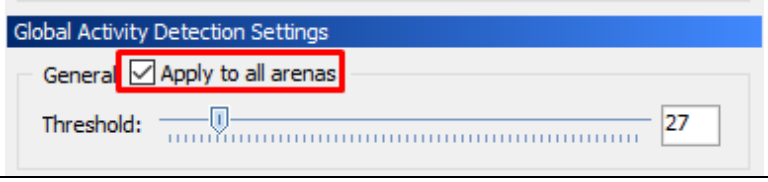
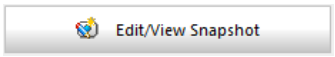
SMARTIO MA	When working with the SMARTIO-MA extension, an Apply to all arenas option is checked by default. Uncheck this option for taking and using a different Global Activity Detection Settings for each arena. 

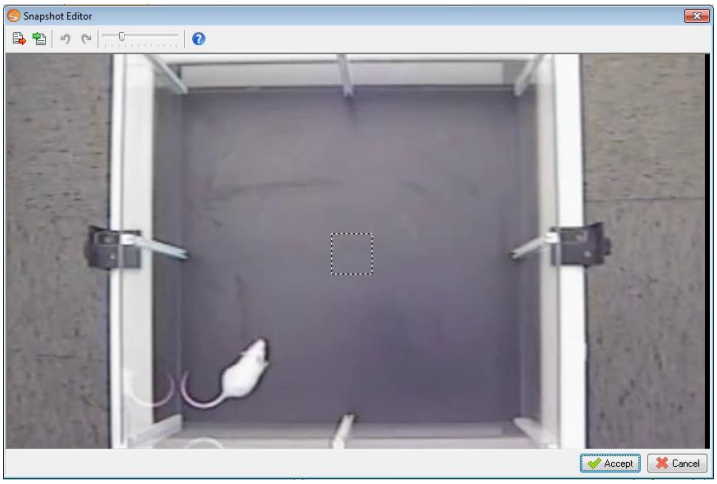
Snapshot editor

This tool is only available for the Automatic Tracking detection modes (Center of mass, 3-points TriWise, color detection). When a digital video source is used to acquire data, it is a strongly recommended to have at least 1 second of the video without the animal inside the box so that a snapshot of the clean box could be taken. Whether this was not possible or whether the video was accidentally recorded without this in mind, SMARTIO provides a Snapshot Editor tool to (1) remove the animal and other artifacts in the snapshot image or (2) to import a snapshot without animal from another video registered in the same experimental conditions.

To do that:

1. If working with a video digital file, use the **Digital Video Control** section to search a frame in which the biggest area of the arena's floor is visible (that is, the animal occupies the smallest area in the arena). Avoid positions in which the animal is reflected in the arena's walls as reflections are more difficult to remove.
2. Click on the **Snapshot** button to capture the current snapshot.
3. Click the arrow button located at the right side of the Snapshot button and select the **Edit/View snapshot** menu option.





4. Adjust the size of the cleaning brush through the toolbar or by moving up or down the wheel in the mouse. Size of the brush is adjustable. Larger brush size may not give optimum results.

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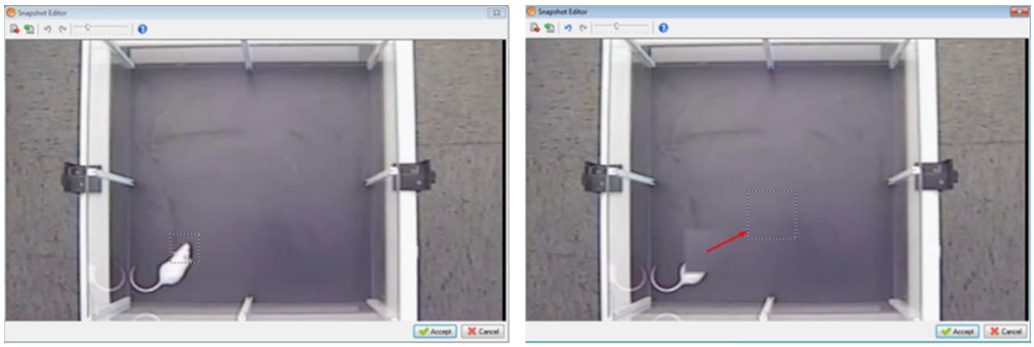
5. Click an area of the image occupied by the animal and, with the left button of the mouse still pressed, move to a clean space of the arena close to the current one.



6. Release the left button of the mouse when a homogeneous surface is obtained.
7. Use the Undo/Redo buttons in the toolbar if the final result is not suitable.

8. Repeat the steps 4 to 7 until the animal is completely removed and the resulting snapshot is clean from imperfections.

9. Export the resulting snapshot if you plan to import it later in the same experiment or in a different one.

	Only the exported snapshots with the same image size as the current digital video file can be imported.

10. Click on the **Accept** button to use the new snapshot for testing the detection.

11. Start a detection test (as explained before in the previous chapters) and adjust the thresholds to get an optimal detection with the new snapshot.

	Snapshots edited manually are stored and retrieved from the experimental file so that it is not needed to edit the snapshot every time the experiment is opened. If a new snapshot is taken the edited snapshot will be lost.

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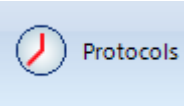
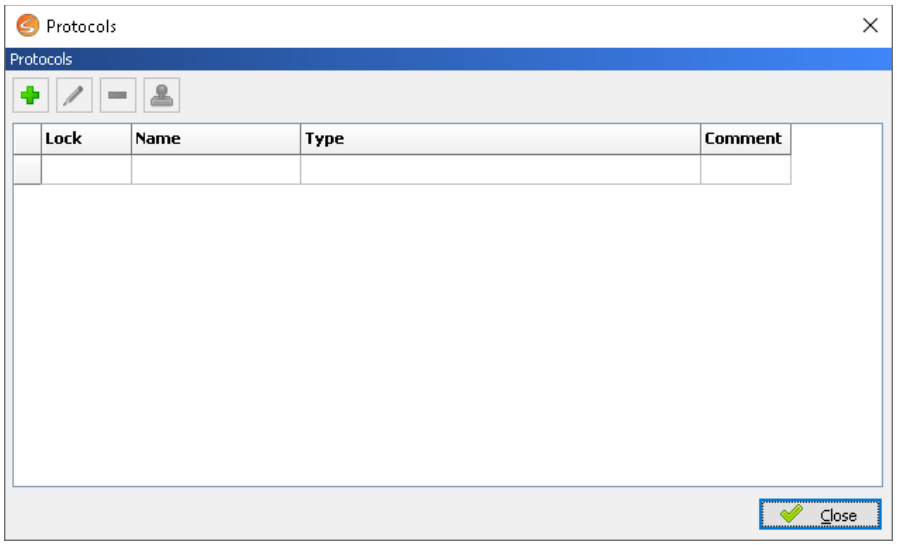
13. PROTOCOLS



A protocol defines the duration of the data acquisition process as well as the conditions that need to be fulfilled to trigger the stimulations.

13.1. PROTOCOLS MANAGEMENT TABLE

When pressing the **Protocols** button on the **Experimentation Assistant** bar**,** the **Protocol** panel is shown providing a Protocols management table.



The **Lock** column indicates whether the protocol is locked or unlocked for edition. A Protocol remains unlocked if it has not been used to acquire data from any Trial; once it has been used at least one time, it becomes locked. For traceability reasons, a locked protocol can be visualized but cannot been edited/modified. If the user would like to introduce a change for using the new modified protocol in futures sessions, the protocol must be first cloned. The clone of the protocol is then available for edition.

The table provides the following tools:


To create a new protocol.


To eliminate a protocol


To edit an existing protocol.


To duplicate/clone an existing a protocol.

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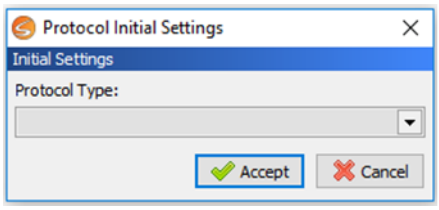
13.2. PROTOCOL CREATION AND INITIAL SETTINGS

To create and edit a protocol:

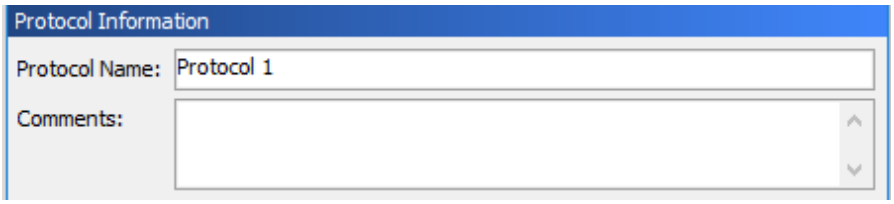
1. Click on the  button to create a new protocol.
2. Select the protocol type from the available list in the **Protocol Initial Settings** panel. The available options are:

    - Time
    - Laser TTL Stimulus



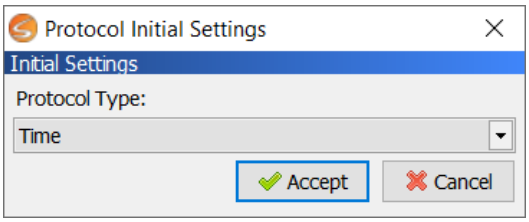
Protocol information

Either the user chooses a Time or a Laser TTL Stimulus protocol, the tab **Protocol Information** section allows to change the name of the protocol and add some comments.



Time protocol edition

The **Time** protocol editor is dedicated to protocols in which only time conditions are present (latency time and total time of acquisition) without the involvement of external variables such as TTL signal triggers.



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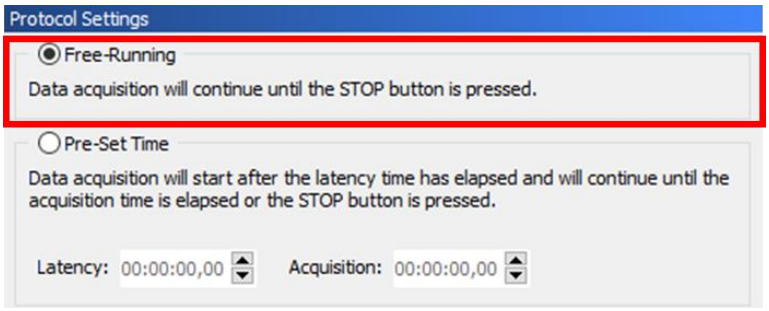
Protocol Settings

After clicking on the **Accept button**, the Time Protocol Editor panel is shown.

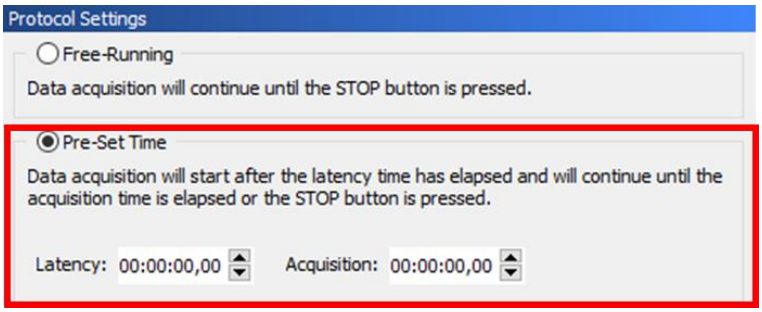
In the **Protocol Settings** section, the users can define the kind of Time protocol they want to design:

- Free Running

In the Free-Running protocol, the user will manually start and stop the tracking process by pressing the START and STOP buttons. Thus, duration of the acquisition period will entirely depend upon the user's on-line commands.



- Preset Time



The Pre-Set Time option allows defining a preconfigured Acquisition period (experiment duration) and a Latency period, which is the time interval before acquisition actually starts. This latency period is intended as a "waiting interval", after the START button is pressed, which can be used to place an animal in the arena or to simply give the subject some time to acclimate to the new environment.

⚠	The latency period is started after the START button is pressed and the animal has been detected in the experimental area.
	Latency and acquisition periods must be set in hh:mm:ss,cc format so, technically, up to 99h 59m 59s, 99c of data acquisition is allowed in this pre-set mode.
	No data is registered during the latency period. In all cases, the track will only begin when the animal is detected into the experimental area.

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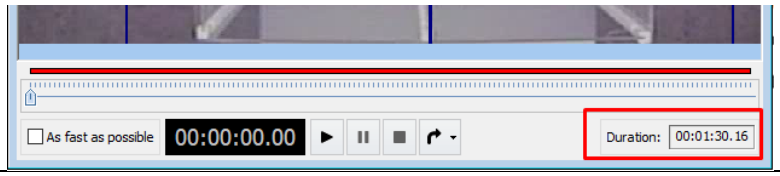
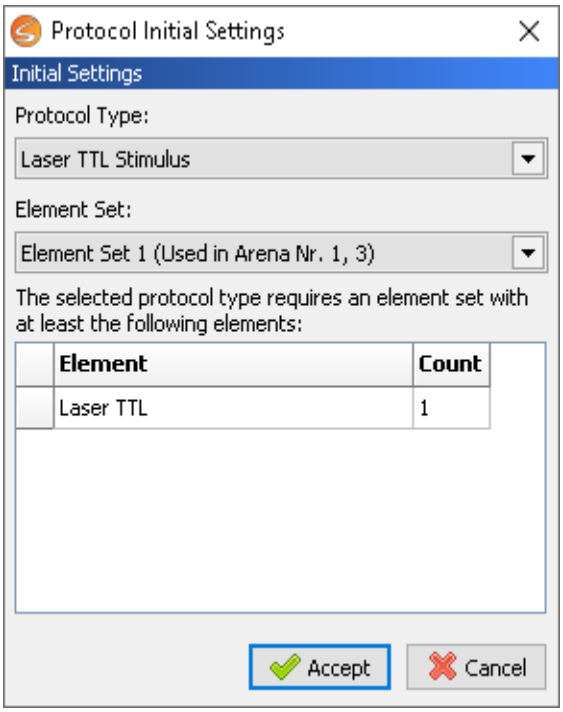
If a digital video file is selected as an image source, the acquisition pre-set time cannot be longer than the total duration of the video. In the Player panel, the maximum acquisition time allowed by the current digital video file is shown.



In Time Protocols, the acquisition process stops automatically when the time set in the **Acquisition** is elapsed (Preset Time Protocols) or when the STOP button is clicked (Free-Running Protocols).

Laser TTL stimulus protocol edition

The **Laser TTL Stimulus** protocol editor allows to design protocols in which both Time conditions and activation of a stimulation from a third-party laser optogenetics stimulator can be controlled. In this last case, the user must select the Element set that would be used in this protocol. The Element set should be previously defined from the Exp. Arena main menu. See chapter 7.2 - HARDWARE DEVICES and chapter 9.2 - ARENA ELEMENT DEFINITION for more information.



Laser TTL stimulus protocol should not be used when the image source is a network camera. Please see chapter 3.5.

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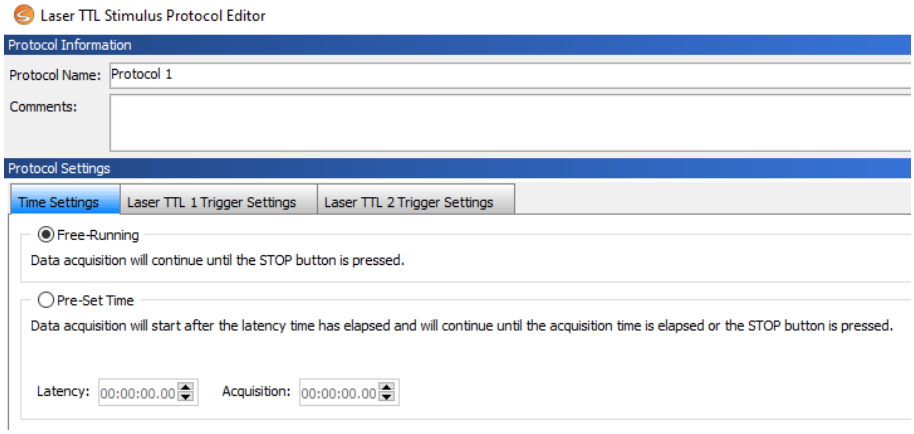
After clicking on the **Accept button**, the Preset Time Protocol Editor panel is shown. In the **Protocol Settings** section, the user can define three aspects of the same protocol:

- Time Settings
- Laser TTL 1 Trigger Settings
- Laser TTL 2 Trigger Settings



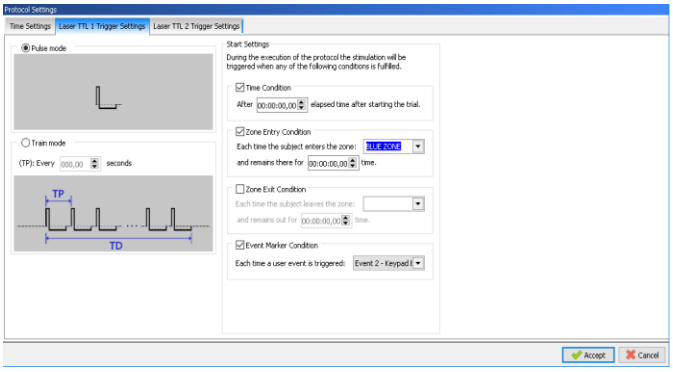
Time Settings

This panel shows the same options than the Time protocol editor: Protocol Information, Free-Running and pre-Set Time. See chapter 13.2 - Time protocol edition for more information.

Laser TTL (N) Trigger Settings

The Laser TTL 1 or 2 Trigger Settings tabs provide the conditions to trigger and manage the laser optogenetics stimulation. The laser optogenetics stimulation can be triggered in two different modes, as a unique pulse or as a train of several continuous pulses:

- Pulse



A unique 50 ms pulse is triggered whenever one of the selected start conditions is fulfilled:

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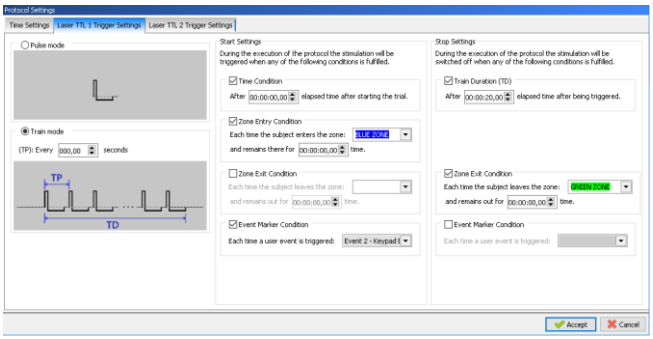
- **Time Condition**: The stimulus will be triggered after a user-defined time (HH:MM:SS.00) elapsed after starting the trial.
- **Zone Entry Condition or Zone Exit Condition**: The stimulus will be triggered each time the subject enters into or exit from a user-defined zone and remains in it or is out of it for at user-defined time (HH:MM:SS.00). The zone must be selected from the available list of options. The available list of zones is updated depending on the Zone Definitions saved in the experimental file (see chapter 11 - ZONES DEFINITION).
- **Event Marker Condition:** The stimulus will be triggered each time the user manually presses a key on the Event marker panel. The key must be selected from the available list of options. We recommend the user to first configuring the Event marker before setting this condition (see chapter 16.3 - EVENT MARKER SETTINGS).

- Train



A train of 50 ms pulses given at specific intervals (period time, TP) is triggered whenever one of the selected start conditions is fulfilled. The available start conditions are the same previously explained for Pulse mode.
The train of pulses is stopped when the selected stop conditions is fulfilled:

- **Train Duration (TD)**: The train of pulses will be stopped once the user-defined time (HH:MM:SS.00) has elapsed after starting the trial.
- **Zone Entry Condition or Zone Exit Condition:** The train of pulses will be stopped when the subject enters into or exit from a user-defined zone and remains in it or is out of it for user-defined time (HH:MM:SS.00). The zone must be selected from the available list of options. The available list of zones is updated depending on the Zone Definitions saved in the experimental file (see chapter 11 - ZONES DEFINITION).
- **Event Marker Condition:** The train of pulses will be stopped each time the user manually presses a key on the Event marker panel. The key must be selected from the available list of options. We recommend the user to first configuring the Event marker before setting this condition (see chapter 16.3 - EVENT MARKER SETTINGS).

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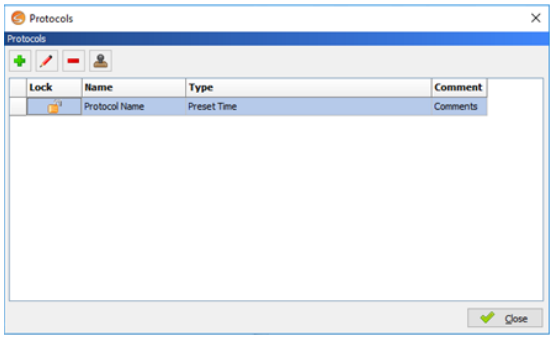
	The duration of the pulse (single or train) is fixed to 50 ms. However, its duration can be modified through the third-party laser optogenetics stimulator used.
	When using a train of pulses, the minimum interval of time between two pulses (TP) is 100 ms.



Once the protocol is edited, click on the **Accept** button. The created protocol will then be listed in the **Protocols table**:

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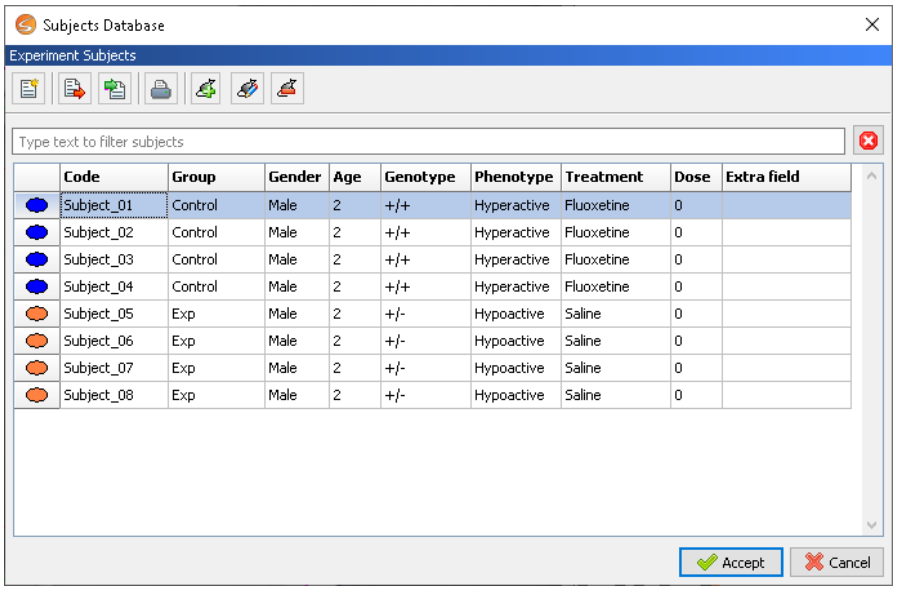
14. SUBJECT DATABASE

Any experiment is carried on over a set of experimental subjects. These subjects must be registered into the experimental file before starting to acquire data from the trials. To create a database of experimental subjects, enter the **Subjects Database** manager by clicking on the **Subjects** button in the **Experimentation Assistant** bar.



Code	Group	Gender	Age	Genotype	Phenotype	Treatment	Dose	Extra field
Subject_01	Control	Male	2	+/+	Hyperactive	Fluoxetine	0	
Subject_02	Control	Male	2	+/+	Hyperactive	Fluoxetine	0	
Subject_03	Control	Male	2	+/+	Hyperactive	Fluoxetine	0	
Subject_04	Control	Male	2	+/+	Hyperactive	Fluoxetine	0	
Subject_05	Exp	Male	2	+/-	Hypoactive	Saline	0	
Subject_06	Exp	Male	2	+/-	Hypoactive	Saline	0	
Subject_07	Exp	Male	2	+/-	Hypoactive	Saline	0	
Subject_08	Exp	Male	2	+/-	Hypoactive	Saline	0	

The table shows important information about the subjects:

- Subject Code: ID or code of the subject
- Subject Group: experimental group related to the subject.
- Color: this field does not represent the physical color of the subject but can be used for identification purposes.
- Gender: gender of the subject. Can be Male or Female.
- Age: age of the subject (the unit is not indicated).
- Genotype: Genotype of the subject (genetic characteristics of the subject), can be wild-type, knock-out for some specific genes, transgenic…
- Phenotype: Phenotype of the subject (physical or physiological characteristics of the subject), can be hyperactive, blind, brown fur color, etc.…
- Treatment: treatment used, if any…
- Dose: dose of the treatment used, if any (the unit is not indicated).
- Extra field: free edition text available to add any other characteristics the user would like to export in the report for analysis.

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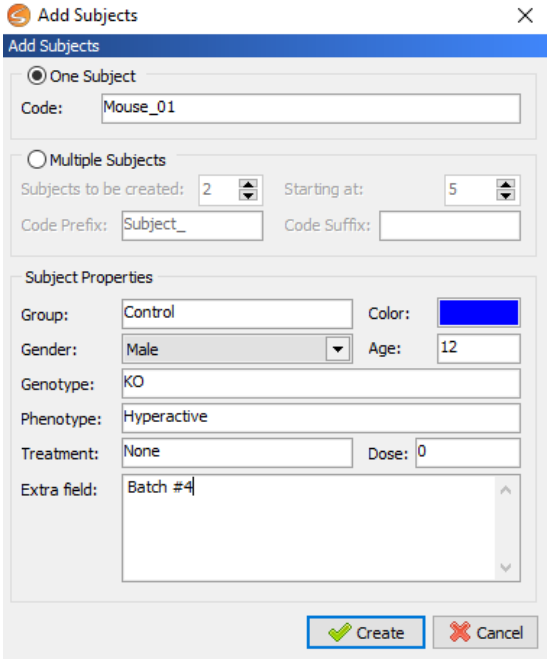
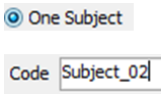
14.1. ADDING NEW SUBJECTS

Click on the **+** button to add new subjects to the database. A panel will be shown allowing to select whether to add one new subject or multiple subjects at the same time.



Adding one new subject

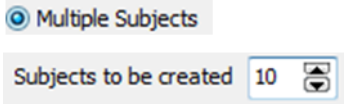
1. With the **One subject** option already selected, enter the subject's code.
2. Fill the rest of the subject's information in the **Subject Properties** section.
3. Click on the **Create** button to add the new subject.



	The new subject to be created cannot have the same code of another subject already belonging to the same group. If that happened, change the code or the group and try again.

Adding a batch of multiple subjects

1. Select the **Multiple subjects** option.
2. Enter the number of subjects to be created between 2 and 999.



3. Every new subject created will have a code composed by three parts combined in sequence:

	A code prefix.

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A code number: which starts at the number specified in the field Starting at.

A code suffix.

4. Fill the rest of the subject's information in the Subject Properties section. Every subject will have the same properties.
5. Click on the **Create** button to add the new subject.

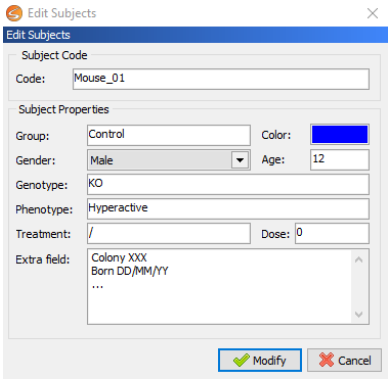
	New subjects cannot have the same code as other subjects that already belong to the same group. If that happens, SMARTIO automatically adds a suffix "(n)" to the subject's code, n being an incremental number used to differentiate them (e.g. "Subject_10" is renamed by "Subject_10 (2)").

14.2. EDITING THE PROPERTIES OF THE SUBJECTS

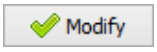
Editing the properties of one subject

1. Double click over the subject or select it and click on the **Modify selected subjects** button.

2. Edit the subject's code or any of the rest of the properties.



3. Click on the **Modify** button to apply the changes.



Editing the properties of multiples subjects

1. Select the subjects to be edited. The combination of the [SHIFT] and [CTRL] keys and the left button of the mouse allows the selection of a variety of ranges very easily.
2. Click on the **Modify selected subjects** button.

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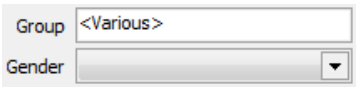
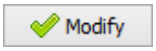
3. The **Edit Subjects** panel shows the properties of all the selected subjects. The value of the common properties is shown directly but heterogeneous properties are shown as "<Various>" or as an empty field.



4. Edit the subject's properties. Enter the values to apply to all the selected subjects at the same time. Leave the rest of the properties unchanged.
5. Click on the **Modify** button to apply the changes.

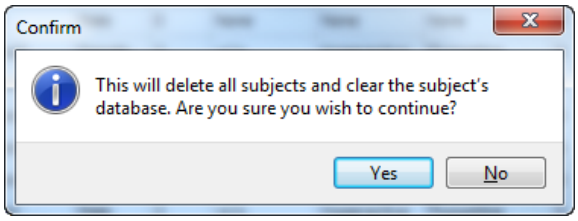


14.3. DELETING SUBJECTS

1. Select the subjects to be deleted. The combination of the [SHIFT] and [CTRL] keys and the left button of the mouse allows the selection of a variety of ranges very easily.
2. Click on the **Delete** selected subjects button.

If multiple subjects are to be deleted, then a confirmation message is shown. Accept the message to definitely delete the subjects.

Click on the **Clear Subjects** button and accept the confirmation message to delete all the subjects at once from the database.



	As with other changes within the subject's database, deleted subjects can be recovered only if the Subjects Database panel is closed through the Cancel button. If the panel is accepted, changes done in the subject's database cannot be undone.

14.4. SAVING THE SUBJECTS DATABASE

To save the changes done within the subject's database, click on the **Accept** button in the **Subjects Database** panel.

Click on the **Cancel** button to lose all the changes.

	All the changes within the subject's database can be recovered only if the Subjects Database panel is closed through the Cancel button. If the panel is accepted, changes done in the subject's database cannot be undone.

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14.5. OTHER TOOLS REGARDING SUBJECTS MANAGEMENT

Sorting the subjects database

Left click column headers for main sort index. Click again on the header to change the sorting direction. Add secondary sort indexes with [SHIFT] + left click.

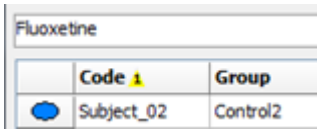
Code 2	Group 1	Gender
Subject_03	Control	Male
Subject_04	Control	Male

Place the cursor on a column-separator line to change the width of the columns.

Searching for subjects in the database

The subject's database includes a useful tool to facilitate searching for subjects with properties matching a specific text.

1. Enter the text to search for in the filter box located just under the subject's table. As keys are being pressed, the subject's table is automatically filtered to show only those subjects with the text inside any of their properties.



2. Click on the **Clear search** button located at the right side of the filter box to cancel the filter.

Exporting and importing the subjects

The complete subject database can be exported to a Microsoft Excel® file.

1. Click on the **Export subject list** button.

2. Select the destination folder and file name and click on the **Save** button.

This file can be edited manually and also imported in a different experimental file.

A2 | Subject_01

	A	B	C	D	E	F	G	H	I	J
1	Code	Color	Group	Sex	Age	Genotype	Phenotyp	Treatment	Dose	ExtraField
2	Subject_01	16744448	Control	Male	2	+/+	Hyperactiv	Fluoxetine	0	
3	Subject_02	16744448	Control	Male	2	+/+	Hyperactiv	Fluoxetine	0	
4	Subject_03	16744448	Control	Male	2	+/+	Hyperactiv	Fluoxetine	0	
5	Subject_04	16744448	Control	Male	2	+/+	Hyperactiv	Fluoxetine	0	
6	Subject_05	4227327	Exp	Male	0	+/+	Hyperactiv	Fluoxetine	0	
7	Subject_06	4227327	Exp	Male	0	+/+	Hyperactiv	Fluoxetine	0	
8	Subject_07	4227327	Exp	Male	0	+/+	Hyperactiv	Fluoxetine	0	
9	Subject_08	4227327	Exp	Male	0	+/+	Hyperactiv	Fluoxetine	0	

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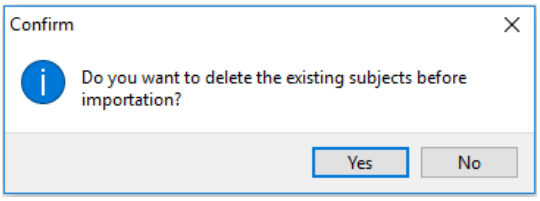
	Some versions of Microsoft Excel® may warn you that the Excel file generated by the exportation of a subject list has a different format than the specified by its extension. In that case, accept the warning message to open the file normally.
	Although manual manipulation can be done in the exported file, please keep in mind that the new information must fulfill the rules of a coherent subject database (e.g. two or more subjects belonging the same group cannot have the same code, color numbers must match the Windows® color palette, gender must be "Male" or "Female" only and age and dose fields must be numbers equal or greater than zero).

To import a previously exported subject list:

1. Click on the **Import subject** list button.
   
2. Locate the folder and file in which the subject list is stored. Then click on the **Open** button.
   The subjects within the file are automatically inserted in the subject database of the experimental file.
3. Click on the **Accept** button to save the subject list in the experimental file.
   

	If the current subject database already contains subjects, a warning message is shown before importing the new list. If the subject database must be cleared before importing the new list, accept the message. If the subject database is not cleared before importing the new list, the subject's code of the imported subjects will be automatically renamed to avoid a duplication of codes in the same group.
	

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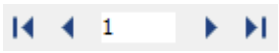
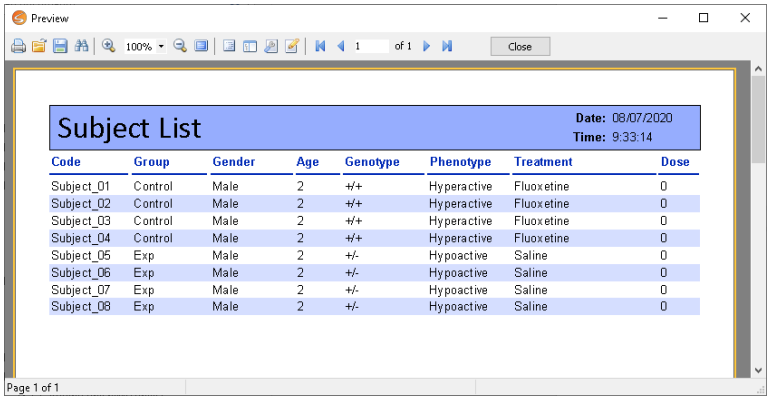
Printing the subjects database

1. Click on the **Print subject list** button.

2. Navigate through the pages of the report using arrow buttons in the **Report Preview** window.





3. Prepare your printer for printing the report.
4. Press the **Print** button to print the report.

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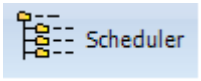
15. SCHEDULER

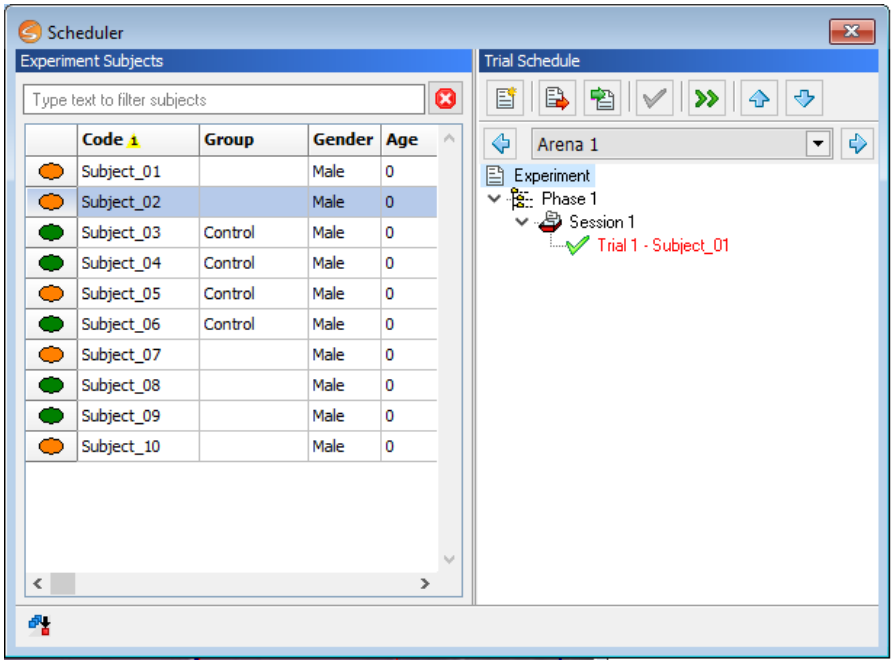
SMARTIO provides a Scheduler tool to set up the plan for the experiment.



The **Scheduler** allows the user to define the different phases, sessions, trials and subjects planned to be executed within the experimental project. All these elements are arranged in a tree-view list starting with the "Experiment" node.

Defining an experimental schedule enables:

- Automatic transition from one subject to the next during the acquisition of tracks without having to enter the name and characteristics of each animal every time the data acquisition starts or finishes.
- Having a list of all the subjects used in the experiment saved, as well as the order in which they have been used (reminder).
- Reuse of lists established in previous experiments with the subjects in the same or different order.
- Combine analysis results of a group of trials and the evolution of the subjects in each group along the time.



The Scheduler panel is split into two sections:

- The experiment subjects list: at the left side of the panel.
- The trial schedule itself: at the right side of the panel.

The trial schedule comes with a predefined plan including a single trial (trial 1) within a session (session 1) and a phase (phase 1). That trial is automatically assigned with the first subject in the database (Subject_01).

The trial is selected by default as "the next trial" to be executed. This property is shown as a green tick at the left side of the trial name. A new clean schedule can be started by clicking on the  button located the left side of the Trial schedule's toolbar.

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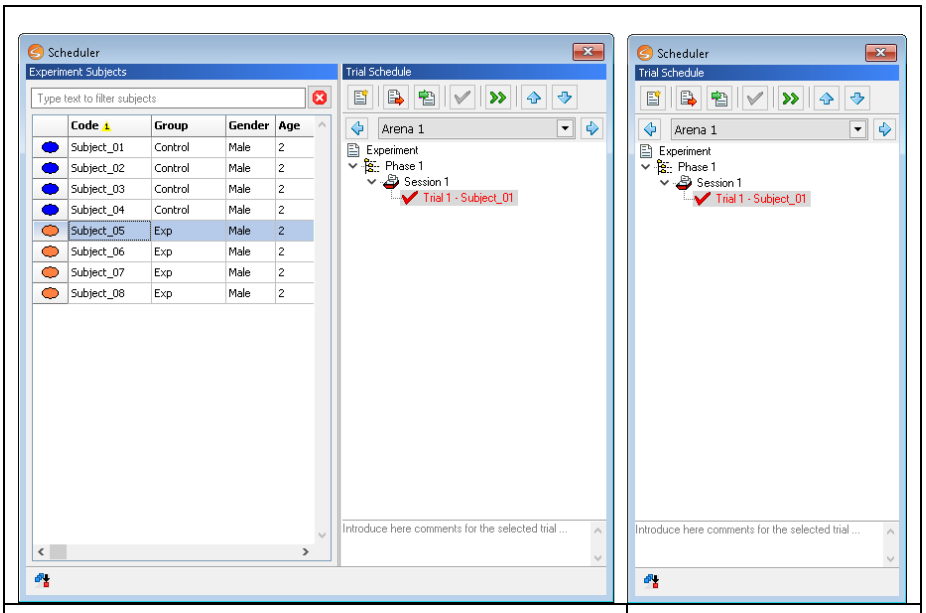
15.1. COMPACT AND EXTENDED VIEW

The **Scheduler** panel is provided as a floating window, meaning that the panel can be kept open while other tasks are running. This is especially useful during data acquisition, since it allows to easily identify which subject is the next to participate in a trial and thus prepare it before starting. Moreover, the trial schedule tree also allows you to inspect the final results of a finished trial by clicking its node. To work with the rest of the windows while the Scheduler panel is shown, compact and expanded views are provided.

- The **Expanded view** (default view) is designed to provide all the information regarding the experiment subjects and plan.
- The **Compact view** is designed to provide only the information regarding the experimental plan (Trial schedule section only).

To switch from the expanded view (default view) to the compact view, click on the **Change view** button located at the left bottom corner of the Scheduler panel.



Expanded view	Compact view

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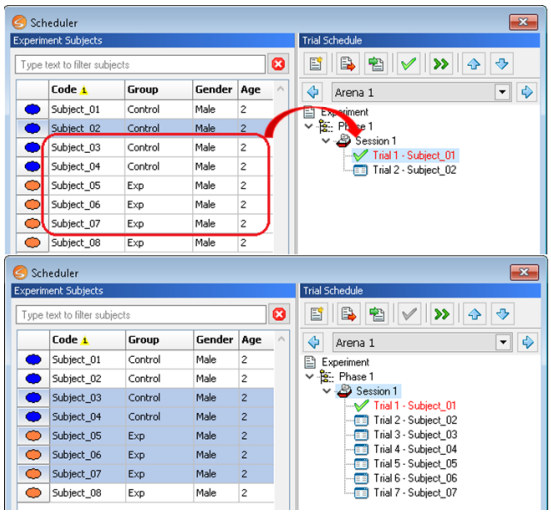
15.2. MANAGING TRIALS

Adding new trials to a session



To add a new trial (or a batch of new trials) to a session:

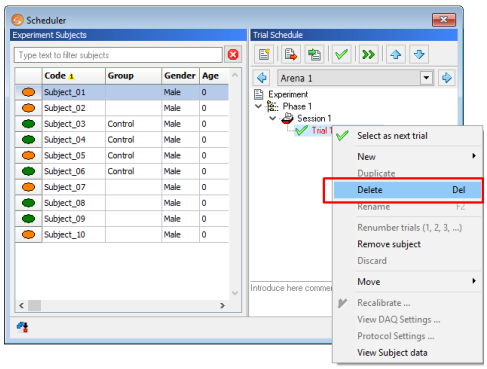
1. Select the subjects that will participate in the trials in the **Experiment Subjects** section. Use the left button of the mouse in combination with the [CTRL] and [SHIFT] keys to select a group of subjects.
2. Drag the selected rows in the table and drop them into the session node at the Trial schedule section.

Trial numbers always start at 1 and are automatically increased by one every time a new trial is added to the session. Different icons are shown in the tree view of the schedule to identify the status of the trials:

	The trial has not been executed, contains no data and is not selected for the next execution.
	The trial is the next one to be executed in the plan. Another trial can be selected as next by means of the same button in the toolbar.
	The trial has been executed and finished. Only these trials can be then analyzed.

Deleting a trial

To delete a trial already defined, right click on the trial's node and select the **Delete** option. Then confirm the deletion message.



Trials can be also deleted quickly by using the [DEL] key.

	If a finished trial is deleted, the data acquired will be lost as well. If the experimental file was saved, the information will not be recovered unless a backup file was saved previously. If a trial was unintentionally deleted, DO NOT SAVE the experimental file, close it and reopen it again to recover the information. However, other changes done since the last saving will be lost.

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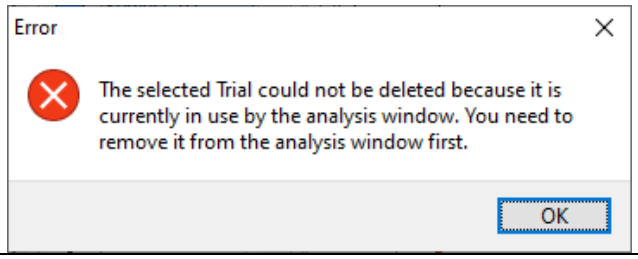
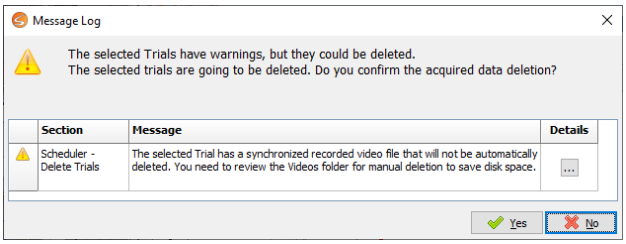
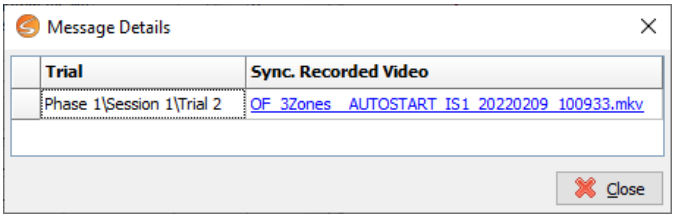
A trial can be deleted under certain conditions, depending on whether it has been acquired or not, there is a synchronized video, is in queue for analysis, etc….

<table>
<tr>
<td>Trial in queue for the analysis

ERROR MESSAGE: The trial cannot be deleted</td>
<td> 
</td>
</tr>
<tr>
<td>The trial is associated with a synchronized video

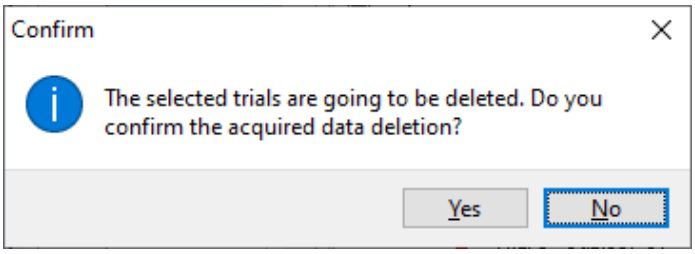
WARNING MESSAGE: The trial can still be deleted</td>
<td> 

Clicking on  the details of the video are shown



Clicking on the name of the video will open the containing folder. Clicking on Yes will delete the trial (not the video). Clicking on No will abort the deletion.</td>
</tr>
<tr>
<td>• The trial has not been acquired
• The trial has been acquired but it is not the analysis queue and there is no synchronized video

CONFIRMATION MESSAGE: The trial can be deleted</td>
<td>A simple confirmation message is displayed:



Clicking on Yes will delete the trial (not the video). Clicking on No will abort the deletion.</td>
</tr>
</table>

	When trying to delete more trials at the same time, take in count that a combination of error, warning and confirmation messages may be shown depending on the different situations.

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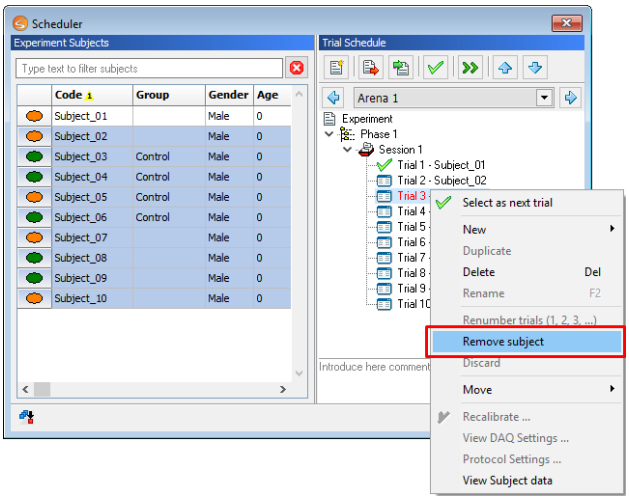
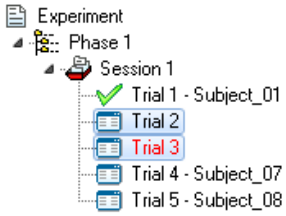
Removing the subject from a trial

1. Select the trial from which the subject should be removed. Use the left button of the mouse in combination with the [CTRL] and [SHIFT] keys to select a group of trials in the schedule's tree.
2. Click with the right button of the mouse over any of the selected trials and select the Remove subject option. Then confirm the removing.



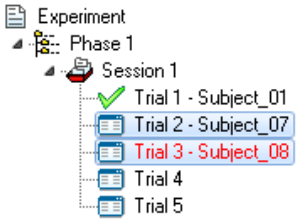
If the subject is removed from a trial, this is still shown in the schedule's tree, but no subject code is shown.



Moving the subjects within a session

1. Select the trial nodes that should be moved within a session. Use the left button of the mouse in combination with the [CTRL] and [SHIFT] keys to select a group of trials in the schedule's tree.
2. Click with the right button of the mouse over any of the selected trials and select the **Move** option. Then select any of the movement options available (Top, Up, Down or Bottom).

The up/down arrow buttons in the **Trial schedule**'s toolbar also facilitates the task of moving the selected trials up and down.



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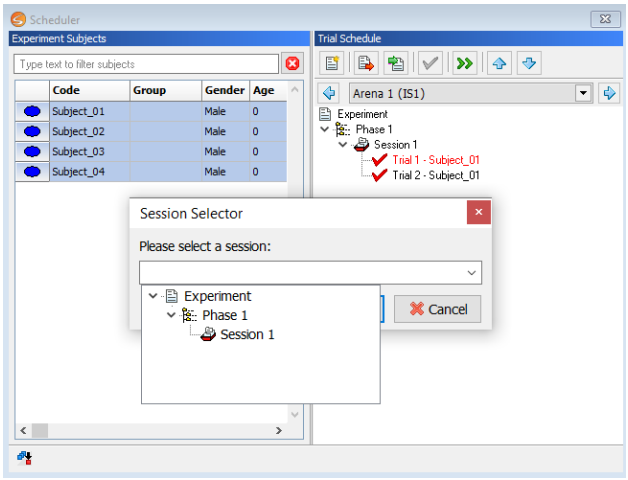
When subjects are moved within the session, the trial numbering is kept - only the subjects are changed from one trial to another. Please note that trials cannot be moved out of the session to which they belong.

Distributing subjects into arenas

SMARTIO MA	An “Arena selector” control is provided for SMARTIO-MA users within the **Trial schedule** section of the **Scheduler** panel to define the trials for each arena independently. 

To facilitate a quick distribution of subjects to the defined arenas, an efficient tool is provided:

1. Select the subjects in the **Experiment Subjects** table that should be distributed within the existing arenas. Use the left button of the mouse in combination with the [CTRL] and [SHIFT] keys to select a group of subjects.
2. Click on the  button
3. Select the session in which the trials will be added



4. The new trials will be automatically inserted after clicking on the **Accept** button.

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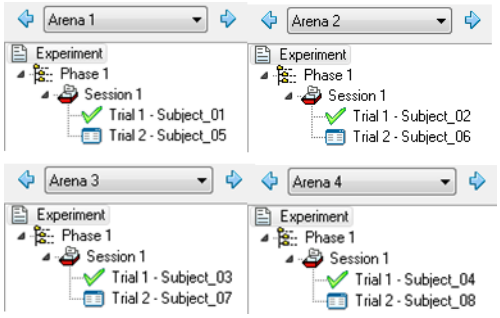
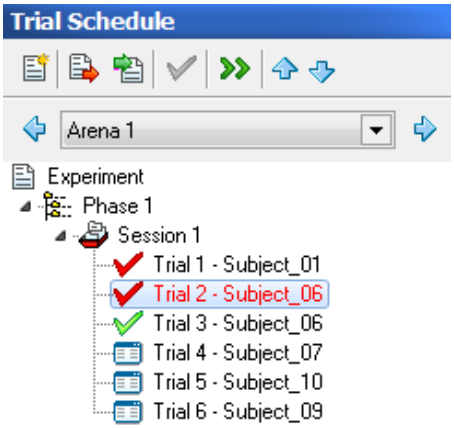
The selected subjects (in the order shown in the table) will be automatically assigned in sequence to a new trial for each arena defined. For example, if 8 subjects are selected (Subject_01 to Subject_08) and distributed along the Session 1 of 4 arenas, the following trials are created:



Trial status



Trial 4 - Subject_07	A trial marked with icon is a programmed trial that remains to be done.
Trial 3 - Subject_06	A trial marked with the icon is the trial selected for the next data acquisition.
Trial 1 - Subject_01	A trial marked with the icon is a trial already used for data acquisition.
Trial 2 - Subject_06	A highlighted trial is a trial selected for the view of the related data in the runtime panel shown in the data acquisition section.

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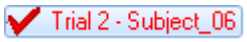
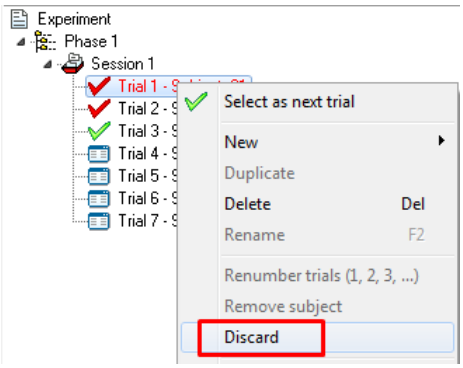
Discarding a trial

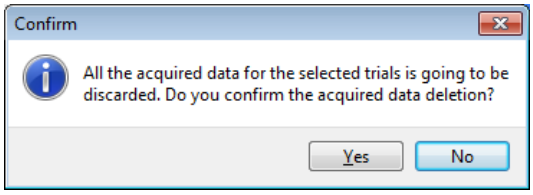
The data registered in a trial can be discarded to allow the selection of the trial for a new data acquisition process. Follow the next steps:

1. Select the trial to discard.
   

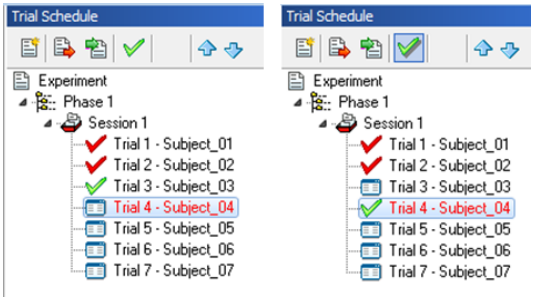
2. Right-click on the trial and select the **Discard** option in the menu.



3. Confirm the previously acquired data deletion.



4. Click on the green mark to select again the trial for data acquisition.



A trial can be discarded under certain conditions, depending on whether there is a synchronized video, is in queue for analysis, etc.…

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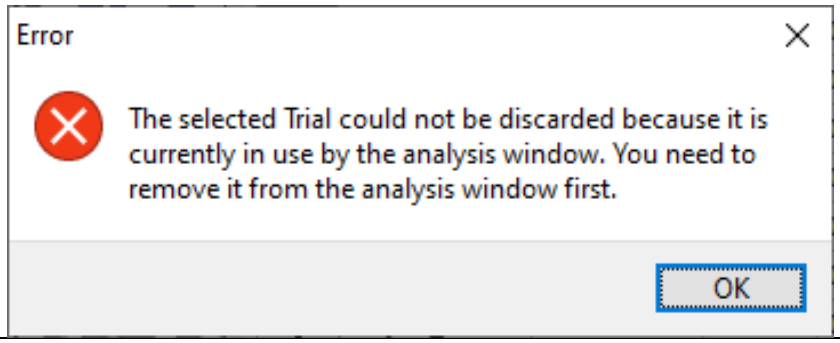
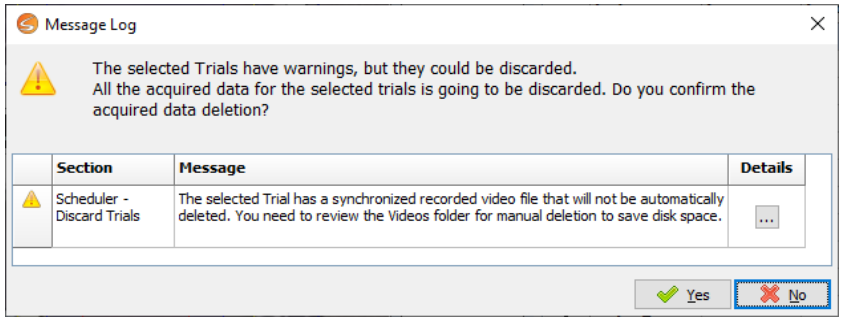
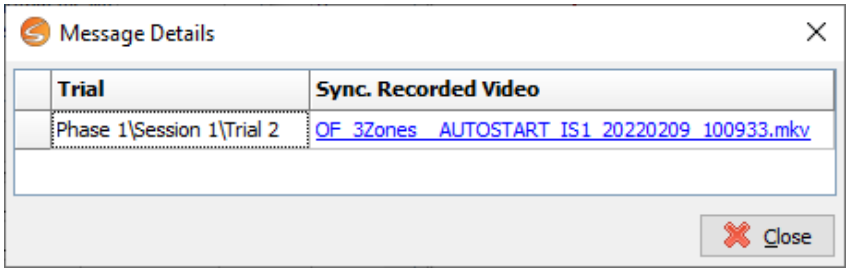
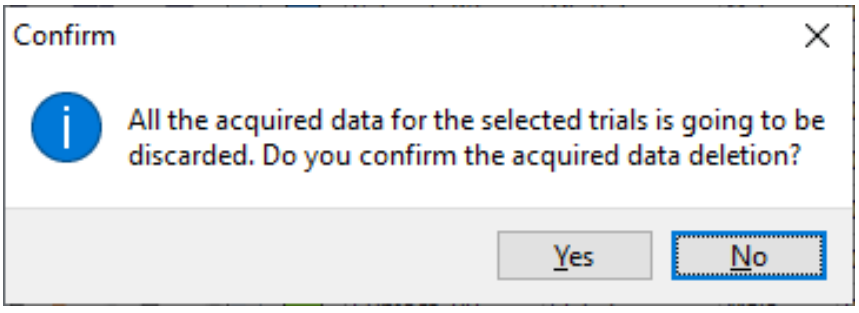
Trial in queue for the analysis **ERROR MESSAGE**: The trial cannot be discarded	
The trial is associated with a synchronized video **WARNING MESSAGE**: The trial can still be discarded	 Clicking on ... the details of the video are shown  Clicking on the name of the video will open the containing folder. Clicking on **Yes** will discard the trial (not the video). Clicking on **No** will abort the discard.
The trial has been acquired but it is not the analysis queue and there is no synchronized video **CONFIRMATION MESSAGE**: The trial can be deleted	A simple confirmation message is displayed:  Clicking on **Yes** will discard the trial (not the video). Clicking on **No** will abort the discard.

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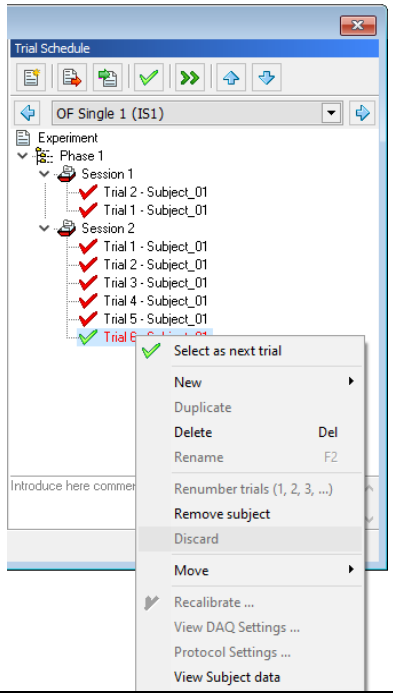
The trial has not been acquired	The **Discard** option is not available in the menu: 

	When trying to discard more trials at the same time, take in count that a combination of error, warning and confirmation messages may be shown depending on the different situations.

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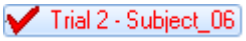
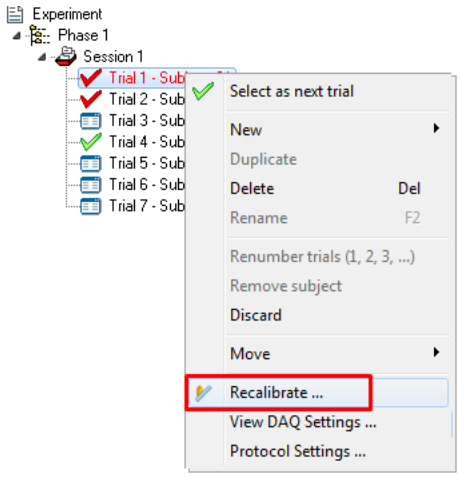
Recalibrating an acquired trial

The calibration used during the data acquisition of a trial is stored with it and used during analysis. It is possible to change the calibration of registered tracks in case the calibration used during data acquisition was incorrect. However, it is recommended, if possible, to discard and acquire the trial again as some detection processes (like TriWise) use calibration to provide more reliable tracking.

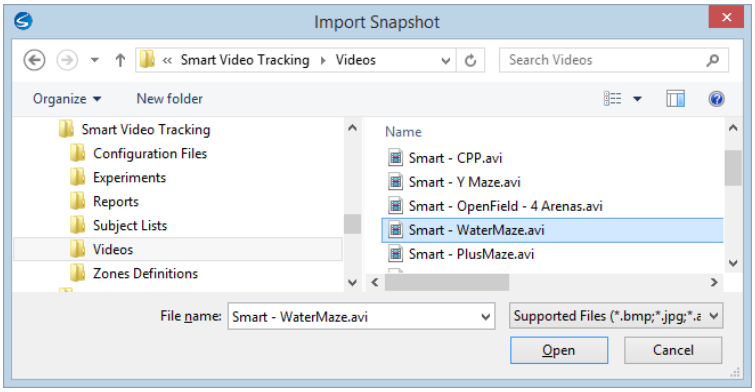
To recalibrate an acquired trial, follow the next steps:

1. Select the trial to recalibrate.
   

2. Right-click in the trial and select the **Recalibrate …** option in the menu.



3. If the trial has not been stored with a calibration reference image, then the selection of a reference image to perform the calibration will be required, otherwise omit this step. It is possible to import a reference image from an image file (.bmp or .jpg) or from a specific frame of a video file.



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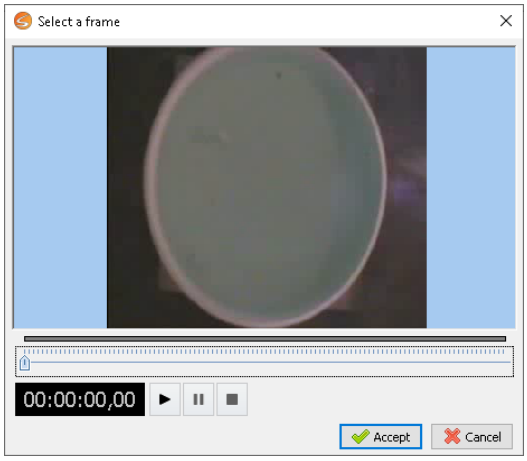
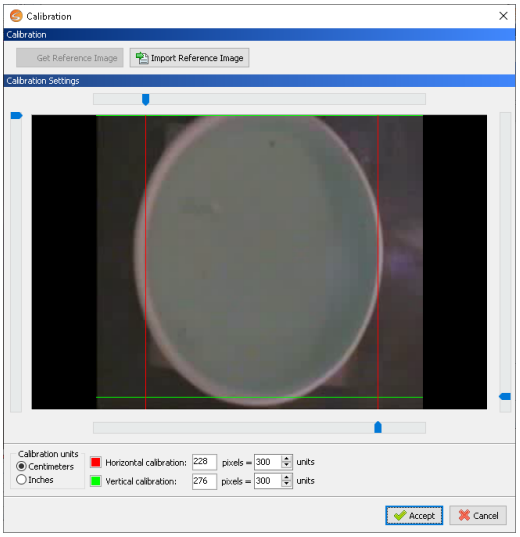
4. In case the reference image is to be imported from a video file, the following dialog allows to specify the frame to be used. To do so, scroll through the video until the desired image is shown and click on the **Accept** button.



5. Once a reference image for calibration is available, the calibration dialog is shown (see <u>chapter 7.1 - Live image source calibration</u> and <u>chapter 7.1 - Video file image calibration</u>)



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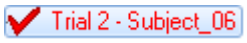
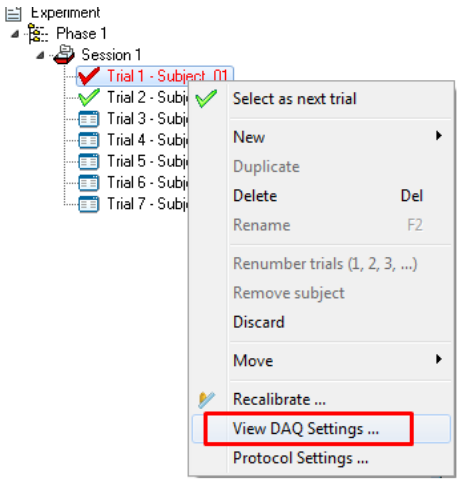
Viewing the settings used during DAQ

Once a trial has been acquired, it is possible to view all the settings that were used during data acquisition. Follow the next steps:

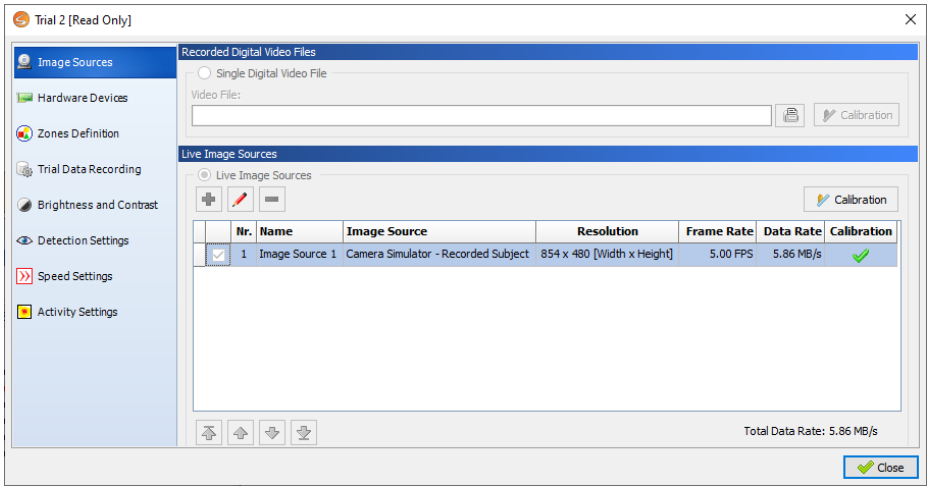
1. Select the trial.



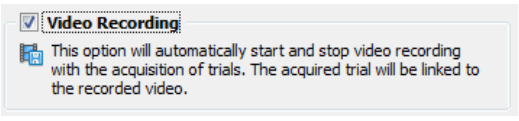
2. Right-click in the trial and select the **View DAQ Settings …** option in the menu.



3. A panel will be shown to review the settings used during DAQ of the selected trial.



This panel provides a view of the settings indicated on the left part of the panel. It also provides the path of the synchronized video file, if the automatic recording has been selected (see chapter 16.2 - RECORDING SETTINGS).



The user cannot modify the settings from this viewer; this window is Read Only, and it is just for a fast visualization/review of the settings associated to the trial.

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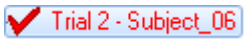
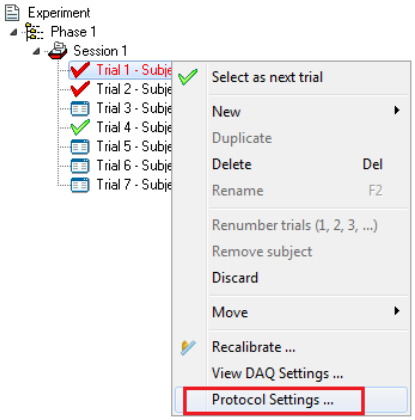
Viewing the protocol settings

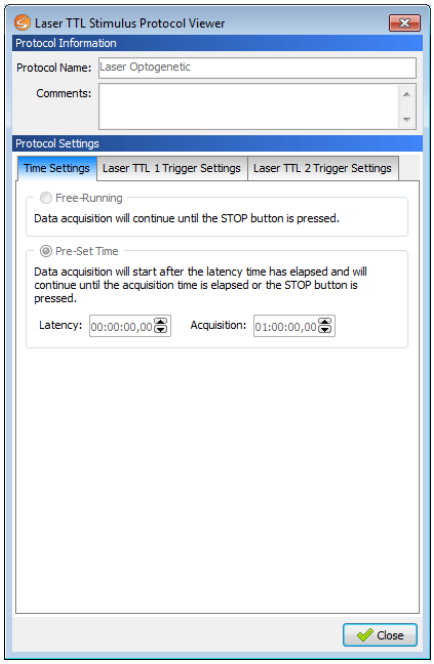
Once a trial has been acquired, it is also possible to view the protocol configurations used during the trial data acquisition. Follow the next steps:

1. Select the trial of which you would like to see its settings.
   

2. Right-click in the trial and select the **Protocol Settings …** option in the menu.



3. A panel will be shown to review the settings used in the protocol associated to the trial. The user cannot modify the settings from this panel, but it is useful to have a fast visualization/review of the settings associated to the registered trial.



	The Protocol Settings…option is only active in the dropdown menu if a session has been registered.

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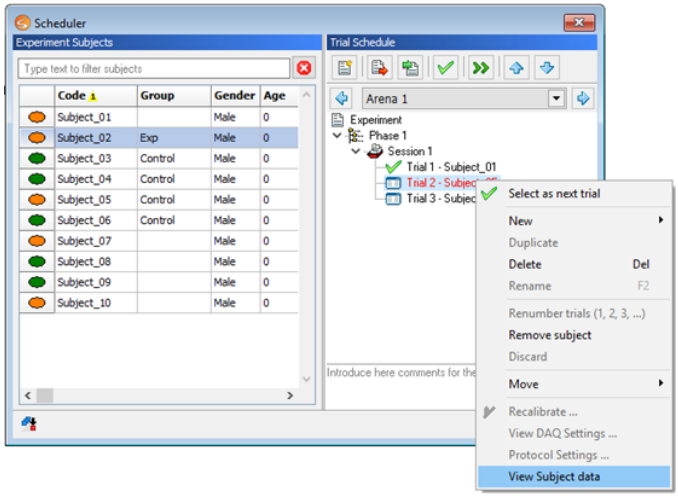
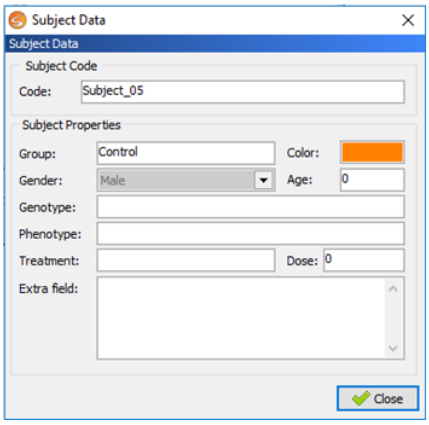
Viewing subject data

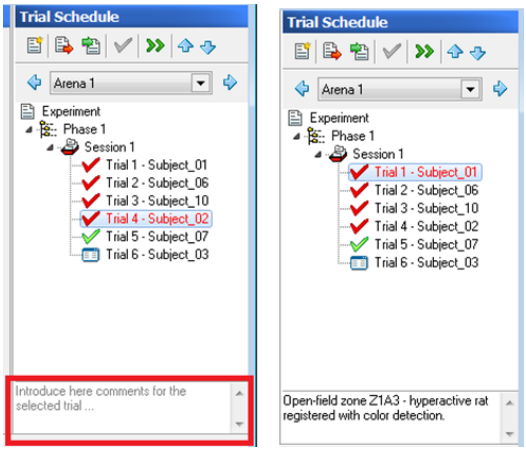
At any moment, it is possible to access the Subject data by using the View Subject data option of the menu.





Trial comments

The Trial Schedule section displays a new section in which the user can write comments about the corresponding trial. The comment can be added, before or after the data acquisition process.



The comments can be retrieved from the data reports generated in Analysis.

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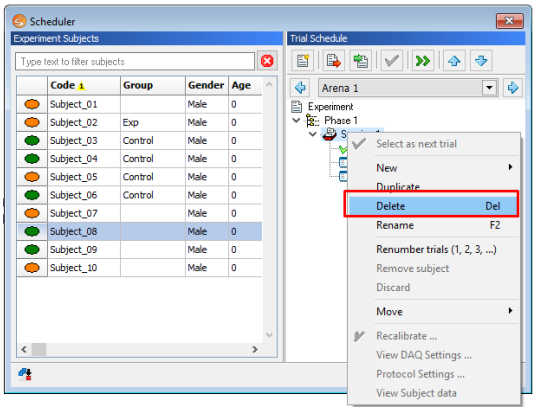
15.3. MANAGING SESSIONS

SMARTIO MA	Although each arena will have its own trials, all arenas will share the same **Phases** and **Sessions**. This means that adding, renaming or deleting **Phases** or **Sessions** affects all arenas, but adding, editing or deleting trials only affects the trials associated to the currently selected arena.

Deleting a session

To delete a session already defined click on the session's node with the right button of the mouse and select the **Delete** option. Then confirm the deletion message.

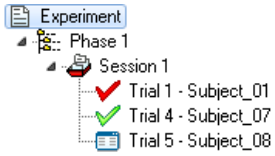


Sessions can be also deleted quickly by using the [DEL] key.

	If a session is deleted, the data acquired within the belonging trials will be lost. If the experimental file was saved that information could not be recovered later unless a backup file was previously saved. If a session was unintentionally deleted, DO NOT SAVE the experimental file. Instead, close it and reopen it again to recover the information. Other changes made since the last saving will be lost.
	A session can only be deleted if all the belonging trials can be removed. Please refer to <u>chapter 15.2 - Deleting a trial</u> for more details on when trials can be deleted.

Renumbering the trials of a session

If some trials are deleted, the numbering sequence of the resting trials within the session can be broken.



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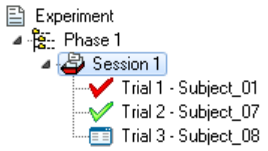
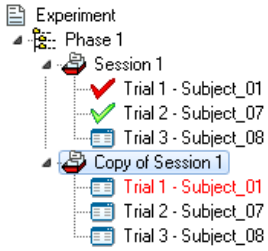
In this example, trials 2 and 3 were deleted so the sequence was broken.

To renumber the trials of the session (to be 1, 2, 3, etc. again), right click with the mouse over the session to be renumbered and select the **Renumber trials** option.



Duplicating a session

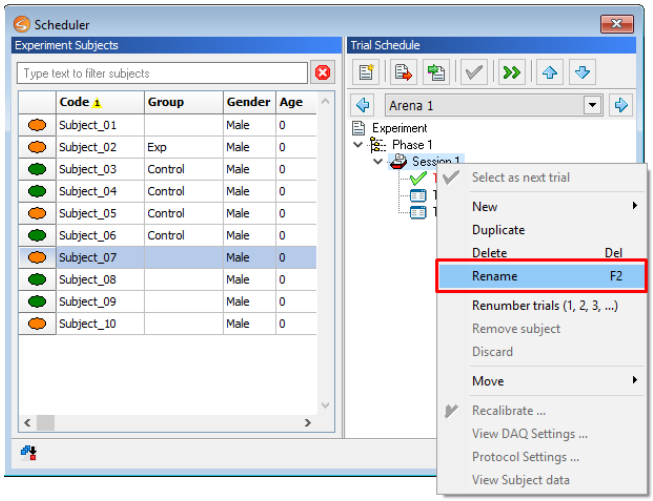
Sessions and their relative trials can be easily duplicated. To do so, right click with the mouse over the session's node and select the **Duplicate** option.



A new session called "Copy of Session X" is automatically inserted at the end of the phase including the same trials and subjects.

Renaming a session

To rename a session right click with the mouse over the session's node and select the **Rename option**. The [F2] key can be also used if the session's node is already selected.



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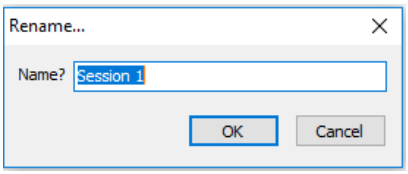
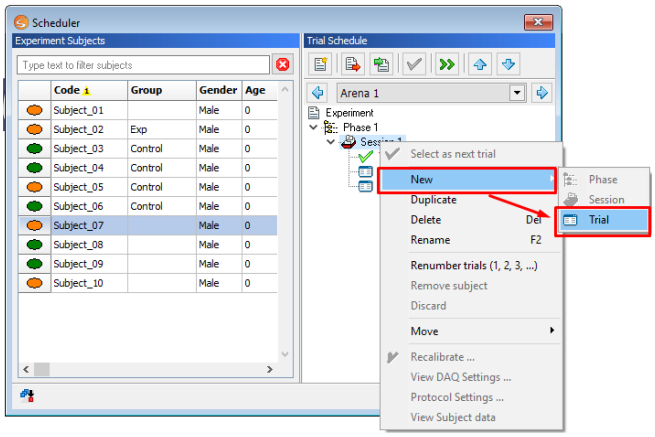
Enter the new name of the session and click on the **Ok** button.



Inserting an empty trial to a session

A new empty trial can be inserted at the end of a session by clicking the session's node with the right button of the mouse and selecting the **New – Trial** option.



Moving the sessions within a phase

1. Select the session.
2. Click with the right button of the mouse over the session's node and select the **Move** option. Then select any of the movement options available (Top, Up, Down or Bottom).

The up/down arrow buttons in the **Trial schedule**'s toolbar also facilitates the task of moving the selected trials up and down.

When sessions are moved within the phase, the session names are kept. If a session number is being used to keep the sequence, the session name may be manually renamed to accommodate for moved sessions within the phase.

Please note that the sessions cannot be moved out of the phase to which they belong.

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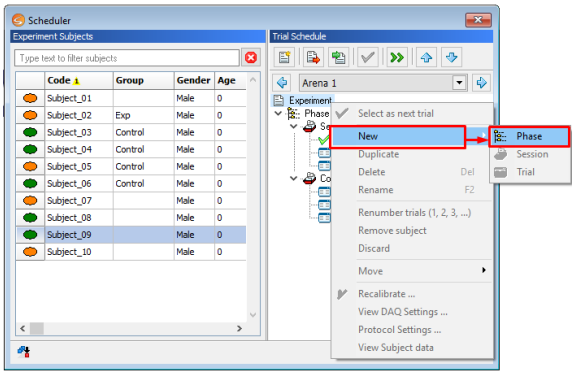
15.4. MANAGING PHASES

SMARTIO MA	Although each arena will have its own trials, all arenas will share the same **Phases** and **Sessions**. This means that adding, renaming or deleting **Phases** or **Sessions** affects all arenas, but adding, editing or deleting trials only affects the trials associated to the currently selected arena.

Inserting an empty phase to the experiment

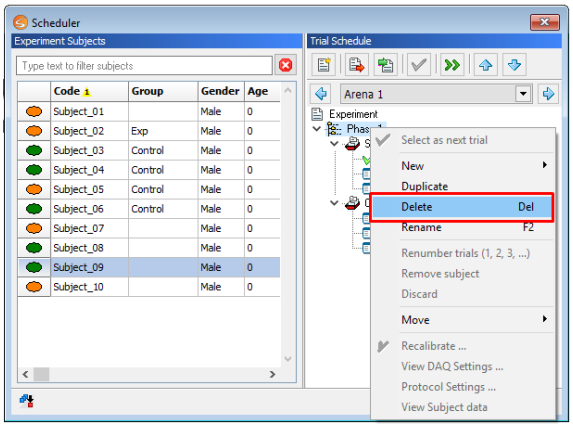
A new empty phase can be inserted at the end of the experimental schedule by clicking the Experiment's node with the right button of the mouse and selecting the **New – Phase** option.



Please refer to the previous chapters for more details on how to insert sessions and trials respectively into the recently created phase.

Deleting a phase

In order to delete a phase already defined right click over the phase's node with the mouse and select the **Delete** option. Then confirm the deletion message.



Phases can be also deleted quickly by using the [DEL] key.

	If a phase is deleted, the data acquired within the belonging sessions and trials will be lost as well. If the experimental file was saved that information could not be recovered later unless a backup file was previously saved. If a phase was unintentionally deleted, DO NOT SAVE the experimental file. Instead, close it and reopen again to recover the information. Other changes done since the last saving will be lost.
	A phase can be deleted only if all the belonging sessions and trials can be removed. Please refer to chapter 15.2 - Deleting a trial and chapter 15.2 - Deleting a session for more details on when trials and sessions can be deleted.

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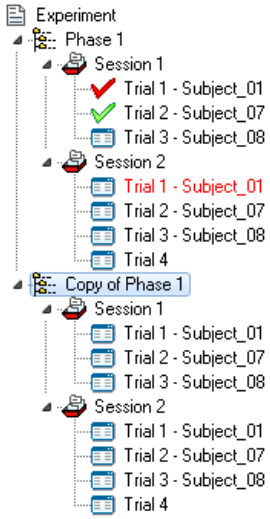
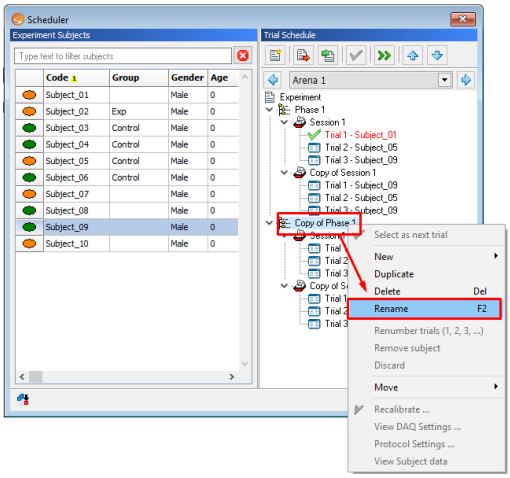
Duplicating a phase

Phases and their relative sessions and trials can be easily duplicated. To do so, right click over the phase's node and select the **Duplicate** option.

A new phase called "Copy of Phase X" is automatically inserted at the end of the experiment including the same sessions, trials and subjects.

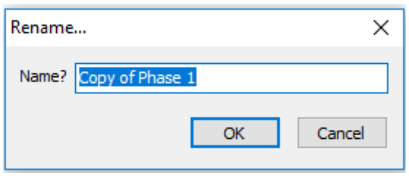


Renaming a phase

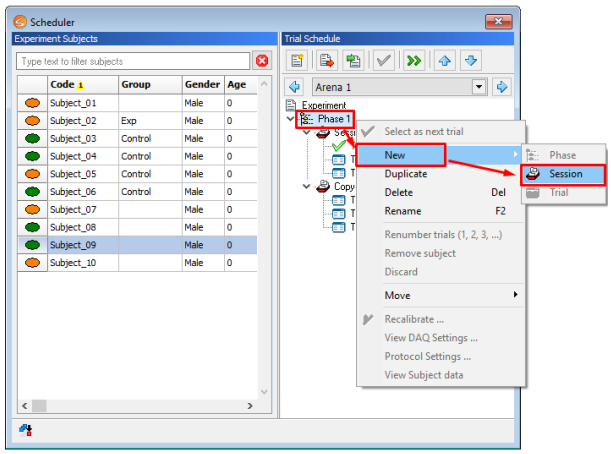


To rename a phase right click with the mouse over the phase's node and select the **Rename** option. The [F2] key can be also used if the phase's node is already selected.

Enter the new name of the phase and press the Ok button.



Inserting an empty session to a phase



A new empty session can be inserted at the end of a phase by clicking the phase's node with the right button of the mouse and selecting the **New – Session** option.

Please refer to the previous chapters for more details on how to insert new trials in a session.

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Moving the phases within the experiment

1. Select the phase.
2. Click with the right button of the mouse over the phase's node and select the **Move** option. Then select any of the movement options available (Top, Up, Down or Bottom).

The up/down arrow buttons in the **Trial schedule**'s toolbar also facilitates the task of moving the selected trials up and down.

When phases are moved within the experiment, the phase names are kept. If the phase number is being used to keep the sequence, the phase name can manually be renamed.

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15.5. EXPORTING AND IMPORTING THE SCHEDULE

	The experiment schedule (only phases and sessions but not trials nor subjects associated) can be exported and stored into external files. This operation is very useful if you want to keep a backup of the work done or if you want to share it within a different experimental file.

To export the experiment schedule:

1. Click on the **Export** button located in the **Trial Schedule**'s toolbar of the panel.
   
2. Enter the name of the exported scheduler file and click on the **Save** button.

Exported scheduler files are stored by default within the "Subject list" folder configured and with the extension *.smeps (SMARTIO Schedule). Please refer to chapter 16.1 - PATH SETTINGS for more details on how to configure the default destination folders.

Exported scheduler files (only phases and sessions but not trials nor subjects associated) can be imported later into the same experimental file or into a different experimental file. To import a previously exported experimental schedule:

Open the experimental file in which the schedule is to be imported.

1. Access the **Scheduler** manager.
2. Press the **Import schedule** button.
   
3. Locate the folder and file in which the scheduler file is stored. Then click on the **Open** button.
4. Accept the confirmation message if you want to delete all the existing phases, sessions and trials (this will result in losing all the acquired data thus far).

The current schedule is automatically applied to the experiment, removing all the existing phases, sessions and trials. However, the experimental subject list is kept.

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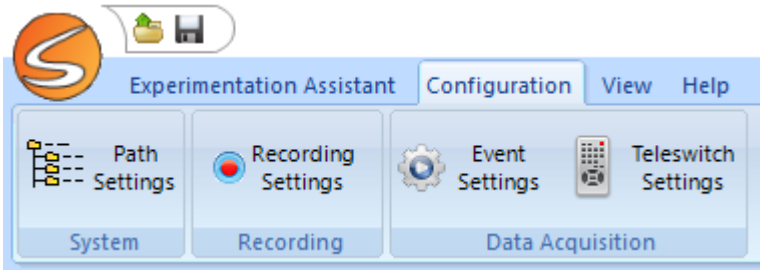
16. ADDITIONAL CONFIGURATION

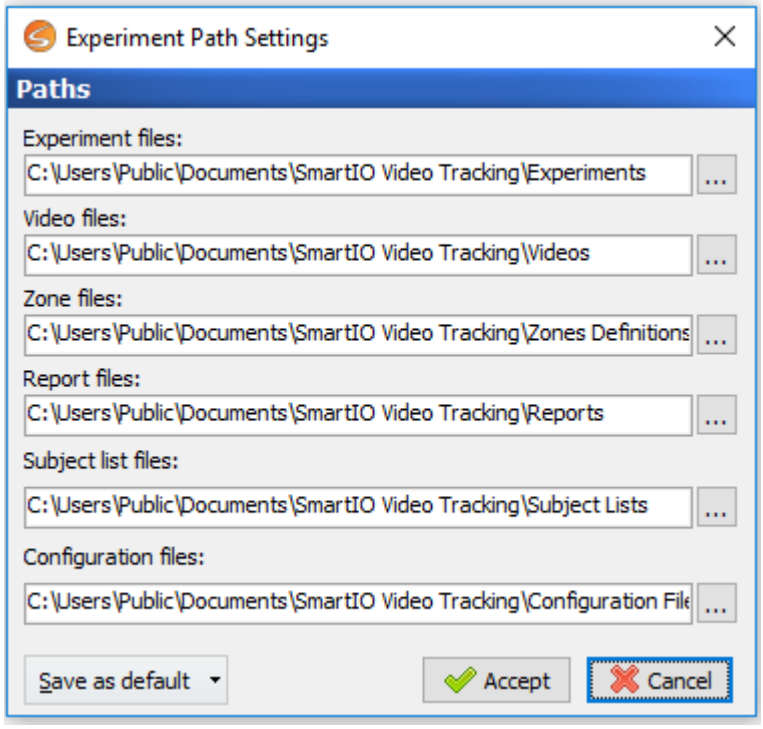
The **Configuration** menu of the SMARTIO main window allows to set up different additional tools.



16.1. PATH SETTINGS

By default, SMARTIO automatically stores the generated files in the Documents folder of the user. The default paths can be easily configured through the Path Settings panel. To do so:

1. Click on the **Path Settings** button.



2. Press the ... button associated to each of the configurable folders.
3. Locate and select the desired folder.
4. Click on the **Accept** button.

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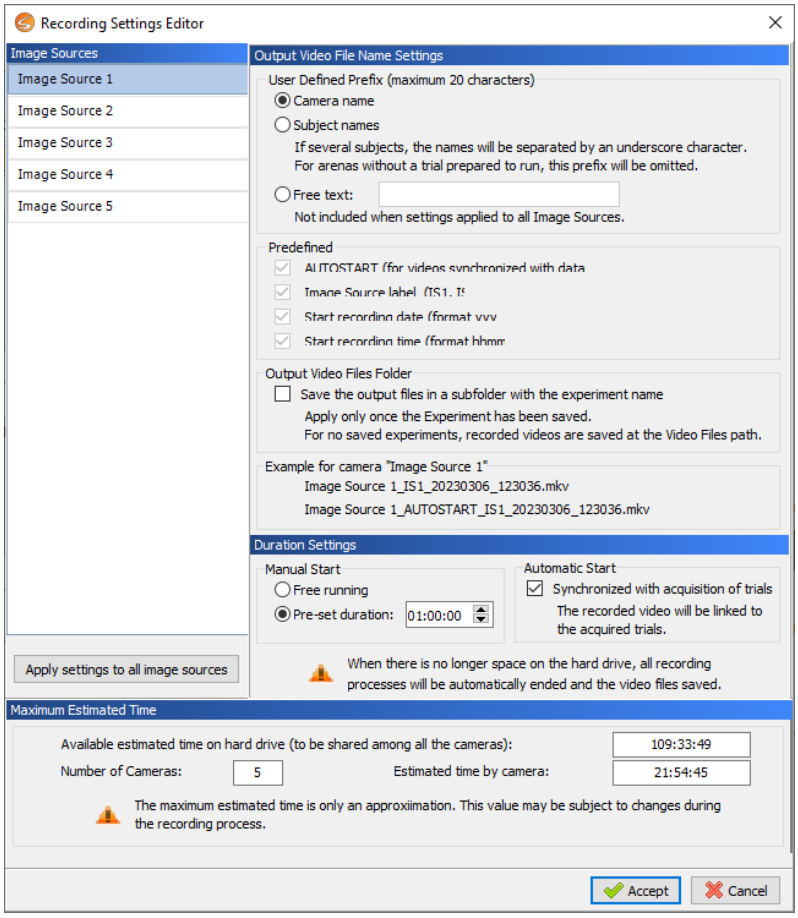
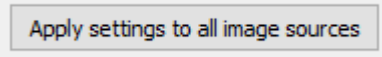
16.2. RECORDING SETTINGS

This window allows the configuration of the video recording settings.



For each image source, settings about the output video file name and duration can be defined. The system will then automatically calculate the maximum time of recording available given the free space on the hard disk.

If more than one camera is present, all these settings may be defined camera per camera or defined once and then applied to all the cameras clicking on the button **Apply settings to all image sources**



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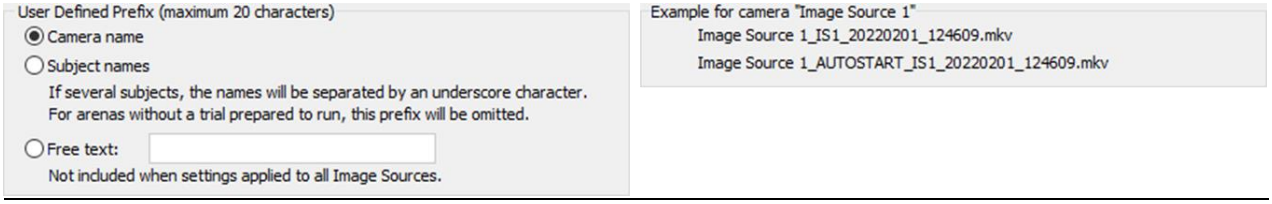
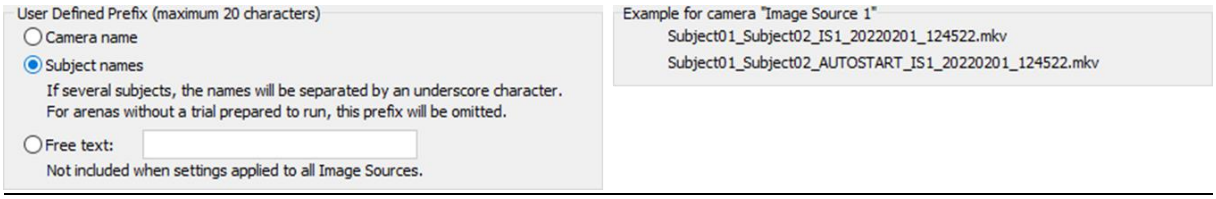
Output Video File Name Settings

The video file name can be defined by

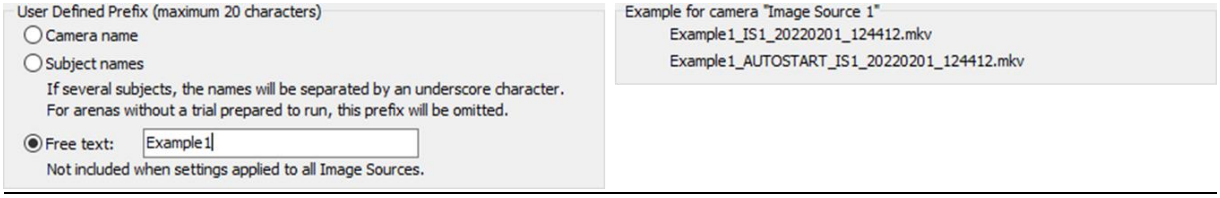
- Camera name: the resulting video will have as a prefix the name of the camera the user defined in the **Device** menu (see chapter 7.1 - Live image source)



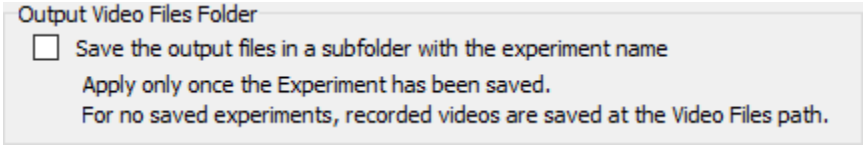
- Subject names: the resulting video will have as a prefix the name of the subject running in the trial (see chapter 14 - SUBJECT DATABASE and chapter 15 - SCHEDULER)



- Free text: the resulting video will have as a prefix a free text introduced by the user in the blank field



The user may also choose to save the video files in a subfolder with the experiment name created under the folder defined in **Path Settings** (see chapter 16.1 - PATH SETTINGS)



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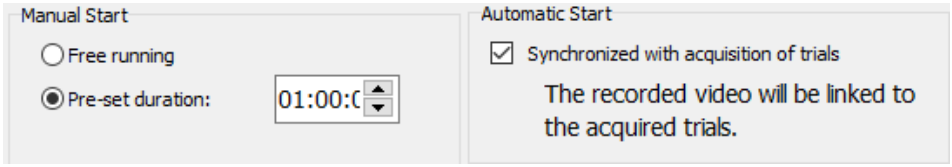
Duration Settings

The video can be manually started or set with an automatic start.



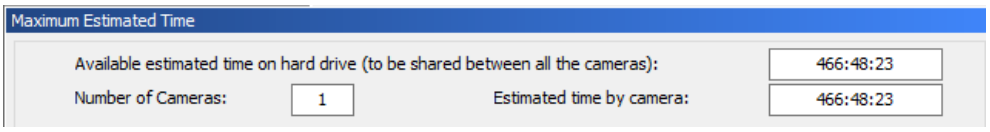
- Manual Start:
    - Free running: the user starts and stops the video manually using the  button (see chapter 8.1 - LIVE IMAGE SOURCE PLAYER PANEL).
    - Preset Duration: the user starts the video manually using the  button (see chapter 8.1 - LIVE IMAGE SOURCE PLAYER PANEL). The recording stops after reaching the user-defined duration.

- Automatic Start: the video is synchronized with the acquisition of the trial. This way, it will start with the start of the data acquisition and stop when data acquisition stops. Moreover, the video file is linked to the acquired trial and its path will be shown in the DAQ Settings of the trial itself.

Maximum Estimated Time

Based on the number and settings of the image sources defined, the system, the remaining space on the local hard disk, SMARTIO will automatically calculate an estimation of the time that each camera can record:

- Available estimated time on hard drive (to be shared among all the cameras): is the total time of recording available on the hard disk
- Number of Cameras: number of cameras the user defined to record videos
- Estimated time by camera: is the total time available divided by the number of cameras and represents the maximum amount of time each camera can record



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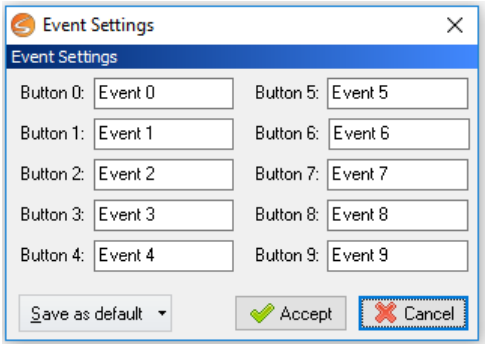
16.3. EVENT MARKER SETTINGS

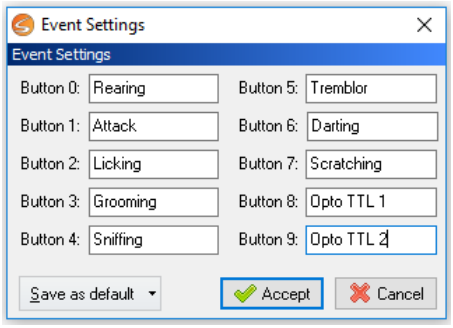
The event marker is an ethological keyboard that allows the manual recording of specific behaviors visualized and recognized by the user. Events are marked by a keystroke on the keyboard. The first step in using the event marker is to define which code will be associated to each event to be recorded. The **Event Settings** panel is available from the **Configuration menu**.



If no code has been defined, SMARTIO will take "Event 0" (zero) as the default for naming the first event, "Event 1" for the second and so on. In this case, this text will be shown on the screen whenever the event file is hard copied.

It is possible to define an associated text (name) related to each one of the events under study. Setting up the Event Marker is as simple as naming the events of interest. This tool can be used for the manual scoring of complex behavioral events or for triggering a stimulation from a third-party laser optogenetics stimulator.

An example is given below.



	A maximum of 10 different events can be defined.

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16.4. TELESWITCH SETTINGS

The Teleswitch unit allows for remote control using the radiofrequency technique for controlling the start and stop of the session without the computer. It is especially useful when the experimental protocol requires the track acquisition process to start at the same time the subject is placed into the experimental area. The remote start of the session in this context can be then achieved using the Teleswitch unit included in the SMARTIO package.

Connecting the Teleswitch

The Teleswitch unit is composed of a remote-control unit and a small USB wireless adapter device. Before connecting the Teleswitch unit to the computer, make sure to install the battery (included) in the back of the unit. Then remove the USB receiver from the back of the unit and plug it into a free USB 2.0 port in your computer.

The recommended distance between the Teleswitch unit and the USB receiver plugged into the computer is 10 meters without any obstacle. An additional USB extension cable is provided to facilitate installing the USB receiver in a position in which the visibility is improved.

If needed, plug one side of the USB extension cable into the free USB port in your computer and the USB receiver into the other side of the cable. Then put the USB receiver in a stable position without any obstacle between it and the Teleswitch unit.

Wait for Microsoft® Windows® to automatically detect and install the new device before configuring it.

⚠	Old serial Teleswitch units provided with SMART v2.5 are also compatible with SMART v3.0 and SMARTIO. Please refer to the SMART v2.5 User's Manual for more details on how to connect the device to your computer.

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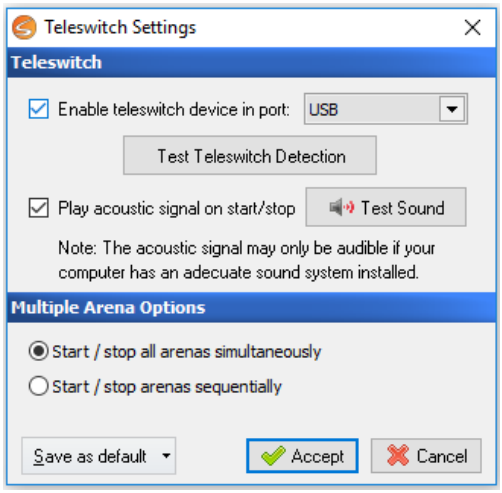
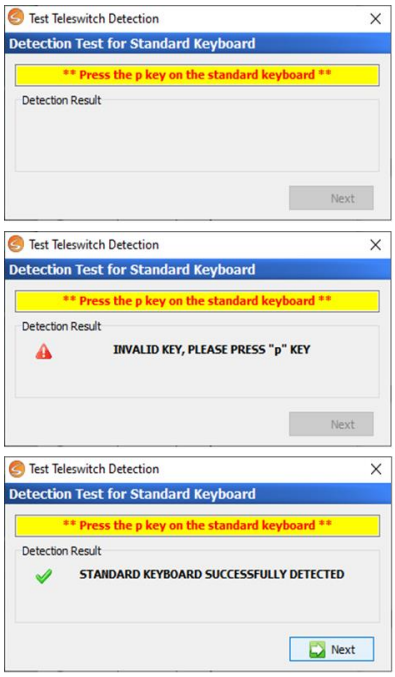
Configuring the Teleswitch

1. Press the **Teleswitch Settings** button.



2. Check the box **Enable Teleswitch device in port** to enable the Teleswitch device.
3. Select the communication port to which your Teleswitch device is connected. By default, a USB port is suggested but old models can be also connected to any of the COM ports available on the computer.
4. Click button Test Teleswitch Detection to open the assistant that allows testing the Teleswitch detection and registering the device. The assistant consists of two steps:

   - The first step requires to press the key for letter “p” at the standard keyboard.



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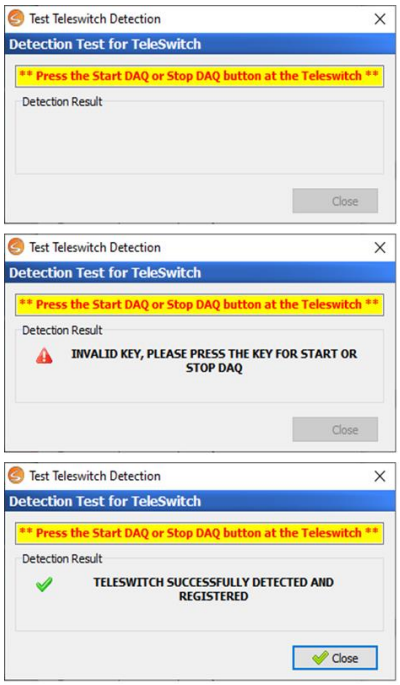
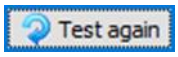
- The second step requests to press the key defined for Start DAQ or Stop DAQ at the Teleswitch.



	The identifier of the standard keyboard must be different from the identifier of the Teleswitch; otherwise, an error message is shown, and the device is not accepted.
	

5. Once a Teleswitch is detected and registered, it is possible to change the device to a different one. To do that, click on the **Test again** button and repeat the previous steps.



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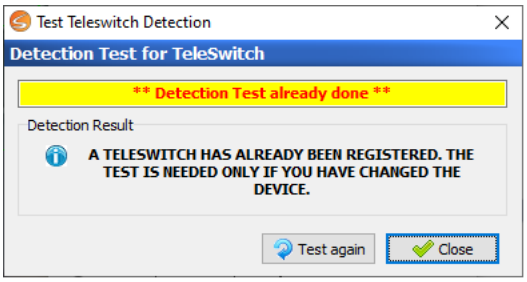

⚠	Execution of this detection test is required if the Teleswitch has been enabled.

6. Check the box **Play acoustic signal on start/stop** to make the computer play an acoustic signal (beep) whenever the trial starts or stops using the Teleswitch unit.
   The **Test Sound** button allows one to test the sound playing before starting any trial.

⚠	This useful tool will help you to make sure that the Teleswitch unit is working fine but may affect the answer of the subjects. Please check this box only if it does not affect within your specific experimental conditions.
	The acoustic signal is played by means of the sound system of your computer so please make sure the sound card and the speakers are properly installed and turned on.

7. To make the Teleswitch settings available for any new experiment, use the option **Save as Default**.

Starting and stopping trials with the Teleswitch

Data acquisition sessions (trials) can be started and stopped using the corresponding buttons of the Teleswitch unit as indicated:

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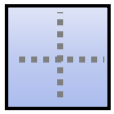
	Please be aware that pressing the “Close Window” key, the close action will be executed over the active window/panel with unpredictable results.
	Please remember that the data acquisition process can only be started if a valid protection key is plugged in or if the trial period has not expired yet.

 **SMARTIO MA**	SMARTIO-MA users are provided with a specific section within the **Teleswitch Settings** panel called **Multiple Arena Options**. The available options allow defining the behavior of SMARTIO when the START and STOP buttons are used during the data acquisition of multiple arena experiments. ▪ **Start / Stop all arenas simultaneously:** Use this option if you want to start or stop all arenas at the same time as the corresponding buttons in the Teleswitch device are pressed. **Start / Stop arenas sequentially:** Use this option if you want to start the arenas starting from the arena 1, then arena 2 and so on. The same applies to stop the arenas (the first arena to be stopped is the arena 1 but, if it is already stopped, then the arena 2 is stopped and so on).

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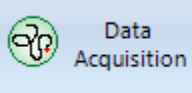
17. DATA ACQUISITION

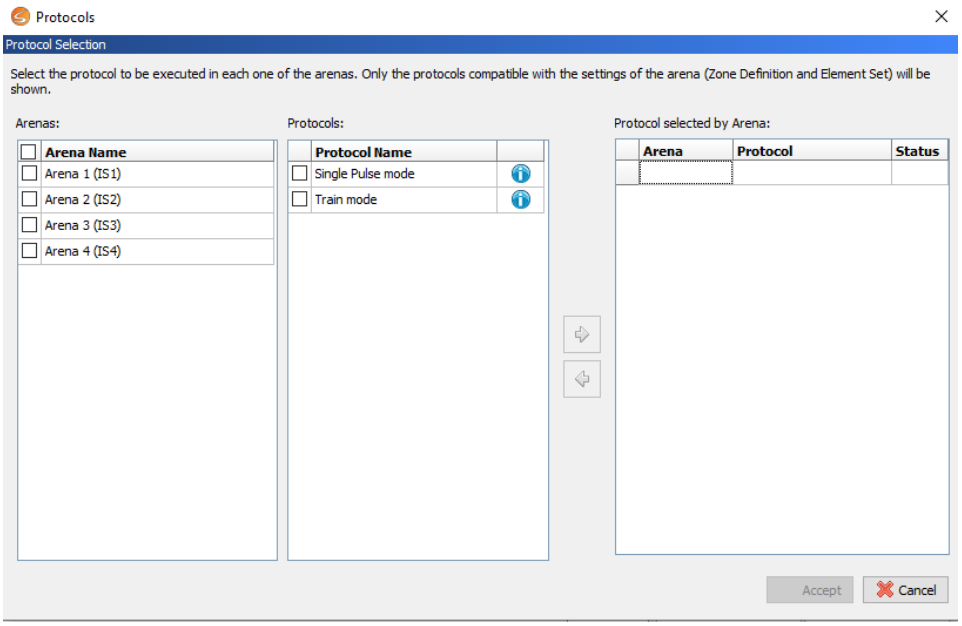


Once the experiment has been configured following the instructions given in the previous chapters, data acquisition mode is available through the **Data Acquisition** button in the **Experimentation Assistant** bar.

⚠	The data acquisition process is closely related to the calibration of the system and the calibration is critical for the further calculations. The first time the data acquisition mode is accessed, SMARTIO checks whether the calibration was done. In case no calibration was done, a warning message is shown preventing you from acquiring data without calibrating the system. See chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration
	Data Acquisition cannot be opened if the Devices panel is open. It is necessary to first close the Devices panel and open the Data Acquisition afterwards.

17.1. ARENA/PROTOCOL ASSOCIATION

Before starting the data acquisition, it is necessary to associate each arena to a protocol.



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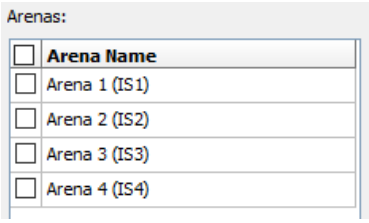
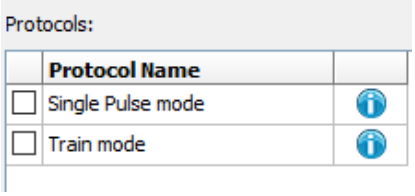
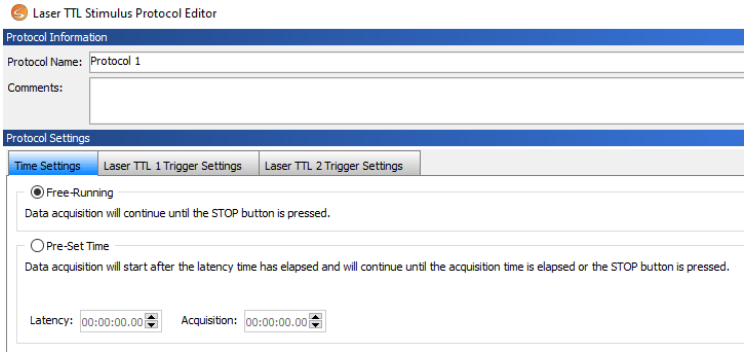
1. Select one or several Arenas in the Arenas section. Use the checkbox at the left of **Arena Name** to select all the Arenas at once.



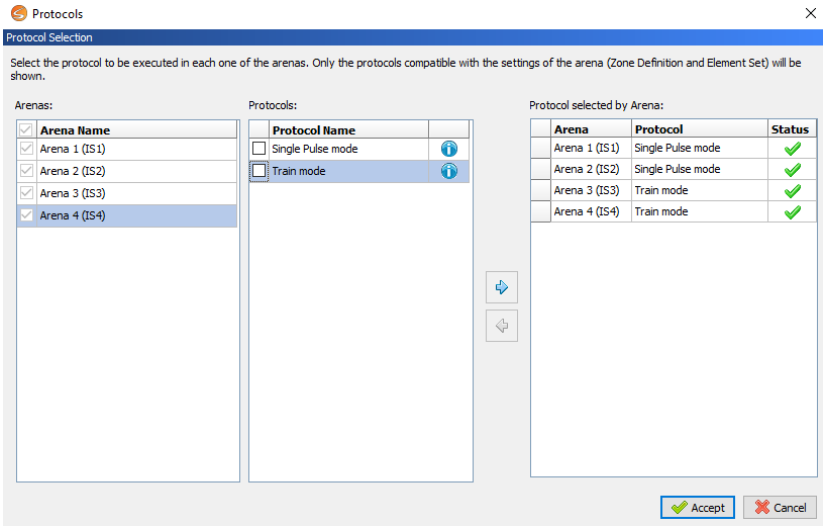
2. Select one Protocol to associate on the **Protocols** section



Click on the information button  to open the Protocol panels for revising the content of a protocol before its selection.



3. Click on the  arrow to complete the association.
4.



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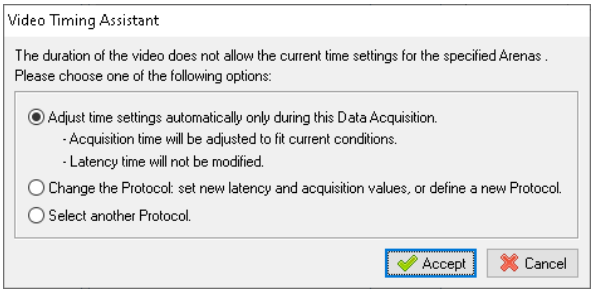
	For data acquisition on a video digital file, SMARTIO checks whether the protocol time settings are compatible with the current selection of the Starting point for each Arena.

The column **Status** will report the compatibility of the association:

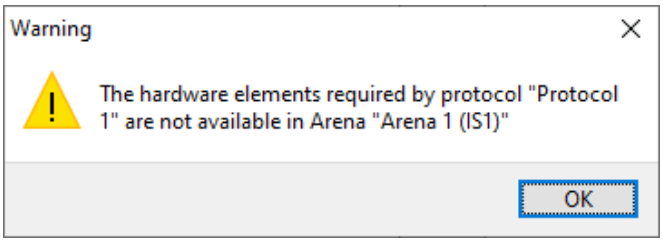
	If the association is compatible, the Status column shows a green check sign.
	If the association is not compatible, the Status column shows a warning sign. The user can click on it to get more information about how to adjust the time settings.



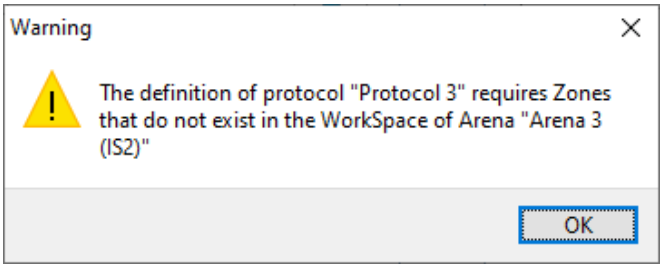
The user can also cancel the association process and change the Starting point for the Arena showing an alarm sign.

SMARTIO will only allow for selection the protocols that are compatible with the selected arenas in terms of Zone Definition and Element Set.

If a protocol that requires a certain number of TTL Elements is associated with an arena that does not have the corresponding number of TTL Elements, the following message will be shown:



If a zone associated to a certain condition to trigger or stop a TTL Element is deleted or renamed, the following message will be shown:



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5. Select an association and click on the LEFT arrow button to undo it.

6. Click on the **Accept** button to enter the data acquisition mode

	The Accept button will only be available when no alarm sign is shown in the Association Status.

The **Data Acquisition** panel is composed by the **Time Control** panel and the **Runtime Viewer** panel

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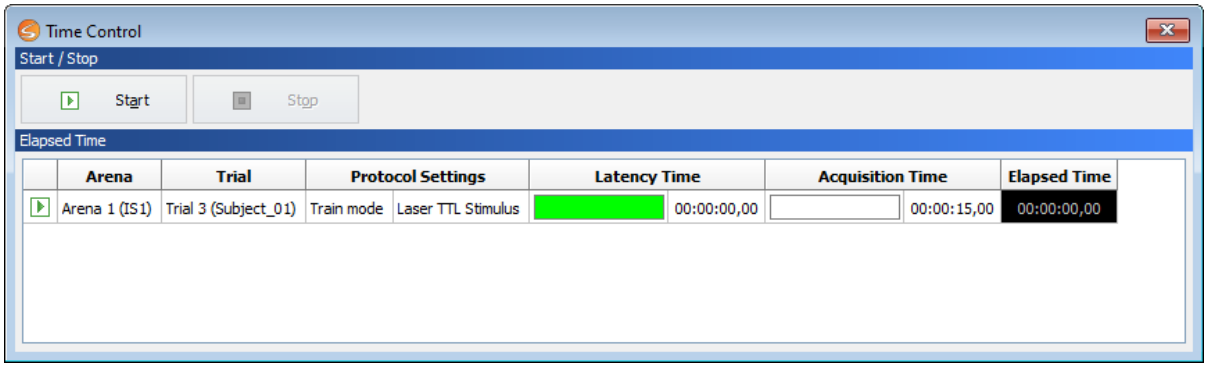
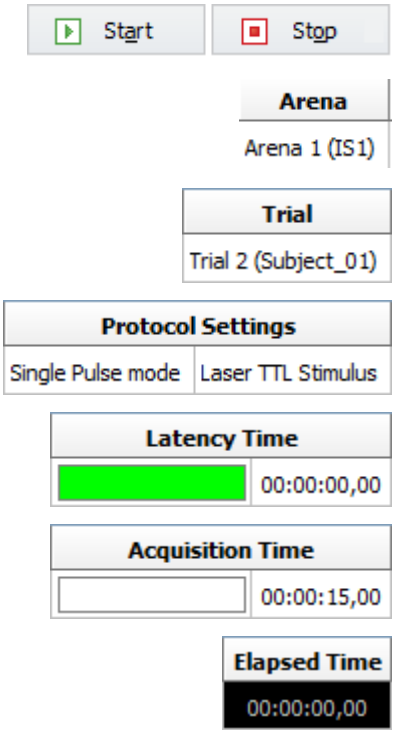
17.2. TIME CONTROL PANEL – START/STOP DATA ACQUISITION



This panel includes:



Start and Stop buttons to control the next trial.

The name of the arena

The trial name (defined in the Schedule panel)

The name and type of protocol

The protocol time settings (defined in the Protocols)

The trial chronometer (HH:MM:SS;00).

Once a trial is finished, either because the user clicks on the **Stop** button (or makes use of the Teleswitch device to stop it) or because the stop time condition is fulfilled, the acquired data is automatically saved. The trial chronometer also stops, and the **Runtime Viewer** panel is no longer refreshing the information, so the last information captured is shown.

If a digital video is used as an image source, the video player also stops reproducing the video.

Finally, the next trial in the **Trial schedule** is automatically selected to be executed.

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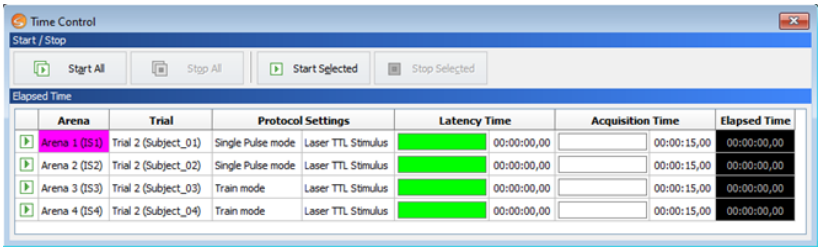
SMARTIO MA

SMARTIO-MA users are provided with specific controls to start and stop arenas either individually or simultaneously.



Within the left column, there are two buttons to individually Start ( ) and Stop ( ) each arena.

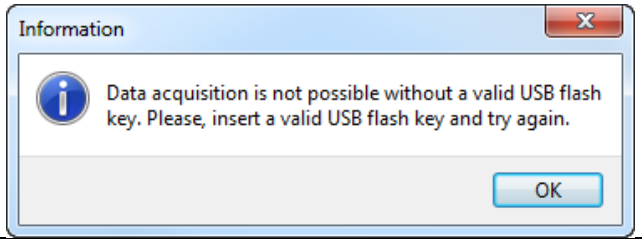
To start or stop multiple arenas simultaneously:

- Click on the **Start All** or **Stop All** button. All arenas prepared for data acquisition will be started, or all arenas acquiring data will be stopped.
- Select the arenas to start or stop in the list using [CTRL] (or in the **Player** using [CTRL] and/or [SHIFT]) and then click on the **Start Selected** or **Stop Selected** button. Selected arenas have a pink background in the Arena cell.

When there is no Trial selected for an Arena, it is shown in the list with blue background, and its Start button is disabled. The same happens if the Arena is not available for DAQ, although in this case, the background is grey.

Running arenas are shown in the **Player** with a green border and name label. Stopped arenas are shown with a pink border and name label.

Data acquisition can only be started if a valid protection key is plugged in or if the trial period has not expired yet. In other case, the following warning message is shown:



Data acquisition cannot be started if the Zones Editor or Protocol sections are active. Close those panels before starting any trial.

SMARTIO will not enable data acquisition unless an animal is detected. If no animal is in the arena, SMARTIO will neither capture data nor count time. The software will keep monitoring the scenario waiting for the animal to be detected. Once SMARTIO detects an animal, it starts the data acquisition.

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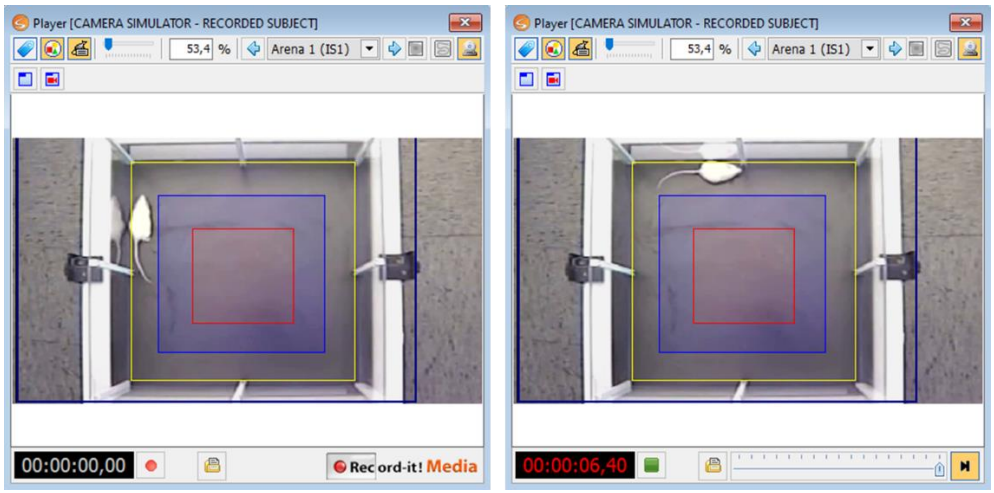
17.3. VIDEO RECORDING AND DATA ACQUISITION

When a live image source is selected, the Player panel provides an embedded module of **Record-IT! Media** for easily recording the image coming from the selected camera.



Video recording and data acquisition can be managed in a different way by the user. Please refer to chapter 16.2 - RECORDING SETTINGS for more details about setting the recording during acquisition and chapter 8 - THE PLAYER PANEL for more details about the player panel options.

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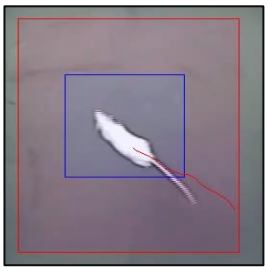
17.4. PLAYER VIEW

During data acquisition, the player provides a view of the experimental arena to visualize the reliability of the tracking process. The tools provided in the player are the same as the ones described in chapter.

- Arena name tag viewer
- Zone definition viewer
- Subject name and coordinate tag viewer.
- Zone opacity tool
- Zoom

Center of mass tracking

The subject track is marked with a red line following the center of mass of the subject under trial.

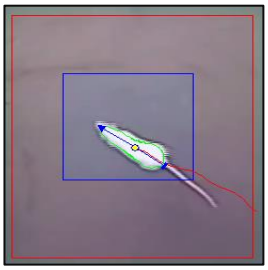


Color tracking

The subject track is marked with a red line following the center of mass of the detected dot of color.

TriWise tracking

The subject track is marked with a red line following the center of mass of the subject. An arrow is shown indicating the direction of the animal body and the position of the head (triangular extremity), the center (circle) and the base tail (short line extremity). A green line is also used to indicate the shape of the dot of pixel detected for each animal and visualized during the Detection test (see chapter 12.3 - TriWise mode detection settings)



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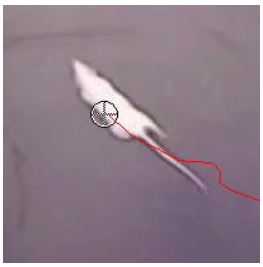
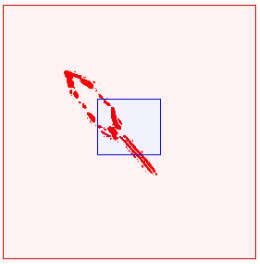
Manual tracking

The pointer becomes a circle to help the user to follow the subject. A red line tracks the movements of the pointer and the video plays while the left button is kept pressed.



Global activity

The subject is shown as the difference between the image in the current frame and the image in the previous frame, numerically materialized in pixel format (red dots).



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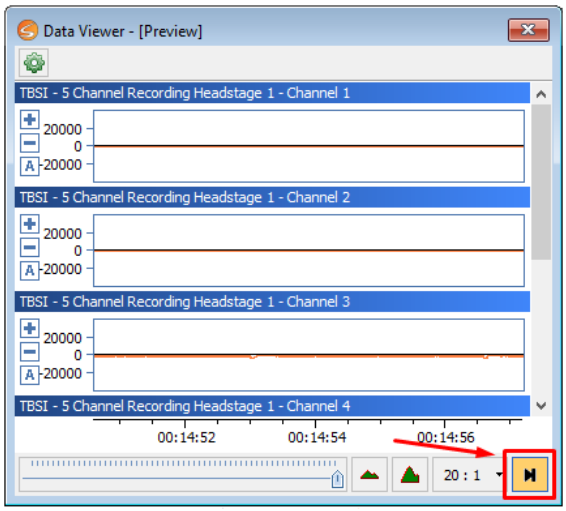
17.5. DATA VIEWER

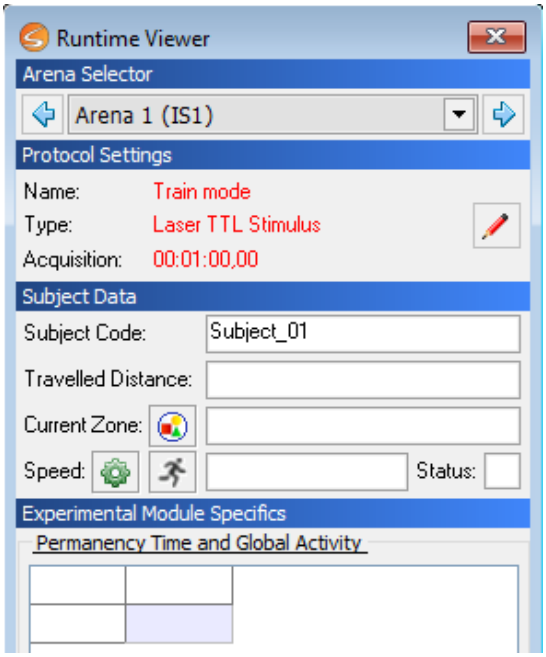
A Data Viewer is shown when SMARTIO is used combined with a third-party laser optogenetic stimulator.

Make sure that the Run button of the Data Viewer is maintained pressed to visualize the current data/event provided by the Viewer.



17.6. THE RUNTIME VIEWER PANEL

This panel shows in real time all the relevant data during the running of the trial and provides additional setting tools.



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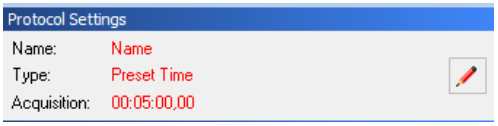
SMARTIO GA	When Global Activity detection is enabled, an extra section will be shown with all the relevant data regarding activity. 
SMARTIO TW	When TriWise detection is enabled, some extra fields will be shown with additional information about rearing, rotations and stretchings. 
SMARTIO MA	An "Arena selector" control is also provided for SMARTIO-MA users within the **Runtime Viewer** panel.  Select the arena (with the selector or clicking directly at the **Player** panel) to review its runtime information.

The panel is divided into 4 sections:

- Protocol Settings
- Subject data
- Trial data (only if GA is enabled)
- Experimental Module Specifics data

Protocol settings

The **Protocol Settings** section provides information about the protocol that has been associated to the trial for the data acquisition.



The following information is displayed:

- **Name**: Name of the protocol
- **Type**: Type of the protocol (Preset Time or laser TTL Stimulus)
- **Acquisition**: total duration of the data acquisition set in the protocol

The protocol selection can be changed without leaving the Data Acquisition section by pressing the Edit button  and changing the Arenas/Protocols associations made in the Protocol panel.

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Subject data

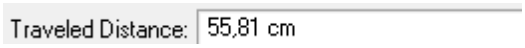
The **Subject Data** section provides information about the subject and the data related to the tracking. The information provided in this section depends on the Detection mode used.

Subject code

The Subject code is the name of the subject assigned to the trial (can be the next or the finished trial selected in the **Schedule** panel).

Traveled distance

The traveled distance is the current distance covered by the subject.

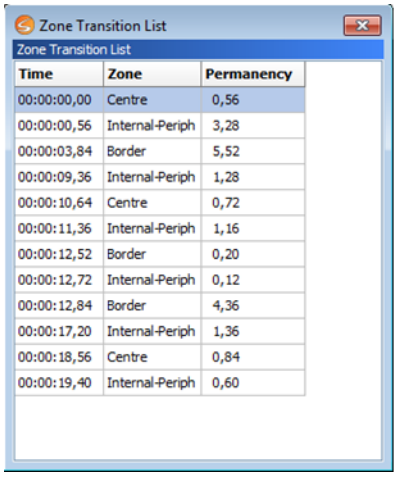


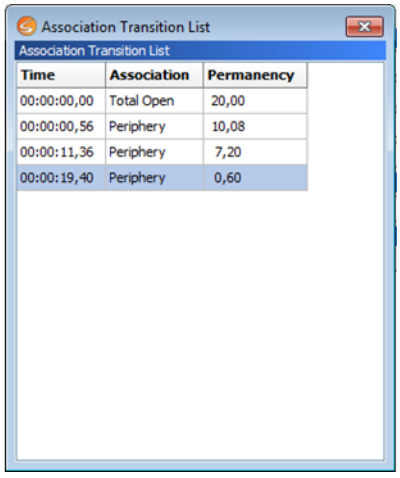
The traveled distance is given in the units selected during the image source calibration process (inch or cm, see chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration). It is only provided when the **Track Detection** option has been marked in the **Trial Data Recording** settings.

Current zone

The current zone displays the name of the zone in which the subject is currently detected (or the name of the last zone occupied in the trial selected in the **Schedule** panel).

The  button shows the **Zone Transition List** and **Association Transition List** panels. These lists are useful tools that log all the zone and association transitions made by the subject during the trial.





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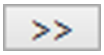
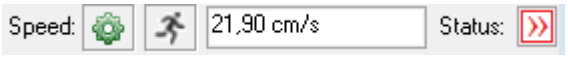
The list is arranged like a table in which each row represents a zone entrance. Each row holds the information of occurrence time, zone/association name and permanence time in seconds. The transitions list is automatically calculated and updated during the execution of the trial and can be reviewed once it has finished.

If a finished trial is being reviewed and a digital video was selected as the image source, a View button is provided to "replay" each transition in the Player panel. Press the Stop button on the Digital Video Control panel to stop the video again. 

SMARTIO MA	An "Arena selector" control is provided for SMARTIO-MA users within each one of the **Transition Lists** panels.  Select the arena (with the selector or clicking directly at the **Player** panel) to review the list of zone/zone association transitions made by the subject specifically in that arena.

Speed and Status

This field shows the speed of the subject currently detected (measured in the units specified during the calibration, cm/s or inch/s, see chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration).



The status field represents the current subject displacement pattern status calculated depending on the speed thresholds adjusted in the **Speed Settings** panel:

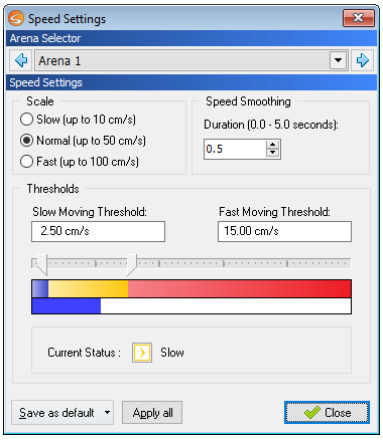
 Represents a RESTING status.

 Represents a SLOW displacement status.

 Represents a FAST displacement status.

Speed settings

The  button leads to the Speed Settings panel for settings the speed thresholds that would define the speed status.



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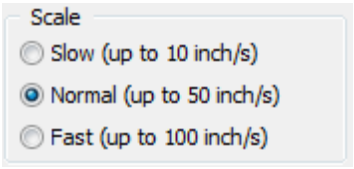
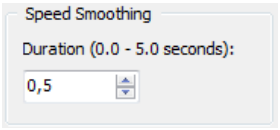
From this panel it is possible to set speed scale, speed smoothing and speed thresholds. The value of each one depends on variables such as the subject size and motor capabilities of the animal strain used:

- **Speed scale:** Allows selecting the maximum speed (in the units used during the calibration process, chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration) the animal can reach.

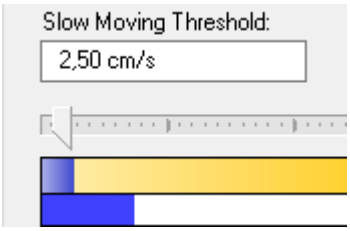


- **Speed Smoothing:** An interval of time (in sec) in which the speed is averaged before determining the punctual speed. This option minimizes the impact that the artifacts in the image can produce on the speed calculations.

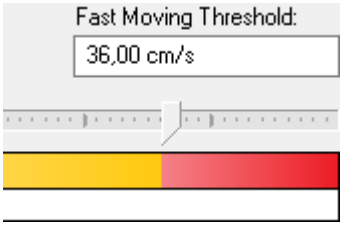


A default value of 0.5 sec is configured. This value is suitable in most cases, but it is possible to adjust it between 0 sec (no smoothing applied; the current speed is exactly the punctual speed) and 5 sec (the punctual speed during the last 5 sec is averaged to calculate the current speed).

- **Speed Thresholds:**
    - Slow moving (resting) threshold: Threshold under which the animal is deemed “immobile”.



    - Fast moving threshold: Threshold above which the animal is deemed to be “moving fast”.



The thresholds can be adjusted by dragging the markers along the horizontal rule. A fine adjustment can be achieved by clicking the rule and using the following combination of keystrokes:

- [LEFT ARROW] / [RIGHT ARROW]: To increase / decrease the “Slow Moving Threshold”.
- [CTRL] + [LEFT ARROW] / [RIGHT ARROW]: To increase / decrease the “Fast Moving Threshold”.
- [SHIFT] + [LEFT ARROW] / [RIGHT ARROW]: To increase / decrease both thresholds at the same time.

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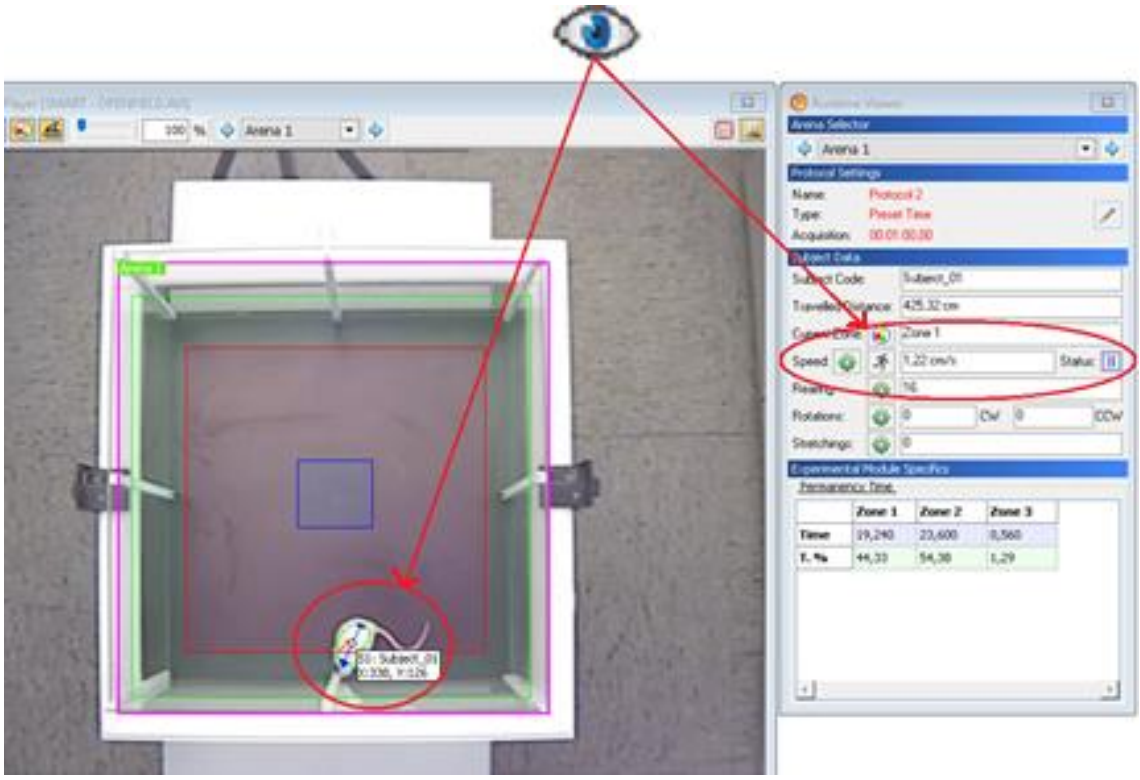
SMARTIO allows changing the values of the thresholds during the tracking as well in order to facilitate the choice of their values depending on the criterion of the user. The values chosen during the detection test and during the acquisition process can be changed afterward during the analysis process for obtaining a new set of data.

⚠	The entire track to which the values of the thresholds have been changed during the tracking process should be reanalyzed again in Analysis mode with the final threshold selections.
	To generate a statistically relevant data set, it is recommended to use the same threshold for all the subjects within the experiment to compare the data between the different experimental groups.

Please find the following recommendations for setting the speed thresholds properly:

- The speed thresholds must be determined by the user using a pilot subject having the same characteristics as the ones that will be used during the experiment.
- Use the detection test mode or a test trial with a pilot animal in the same conditions in which the experiment will be performed.
- Look at the behavior of the animal. At the same time, look at the range of current speed displayed by SMARTIO each time you consider the animal as immobile (resting), moving slow or moving fast.

In the example below, the mouse was considered as “resting” when the current speed was always below 10 inch/s. The mouse was considered “moving slowly” when the range of the current speed was equal or greater than 10 and lower than 35 inch/s. Finally, the mouse was considered “moving fast” when the speed was equal or greater than 35 inch/s.



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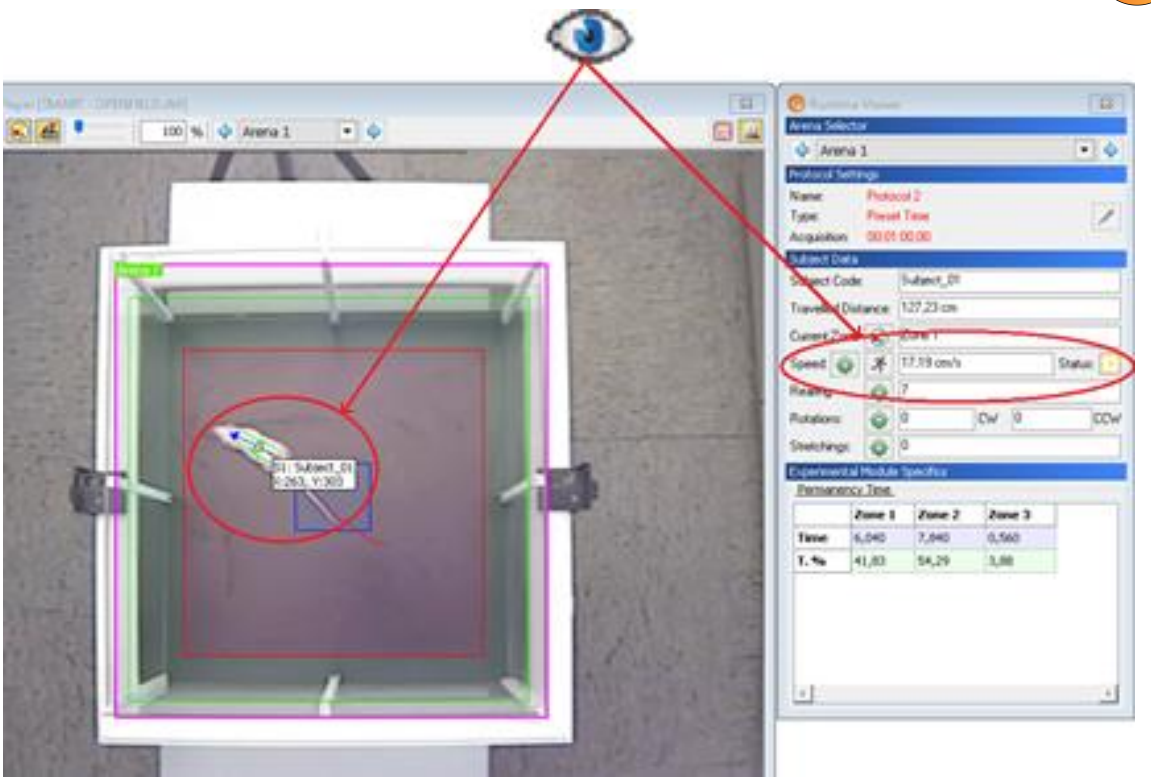

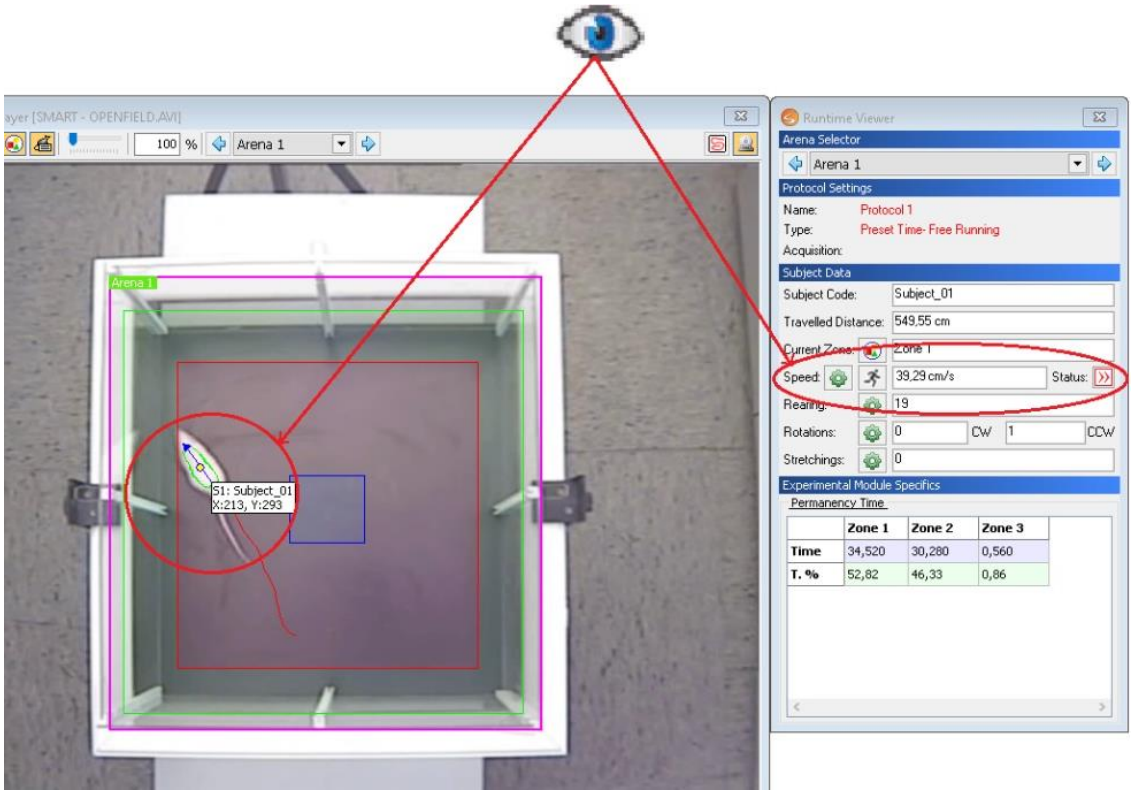
Arena 1
100 %
Runtime Viewer
Arena Selector
Arena 1
Protocol Settings
Name: Protocol 1
Type: Preset Time- Free Running
Acquisition:
Subject Data
Subject Code: Subject_01
Travelled Distance: 549,55 cm
Current Zone: Zone 1
Speed: 39,29 cm/s
Status:
Rearing: 19
Rotations: 0 CW 1 CCW
Stretchings: 0
Experimental Module Specifics
Permanency Time
Zone 1 Zone 2 Zone 3
Time 34,520 30,280 0,560
T. % 52,82 46,33 0,86
S1: Subject_01
X:213, Y:293

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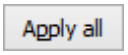
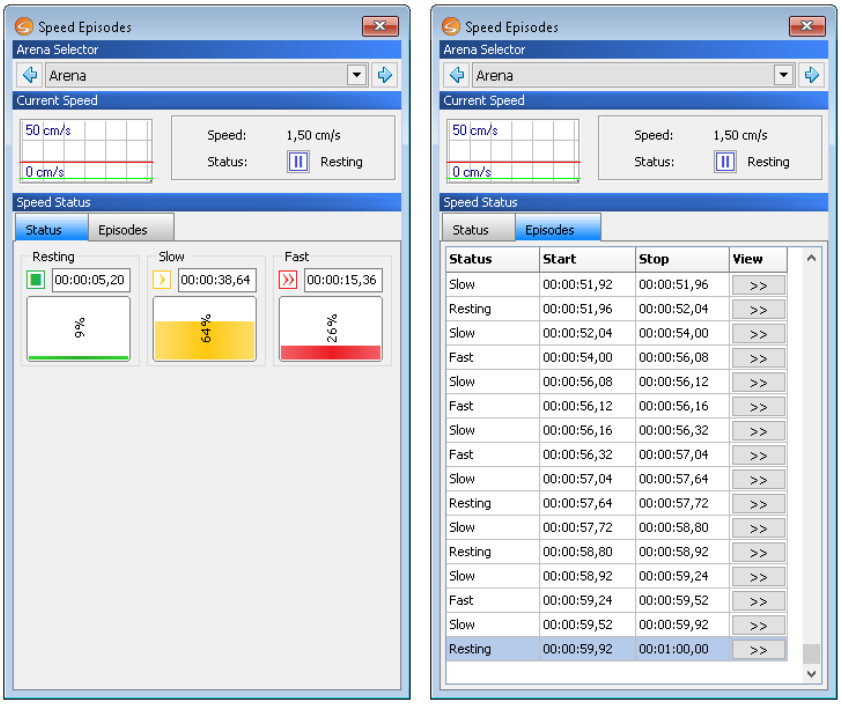
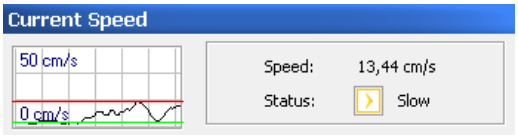
SMARTIO MA	An “Arena selector” control is provided for SMARTIO-MA users within the **Speed Settings** panel to adjust the speed thresholds for each arena independently.  Select the arena to configure (with the selector or clicking directly at the **Player** panel) and adjust the parameters freely.  In order to apply the new settings to all the arenas simultaneously, press the button **Apply all**..

Speed episodes details

The **Speed Episodes Details** panel can be accessed through the  button. This panel shows additional information about the subject displacement pattern, such as the time the subject has been in the different speed pattern and the list of speed episodes.



- **Current Speed:** This section shows the current speed and the current speed status. The scope shows the speed throughout time and the current speed threshold values.



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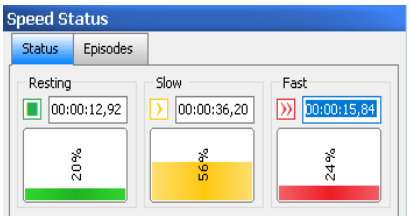
The status field represents the current subject displacement pattern status calculated depending on the speed thresholds adjusted in the Speed Settings panel:

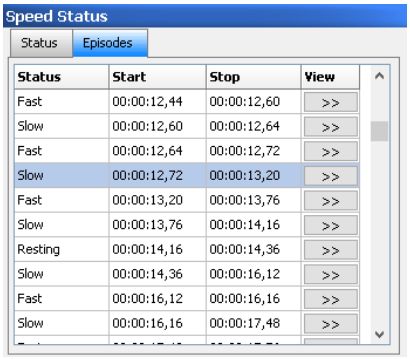
Represents a RESTING status.

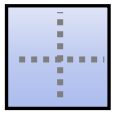
Represents a SLOW displacement status.

Represents a FAST displacement status.

- **Speed Status:** This section shows the time and its percentage that the subject has been in the different displacement pattern status. A table shows the history of all the different speed episodes throughout the trial. If a finished trial is being reviewed (by selecting it on the Scheduler's tree) and a digital video was selected as the image source, a **View** button is provided to "replay" each change on the speed status in the **Player** panel.

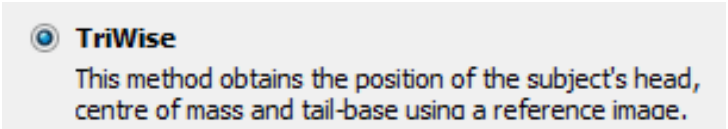




 **SMARTIO MA**	An "Arena selector" control is provided for SMARTIO-MA users within the **Speed Episodes Details** panel to review the speed episodes details for each arena independently.  Select the arena to configure (with the selector or clicking directly at the **Player** panel) and adjust the parameters freely.

Rearing

This data is only displayed when the **TriWise tracking mode** is selected in the **Detection Settings** panel.



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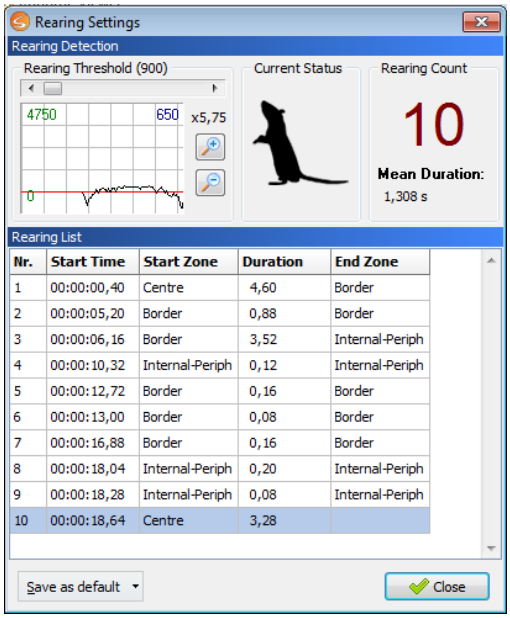
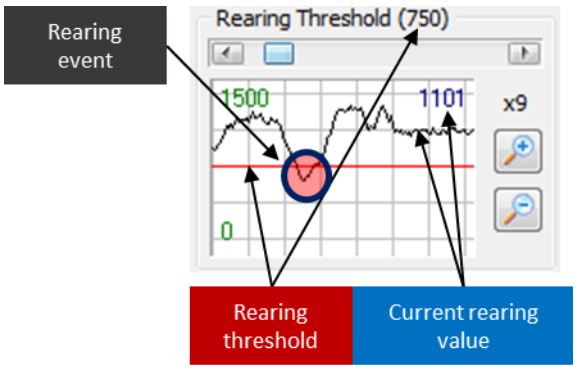
The rearing data represents an estimation of the animal's vertical activity. The rearing behavior is considered as an index of animal exploration.

To configure the rearing detection threshold, press the  button. The **Rearing Settings** panel is shown:



During data acquisition, the graph shows the current "rearing value" and its history. The "rearing value" represents the inverted height of the subject. The higher the rearing value, the lower the subject. If the rearing value is lower than a user-defined threshold, the subject is considered in a rearing event. The threshold value mainly depends on the detected animal's size in normal conditions and during the rearing events.



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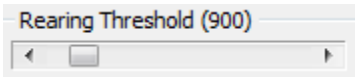
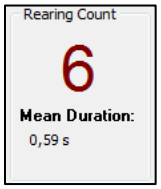


To detect a rearing event, the rearing threshold must be set correctly using the scrollbar in the **Rearing Threshold** box. It is considered that the subject is rearing when the rearing value is lower than the rearing threshold. To accurately detect rearing events, set the rearing threshold (red line) so that it is above the rearing value (black line) while the subject is rearing, but below the rearing value while the subject is not rearing.



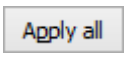
Rearing List

Nr.	Start Time	Start Zone	Duration	End Zone
15	00:00:32,68	Border	2,04	Border
16	00:00:34,92	Internal-Periph	0,32	Internal-Periph
17	00:00:35,72	Centre	2,32	Centre

The detected rearing events are also shown in the Rearing List table. A counter at the top of the panel shows how many rearing events were detected and the average duration of all the events.

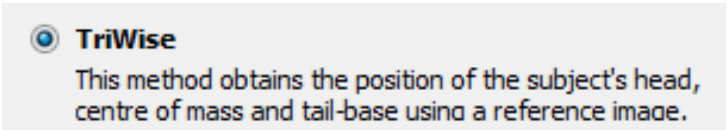
The Rearing List table displays the following information:

- **Nr**: the sequence number of the rearing event starting at 1.
- **Start Time**: the relative time in which the rearing event is detected.
- **Start Zone**: the name of the zone in which the subject is detected when the rearing event started.
- **Duration**: the total duration (in sec) of the rearing event. A rearing event ends when the rearing value is higher than the rearing threshold.
- **End Zone**: the name of the zone in which the subject is detected when the rearing event ended.

SMARTIO MA	An "Arena selector" control is provided for SMARTIO-MA users within the **Rearing Settings** panel to adjust the rearing threshold for each arena independently.  Select the arena to configure (with the selector or clicking directly at the **Player** panel) and adjust the parameters freely.  In order to apply the new settings to all the arenas simultaneously, press the button **Apply all**..

Rotation

This data is only displayed when the **TriWise tracking mode** is selected in the **Detection Settings** panel.



This tool automatically detects the subject's rotation events. The total number of CW (clockwise) and CCW (counterclockwise) rotations are shown in the **Rotation section**.

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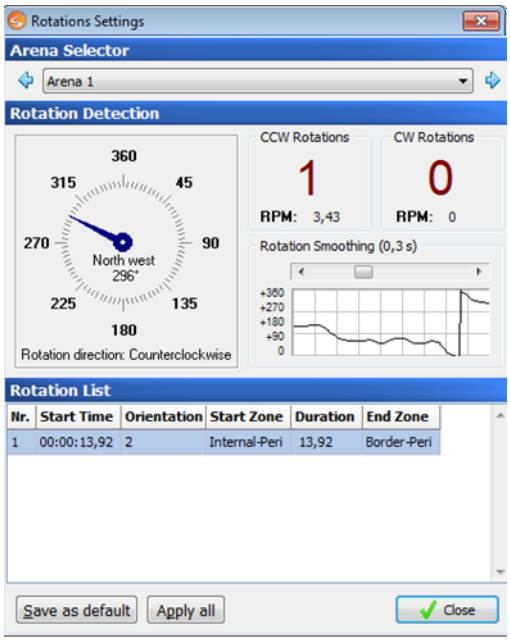
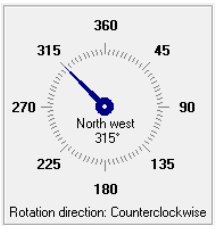
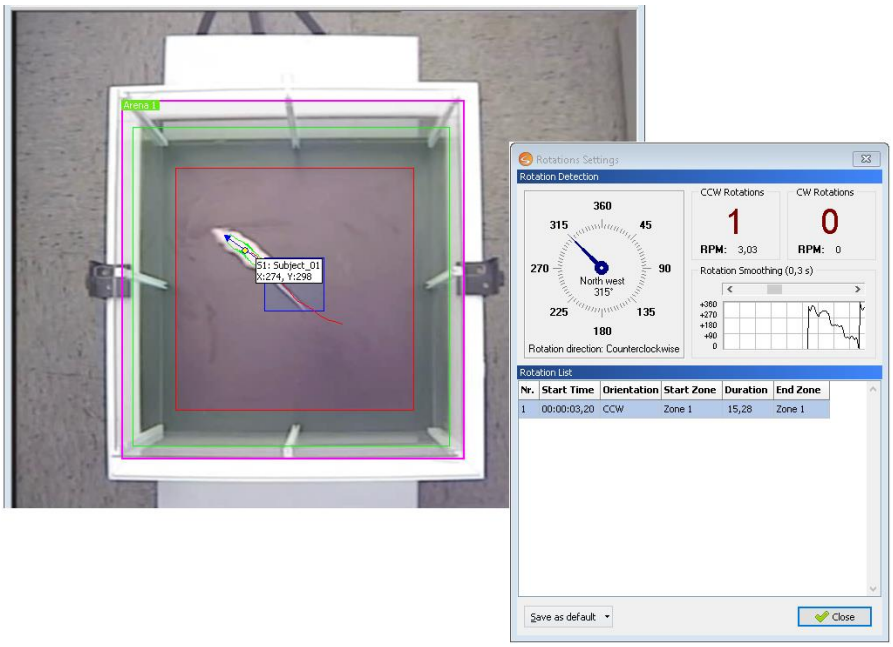
To configure the rotation detection threshold, press the  button. The **Rotation Settings** panel is shown:



During data acquisition, the compass represents the current orientation in degrees of the subject. It is possible to read the precise value of the current orientation and its cardinal orientation inside the compass. A single rotation smoothing value must be set. This value mainly depends on the subject activity (quick small changes in orientation) and the quality of the image.





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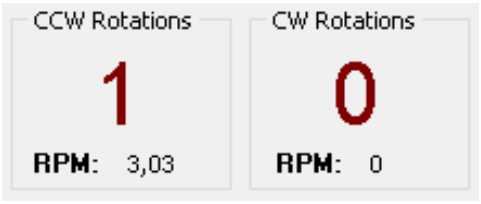
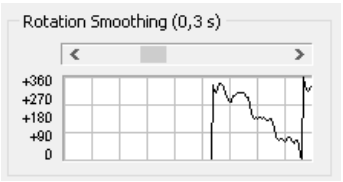




The graph shows the history of the orientation in degrees. It is possible to configure the smoothing of the orientation by moving the scrollbar in the **Rotation Smoothing** box. The smoothing is configured in seconds. The current orientation is calculated as the current orientation averaged with the samples of the last seconds configured by the scrollbar in the Rotation smoothing box. A rotation is detected when the subject's orientation passes through all cardinal points in strict order. If the order is "broken", no rotation is considered.

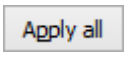
Rotation List

Nr.	Start Time	Orientation	Start Zone	Duration	End Zone
1	00:00:13,92	CCW	Internal-Peri	13,92	Border-Peri

The rotations detected are also shown in the Rotation List table. Two counters at the top of the panel show the amount of counterclockwise and clockwise rotations and their RPM (rotations per minute).

The Rearing List table displays the following information:

- **Nr**: the sequence number of the rotation event starting at 1.
- **Start Time**: the relative time in which the rotation event is detected.
- **Start Zone**: the name of the zone in which the subject is detected when the rotation event started.
- **Duration**: the total duration (in sec) of the rotation event.
- **End Zone**: the name of the zone in which the subject is detected when the rotation event ended.

SMARTIO MA	An "Arena selector" control is provided for SMARTIO-MA users within the **Rotation Settings** panel to adjust the rotation smoothing for each arena independently.  Select the arena to configure (with the selector or clicking directly at the **Player** panel) and adjust the parameters freely.  In order to apply the new settings to all the arenas simultaneously, press the button **Apply all**..

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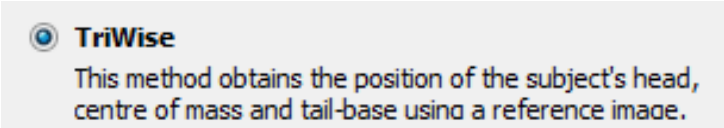
Stretching

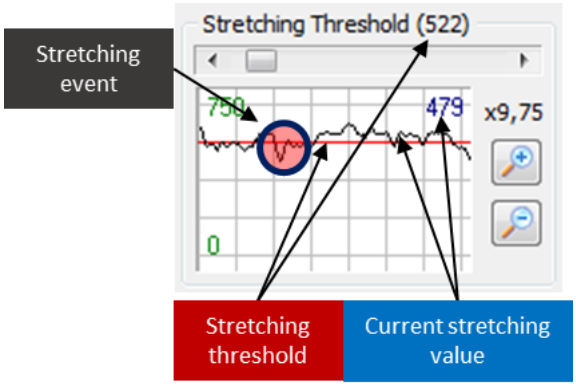
This data is only displayed when the **TriWise tracking mode** is selected in the **Detection Settings** panel.



This tool automatically detects animal's stretching events. The total number of stretching-behavior is shown in the **Stretching section**.

To configure the stretching detection threshold, press the  button. The **Stretching Settings** panel is shown:

During data acquisition, the graph shows the current "stretching value" and its history. The "stretching value" represents the total length of the subject's body (from head to tail). The higher the stretching value, the longer the subject's body. If the stretching value is higher than a user-defined threshold, the subject is considered in a stretching event. The threshold value mainly depends on the detected animal's size in normal conditions and during the rearing events.



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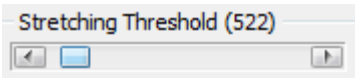
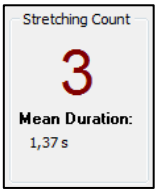


To detect a stretching event, the stretching threshold must be set correctly using the scrollbar in the Stretching Threshold box. It is considered that the subject is stretching when the stretching value is higher than the stretching threshold. To correctly detect stretching events, set the stretching threshold (red line) so that it is below the stretching value (black line) while the subject is stretching, but above the stretching value while the subject is not stretching.



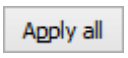
Stretching List

Nr.	Start Time	Start Zone	Duration	End Zone
1	00:00:00,40	Internal-Periph	0,40	Border-Periphe
2	00:00:00,40	Border-Periphe	0,40	Border-Periphe
3	00:00:03,32	Border-Periphe	3,32	Border-Periphe

The detected stretching events are also shown in the Stretching List table. A counter at the top of the panel shows how many stretching events were detected to the moment and the average duration of all stretching.

The Stretching List table displays the following information:

- **Nr**: the sequence number of the stretching event starting at 1.
- **Start Time**: the relative time in which the stretching event is detected.
- **Start Zone**: the name of the zone in which the subject is detected when the stretching event started.
- **Duration**: the total duration (in sec) of the stretching event. A rearing event ends when the stretching value is higher than the stretching threshold.
- **End Zone**: the name of the zone in which the subject is detected when the stretching event ended.

SMARTIO MA	An "Arena selector" control is provided for SMARTIO-MA users within the **Stretching Settings** panel to adjust the stretching threshold for each arena independently.  Select the arena to configure (with the selector or clicking directly at the **Player** panel) and adjust the parameters freely.  In order to apply the new settings to all the arenas simultaneously, press the button **Apply all**..

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Trial data

This data is only displayed when the **Activity Detection mode** is selected in the **Detection Settings** panel.

This option provides additional runtime information and adjustments related to animal activity patterns.

Activity and Status

The global activity of the subject recorded in the trial is shown in the **Activity box**. The data are given in the units specified during the calibration, cm^2/s or $inch^2/s$ (chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration).

The activity status field represents the level of the subject immobility/activity depending on the activity thresholds adjusted in the **Activity Settings** panel:

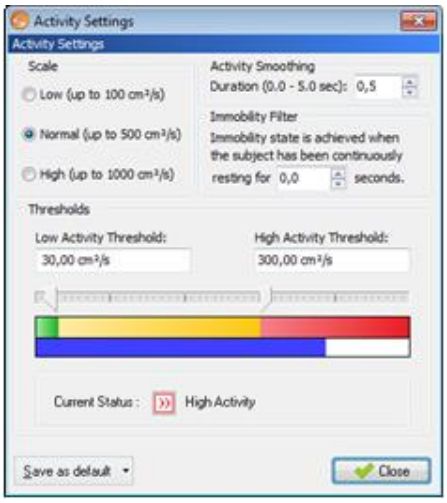
Represents immobility

Represents a low activity level.

Represents a high activity.

Activity settings

The  button displays the **Activity Settings** panel for setting the Activity thresholds.



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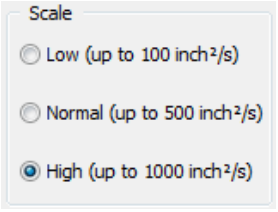
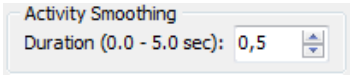
From this panel it is possible to set activity scale, activity smoothing, immobility filter and activity thresholds. The value of each one depends on variables such as the subject size and motor capabilities of the animal strain used and the specific conditions of your experiment:

- **Activity scale**: Allows selecting the maximum activity level (in the square units used during the calibration process chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration) the animal can reach.



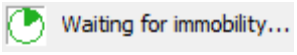
- **Activity Smoothing:** An interval of time (in sec) in which the activity level is averaged before determining the punctual activity. This option minimizes the impact that the artifacts in the image can produce on the activity calculations.



A default value of 0.5 sec is configured. This value is suitable in most cases, but it is possible to adjust it between zero sec (no smoothing applied; the current activity level is exactly the punctual activity) and 5 sec (the punctual activity during the last 5 sec is averaged to calculate the current activity level).

- **Immobility Filter:** An interval of time (in sec) the animal must remain under a "Low Activity" status to be considered effectively as an "immobility" episode.

When this value is >0, each time the animal's activity goes below the Low Activity threshold, an immobility detection period starts. It is reflected with a **Waiting for immobility** sign in the **Current Status** field of the **Activity Settings** panel.



If the animal remains under the Low Activity threshold status during a time equal or longer than the configured in the **Immobility filter** field, then the sign changes to Immobile.

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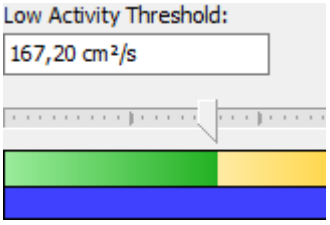
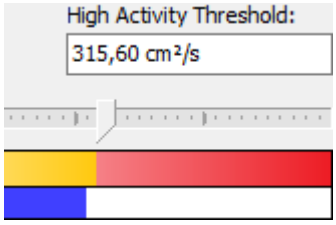
- **Activity Thresholds:**
    - Low activity threshold: Threshold under which the “immobility” state is evaluated.



    - High activity threshold: Threshold above which the animal is deemed to be “highly active”.



The thresholds can be adjusted by dragging the markers along the horizontal rule. A fine adjustment can be achieved by clicking the rule and using then the following combination keystrokes:

- [LEFT ARROW] / [RIGHT ARROW]: To increase / decrease the “Low Activity Threshold”.
- [CTRL] + [LEFT ARROW] / [RIGHT ARROW]: To increase / decrease the “High Activity Threshold”.
- [SHIFT] + [LEFT ARROW] / [RIGHT ARROW]: To increase / decrease both thresholds at the same time.

SMARTIO allows changing the values of the thresholds during the tracking as well to facilitate the choice of their values depending on the criterion of the user. The values chosen during the detection test and during the acquisition process can be changed afterward during the analysis process for obtaining a new set of data.

	The entire trial to which the values of the thresholds have been changed during the data acquisition process should be reanalyzed again in Analysis mode with your final choice of thresholds.
	To generate a statistically relevant data set, it is recommended to use the same threshold for all the subjects within the experiment to compare the data between the different experimental groups.

Here are some recommendations for setting the activity thresholds properly:

- Before starting the data acquisition, perform a detection test with a pilot animal and have a look on the Activity Settings panel during animal immobility and activity, depending on your own criteria.
- If the experiment is videotaped, the video can be analyzed again using a different threshold.

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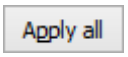
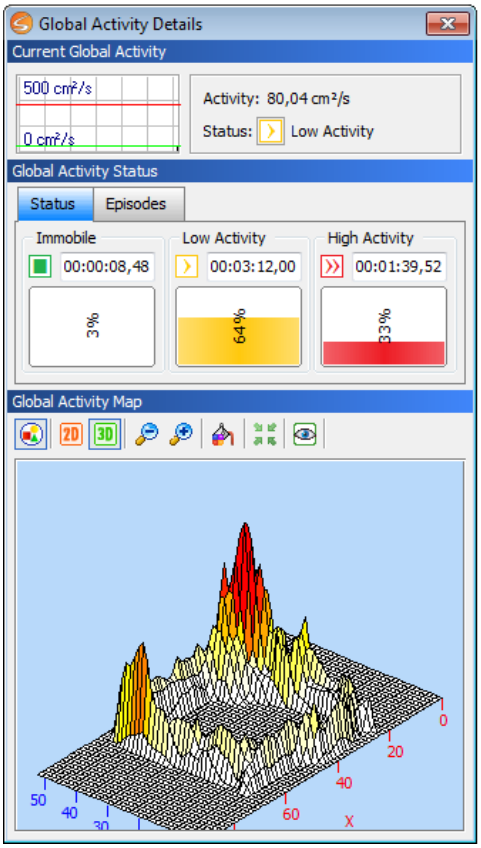
- In the Analysis module, it will be possible to adjust the time threshold for excluding any immobility episodes which duration is lower to the defined threshold. This time threshold allows eliminating any non-specific pause of activity which cannot be considered a true immobility episode.

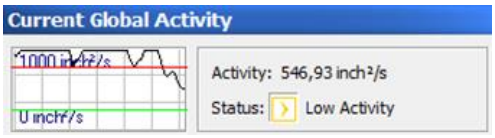
SMARTIO MA	An "Arena selector" control is provided for SMARTIO-MA users within the **Activity Settings** panel to adjust the activity thresholds for each arena independently.  Select the arena to configure (with the selector or clicking directly at the **Player** panel) and adjust the parameters freely.  In order to apply the new settings to all the arenas simultaneously, press the button **Apply all**..

Global activity details



The **Global Activity Details** panel can be accessed through the  . This panel shows additional information about the subject global activity such as the time the subject has been in the different activity statuses, the list of activity episodes and the total accumulated activity map.

- **Current Global Activity:** This section shows the current global activity and the current global activity status. The scope shows the global activity throughout time and the current activity threshold values.



The status field represents the level of the subject general activity depending on the activity thresholds adjusted in the Activity Settings panel:

Represents immobility

Represents a low activity level.

Represents a high activity.

	The global activity information gathered during the data acquisition is stored into the experiment file with a precision of 1 minute so that it may be re-analyzed later.

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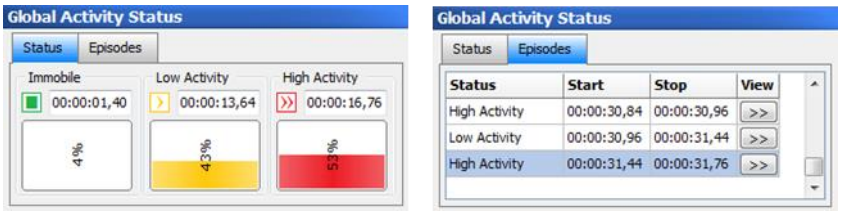
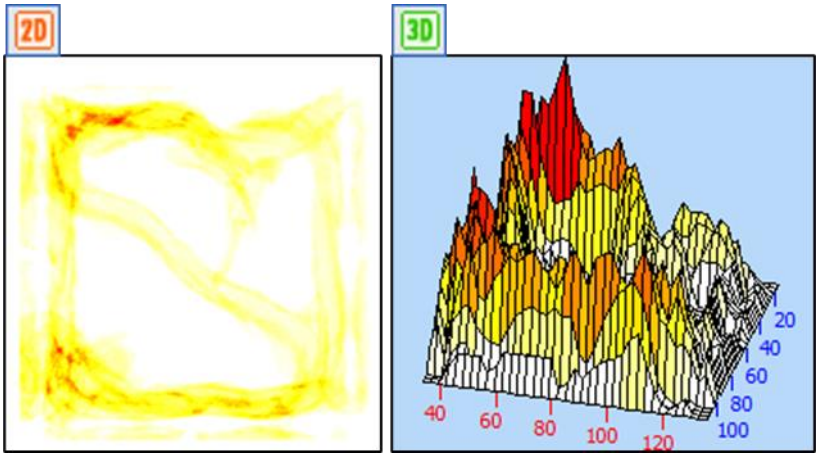
- **Global activity status:** This section shows the time and the time percentage that the subject has been in the different activity states. A table shows the history of all the different activity episodes throughout the trial. If a finished trial is being reviewed (by selecting it on the Scheduler's tree) and a digital video was selected as the image source, a **View** button is provided to "replay" each change on the activity status in the **Player** panel.



- **Global activity map:** The Global Activity Map tool is a basic tool showing a visual resume of the zones of maximal activity achieved along the acquisition process. This tool does not generate any numeric data that could be used for calculation or statistics. It is strictly for illustration purposes only.
  The activity map is based on the detection of changes of pixels occurring between two consecutive frames into the entire captured image (the distribution of activity does not take in count the zones drawn by the user). The redder the zone, the more changes in pixels have been registered there; a yellow zone indicates a low amount of changes while a white zone indicates no changes in pixel (meaning, no activity).
  When the **Global Activity Map** is opened during the data acquisition, the image is automatically updated every second to represent the map of total accumulated activity.
  The Global Activity Map may be visualized in 2D or 3D by selecting the respective buttons. The 3D map may also be rotated to visualize it from different angles.



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 Change the background color

 Zoom in / Zoom out (only 3D map)

 Restore the 3D map to its original position

 Launches the **Activity Map Viewer** tool to analyze the activity map in detail and export the image

 Export the map as a .bmp file

Experimental module specifics data

The **Experimental Module Specifics** section in the Runtime Viewer panel provides summary information about the time and activity registered in each user-defined zone and/or associations considered as zone of interest in the **Zone and association management** tool (see chapter 11.2 - THE ZONES EDITOR TOOL).

Experimental Module Specifics

Permanency Time and Global Activity

	Border	Centre	Periphery	Total
Time	40,680	6,360	58,600	64,960
T. %	62,62	9,79	90,21	100,00
Activity	5155,88	1388,55	9314,21	10702,76
A. %	48,17	12,97	87,03	100,00

The following data are provided when the data acquisition process is done using any of the 3 tracking modes available (center of mass, 3-point TriWise, and color detection).

- **Permanency Time**: the accumulated time (in seconds) the subject spent in each of the defined zones and associations. The percentage of the total time spent in each zone and association is also shown.
  Permanency times and percentages per zone are shown in the **Time** and **T. %** rows in the table.
- **Global Activity**: the accumulated activity (measured in the units specified during the calibration, see chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration) the subject made into each of the defined zones and associations. The percentage of the total activity made into each zone and association is also shown.
  Accumulated activity and percentages per zone are shown in the **Activity** and **A. %** rows in the table.

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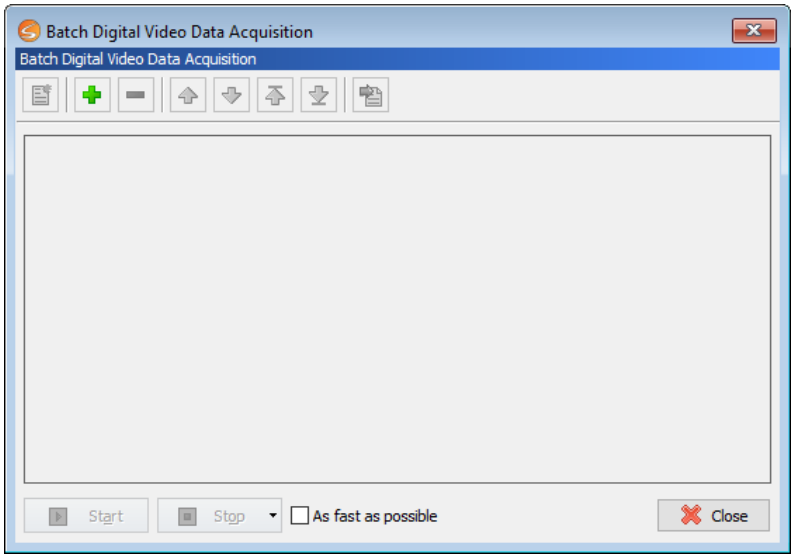
17.7. BATCH DIGITAL VIDEO DATA ACQUISITION

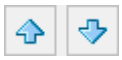
Once a new Batch Data Acquisition experiment is started (see chapter 6.1 - Starting a new experiment for Batch Data Acquisition), the following window will be shown:



From this window, it will be possible to add the videos to be analyzed.

Clear current list.

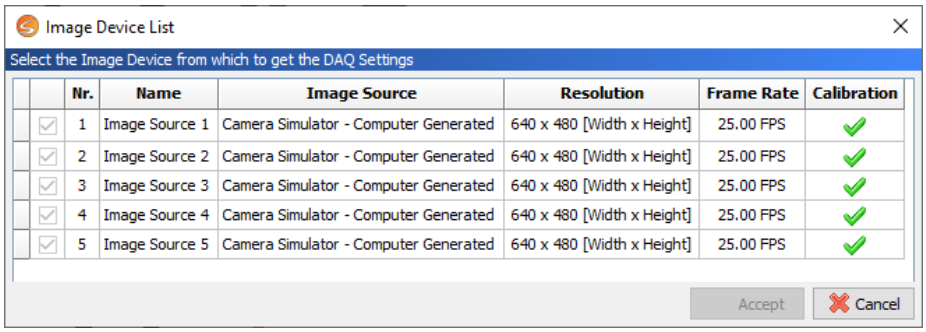
Add/Remove the selected videos.



Move up/down the selected videos.

Move top/bottom the selected videos.

Import DAQ Settings (see chapter 6.2 - Importing and exporting data acquisition settings). When selecting a DAQ Settings file containing settings from more than one image source, the following panel will allow to select the settings of a specific image source to apply.



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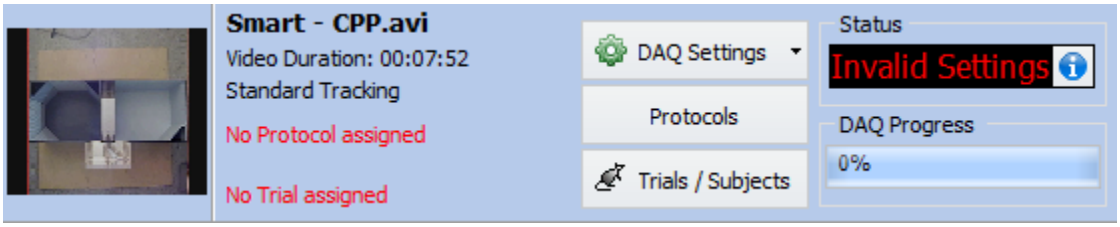
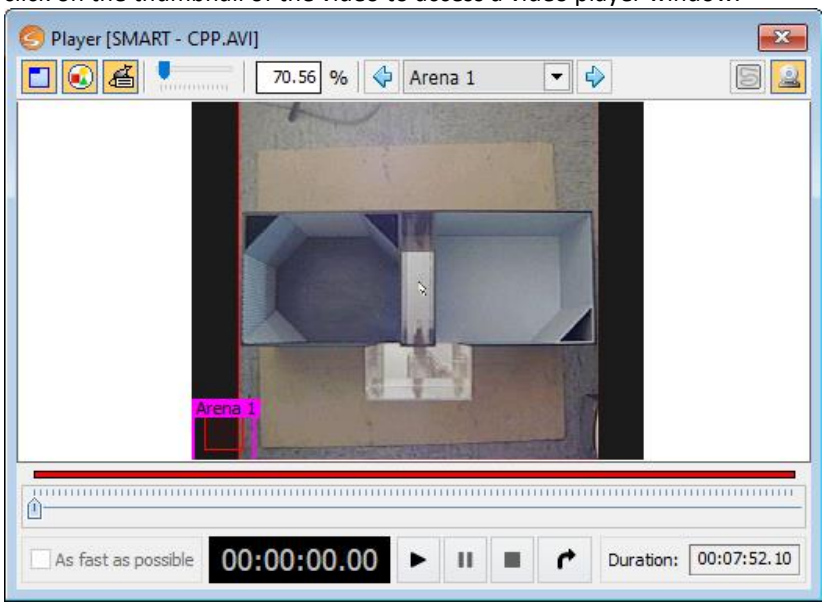
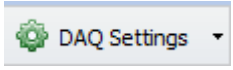
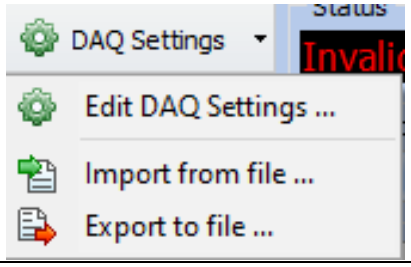
Once added the videos, several options will be available for each one:



	Double click on the thumbnail of the video to access a video player window.  Use the  button to set a starting point for each video.
Smart - CPP.avi Video Duration: 00:07:52 Standard Tracking	This section shows general info about the video (name, duration and tracking mode).
No Protocol assigned No Trial assigned	This section shows the status of protocol assignation and trial assignation.
 	Allows to check the DAQ settings and modify some of them (see chapter 17.7 - Editing the DAQ Settings in Batch Mode Acquisition). Using the lateral arrow, it is also possible to import/export the DAQ settings. 

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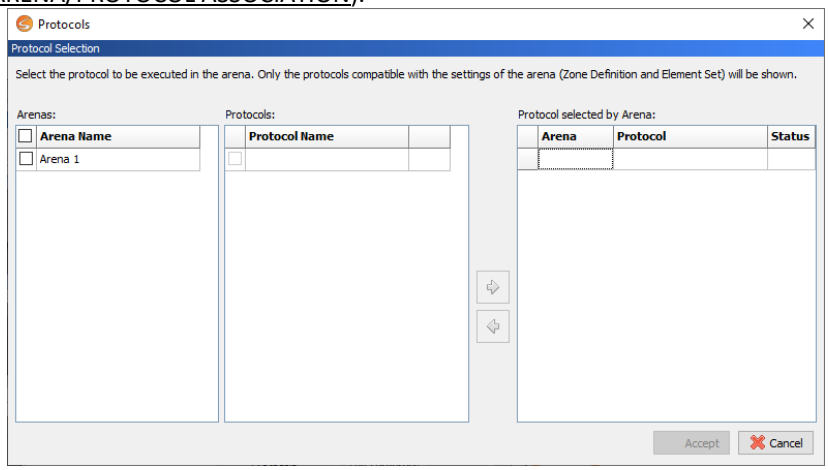
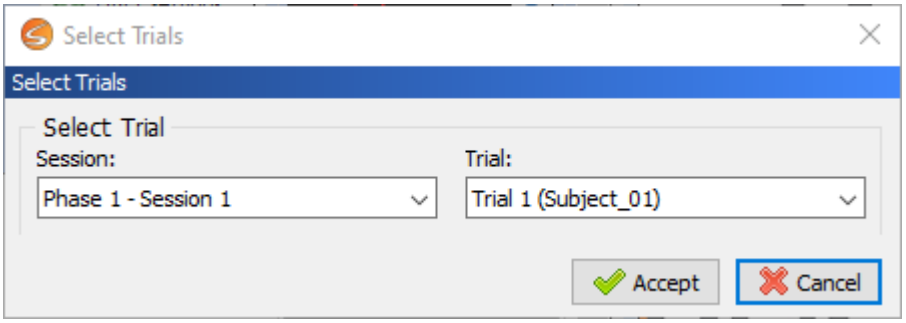
Protocols	Allows to assign a protocol to this video. At least one protocol must have been created or imported before trying to assign one to the video (see chapter 13 - PROTOCOLS and chapter 17.1 - ARENA/PROTOCOL ASSOCIATION). 
Trials / Subjects	Allows to assign a subject to a trial. The scheduler must be set in order to assign a subject to a trial (see chapter 15 - SCHEDULER). 
Status Invalid Settings	Current status of the video. Click on  to show detailed information of the settings that need to be reviewed before starting. Once the protocol and the trial are correctly assigned and the DAQ settings are ready, this window turns into the following: 
DAQ Progress 0%	Shows the progress of the data acquisition.

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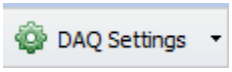
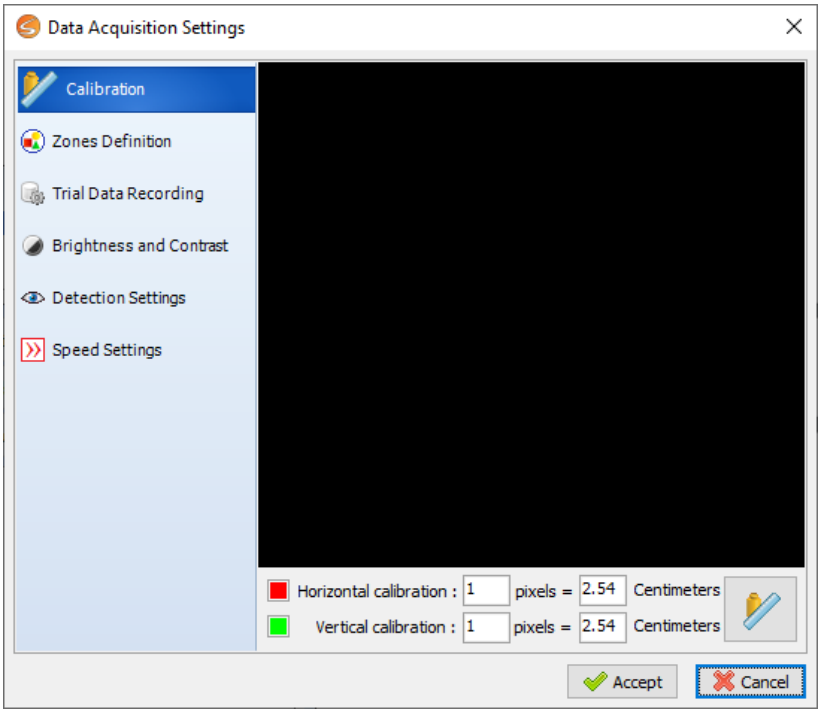
Editing the DAQ Settings in Batch Mode Acquisition



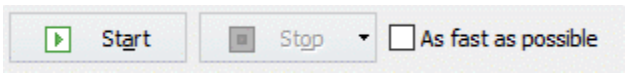
In the Batch Data Acquisition view, it is possible to check the DAQ Settings and modify some of them using the button



- **Calibration:** allows to edit the calibration. Click on  to access the Video File Image calibration panel (see chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration).
- **Zones Definition:** allows to check the zones definition associated to the DAQ settings loaded (see chapter 11 - ZONES DEFINITION).
- **Trial Data Recording:** allows to edit the detection modes (see chapter 12.3 - DETECTION SETTINGS).
- **Brightness and Contrast:** allows to edit the brightness and contrast settings (see chapter). Can be edited independently per each arena (see chapter 12.2 - BRIGHTNESS AND CONTRAST).
- **Detection Settings:** allows to edit the track detection settings (threshold and erosion) edit the snapshot and apply the anti-vibration filter (chapter 20.4 - Filtering and smoothing techniques in SMARTIO). Can be edited independently for each arena.
- **Speed settings:** allows to edit the speed settings (see chapter 17.6 - Speed settings). Can be edited independently for each arena.

Once the DAQ Settings have been correctly set and each video shows the 

message, it will be possible to start the batch acquisition using the **Start** button.



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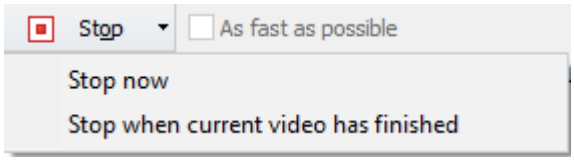
Check the **As fast as possible** box to run the analysis at the maximum speed available. It is possible to stop the batch data acquisition using the button **Stop**. Clicking on the lateral arrow at the side of the Stop button, it is also possible to choose whether to stop immediately or before starting the following video.



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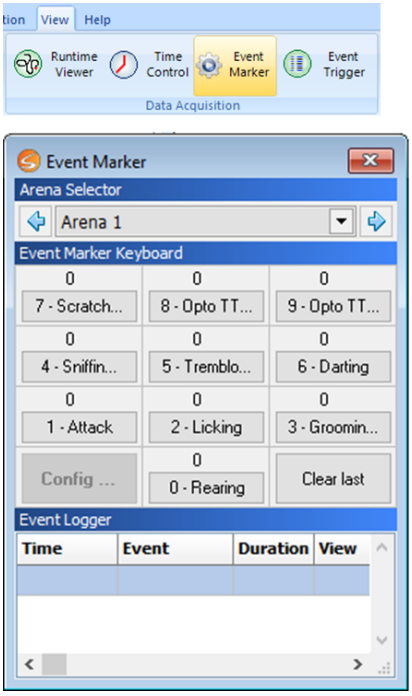
17.8. RECORDING MANUAL EVENTS

SMARTIO provides the **Event Marker** tool to record events of interest which have happened during the execution of a trial.

To display the **Event Marker** acquisition panel, click on the **Event Marker** option of the **View** main menu.



The **Event Marker** panel presents two sections:

- The **Event Marker Keyboard**: provided with a button for each kind of event to record and the number of occurrences of each one.
- The **Event Logger table**: shows the list of the manually scored events with the following information:
    - Time: the relative time (in hh:mm:ss,c) in which the event of interest started.
    - Event: the name of the event of interest recorded, as defined in the **Event Settings** panel.
    - Duration: the duration (in sec) of the event of interest recorded.

To record events of interest with the help of the Event Marker tool:

1. Show the **Event Marker** panel.

2. Configure the events of interest by mean of the **Event Settings** panel (for more details, see chapter 16.3 - EVENT MARKER SETTINGS).
3. Make sure that the numeric keypad is enabled ([NUM LOCK] key must be pressed).

4. Configure and start the trial (data acquisition).

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5. Press the numeric keys depending on the kind of event visually identified each time the events occur. Keep the key pressed throughout the whole duration of the event to be marked.

SMARTIO MA	An "Arena selector" control is provided for SMARTIO-MA users within the **Event Marker** panel.  Select the arena (with the selector or clicking directly at the **Player** panel) to review the information related to the recorded events and to record additional events belonging specifically to that arena.

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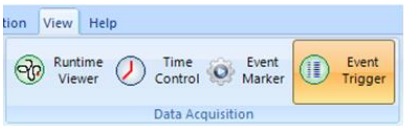
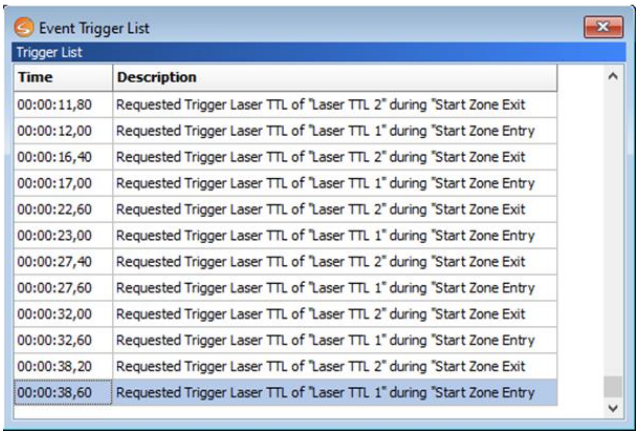
17.9. RECORDING TRIGGER EVENTS

SMARTIO provides the **Event Trigger** viewer to visualize the stimulations that have been triggered during the executing of a trial. Click on the **Event Trigger** option of the **View** main menu.





A Log table will be displayed showing the triggering events occurring during the trial. The triggering events can be, for example, third-party laser optogenetic stimulator triggered by the conditions defined by the protocol (time elapsed, entry into/exit from a zone, event marker manual key pressing).

The **Event Trigger List** shows the list of the recorded trigger events with the following information:

- **Time**: the relative time (in hh:mm:ss,c) in which the event of interest started.
- **Description**: description of the action that led to the trigger of the stimulation.

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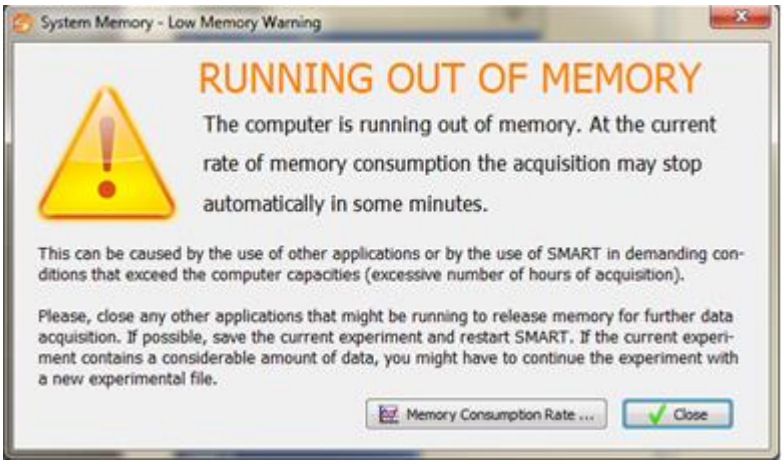
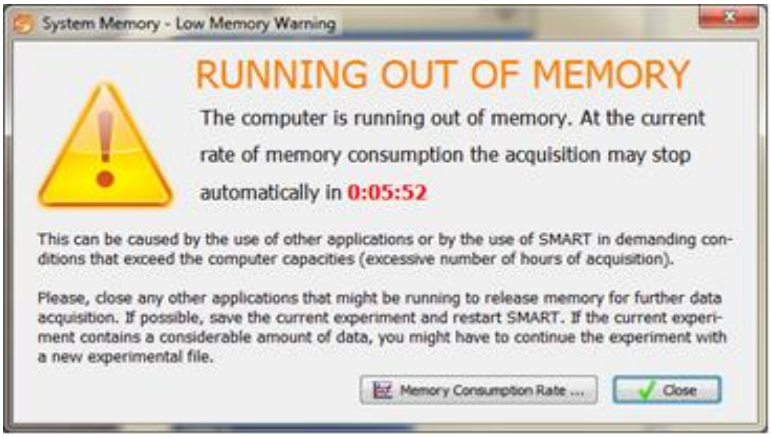
17.10. COMPUTER RESOURCES MONITORING

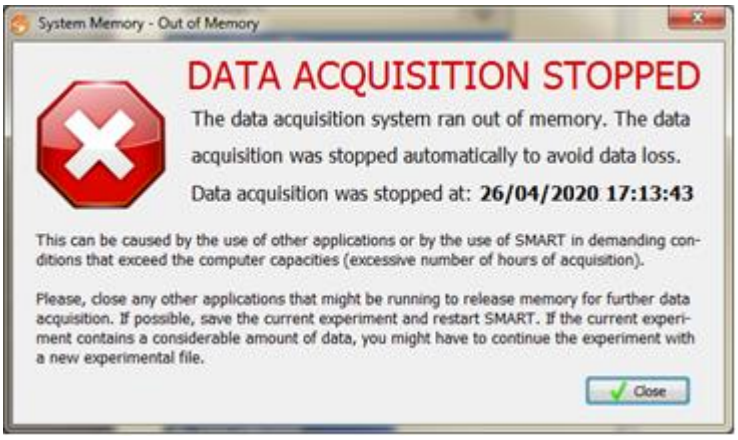
SMARTIO constantly monitors the computer's resources during data acquisition in order to avoid undesirable data loss. If the RAM memory reaches critical levels, SMARTIO shows a warning message with the following message:



This message will automatically disappear if memory level is restored again but, if the critical situation persists and gets worse, SMARTIO automatically will stop the running trials in all arenas.



In this case, the following message is shown including the time and date at which the trials were stopped, and a log file is generated within the SMARTIO installation folder.



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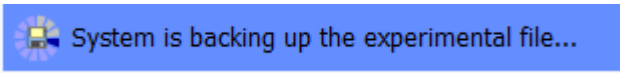
17.11. DATA BACKUP

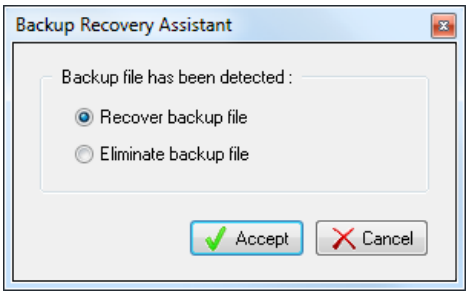
SMARTIO has a built-in automatic data backup system designed to prevent experimental data loss, especially in conditions in which long data acquisition sessions (several hours) are executed. During these hours a problem with the operating system or a power supply failure could ruin all the data acquired since the problem occurred. To minimize this undesirable condition, the backup system is periodically and transparently launched every 5 minutes during acquisition and every time a panel is closed. SMARTIO stores a copy of all the information in the experiment file: from the configuration to the data acquired during the acquisition process.



When the system is doing a backup, the following message is shown in the left part of the SMARTIO footer.

	The system generates a pair of files called *Experiment.~bak* and *Experiment.ldb* to store the backup information. DO NOT remove these files unless you are absolutely sure that they are no longer needed.
	As the backup system is launched every 5 minutes (and not sooner), all the information acquired during the last 5 minutes may be lost if the system crashes. If that information is critical for your needs, make sure to save the file manually in a shorter interval of time.

In case of a computer or software crash, the backup system will detect the incident during the following execution of the software and will provide a mechanism to recover or discard the backed-up information.



If the **Recover backup file** option is selected, the backed-up information will be automatically recovered and opened as a new experiment file. Thus, a new manual save is needed.

If the **Eliminate backup file** option is selected, the backed-up information will be deleted.

	Select this option only if you are absolutely sure that the backed-up information is no longer useful. Please confirm with other users whom the use of SMARTIO is shared to avoid loss of data belonging to others.

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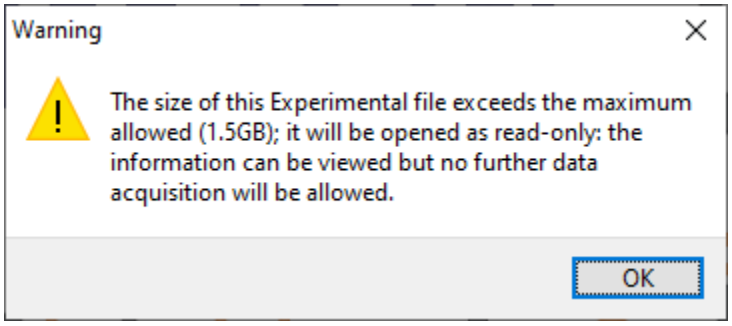
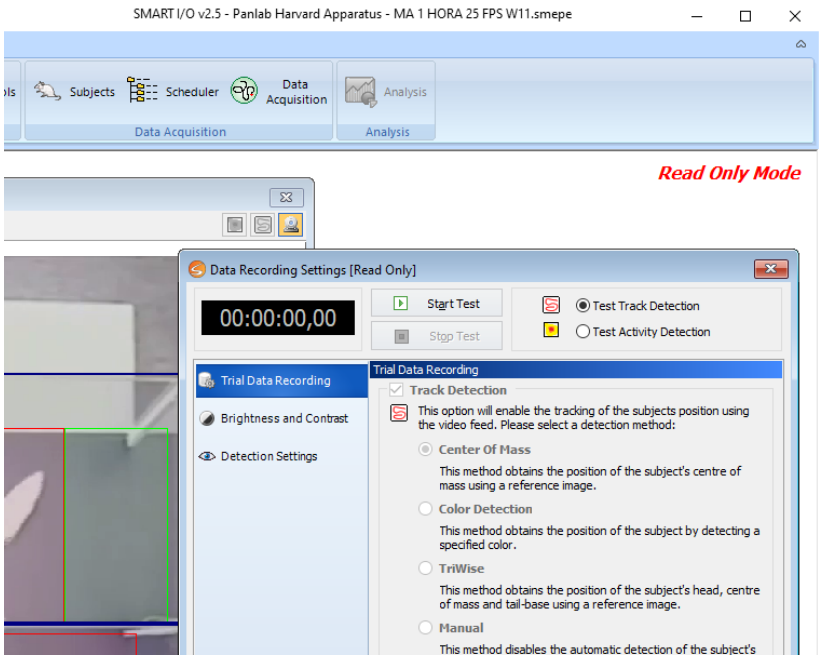
18. READ-ONLY MODE

SMARTIO can only manage experimental files of a limited size. The size of the experimental file depends on many factors, such as number of trials acquired and camera settings. The limit size under which it is still possible to acquire data is 1.5 GB.

When trying opening an experiment bigger than 1.5 size, SMARTIO will open it in **Read-only** mode, a mode in which is still possible to analyze acquired trials, view the protocols, the list of subjects, the scheduler and the runtime panels for each trial selected in the scheduler- However it will not be possible to acquire new data.



When in read-only mode, a red message will be shown in the main window of SMARTIO, and the head bars of all panels will show “Read only”.



In read-only mode, it will still be possible to create and save new Zones Definitions (see chapter 11 - ZONES DEFINITION), allowing to analyze the acquired trials under with different zones configurations different from the ones using during the acquisition.

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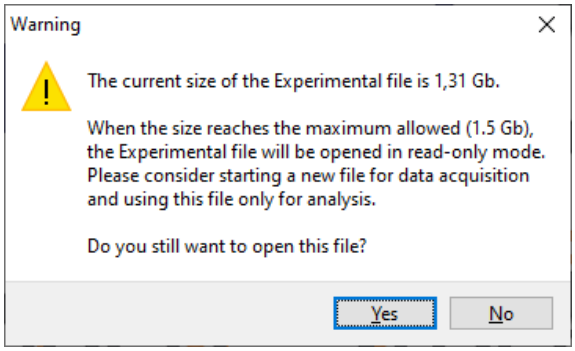
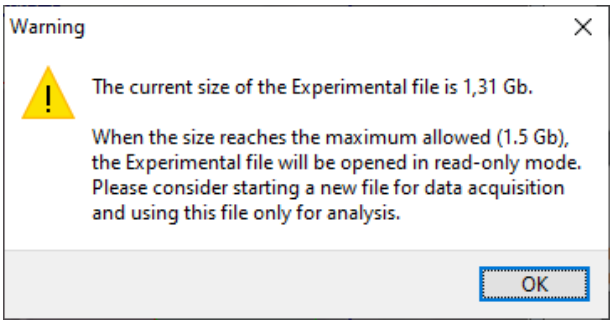
When opening a file right under the 1.5 GB size limit, the following warning message will be shown:



When starting the acquisition of new data in a file that is right under the 1.5 GB size limit, the following warning message will be shown:



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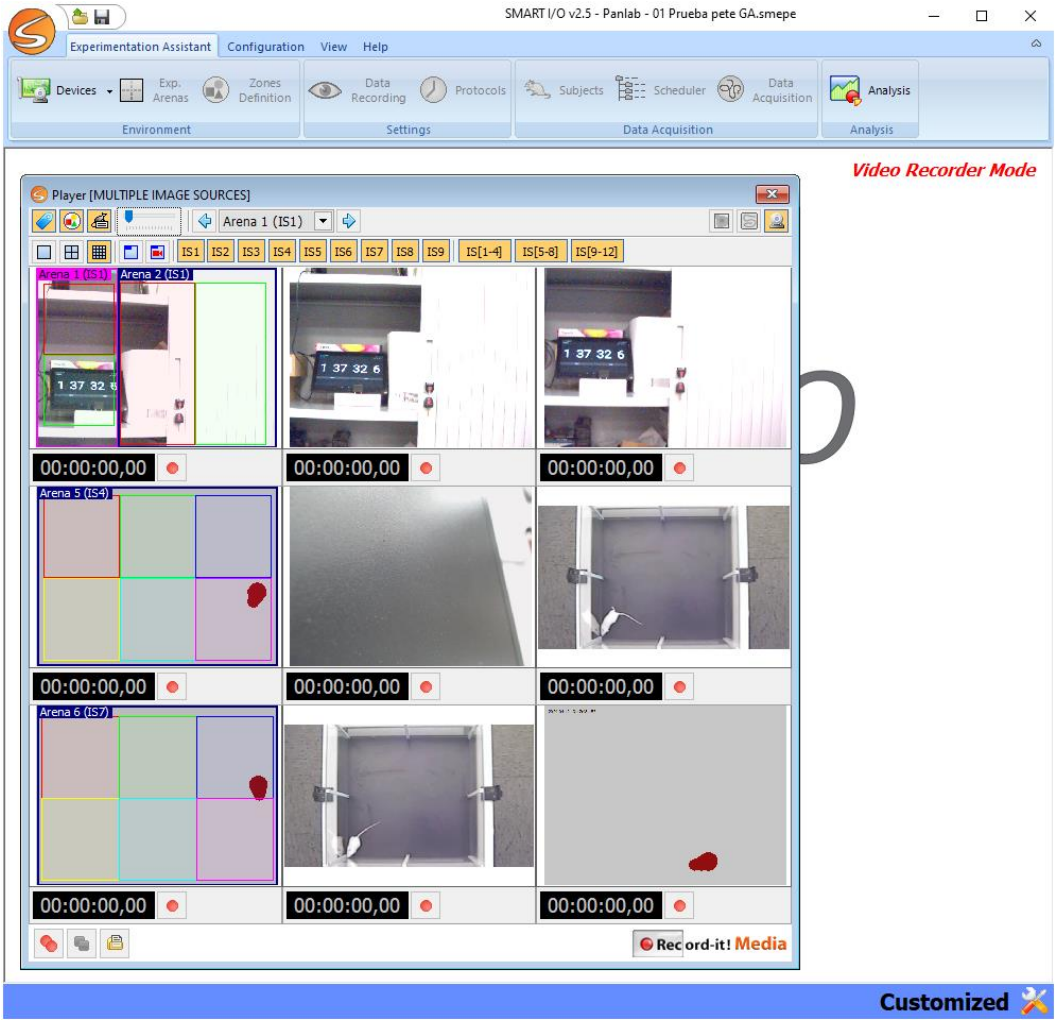
19. VIDEO RECORDER MODE

SMARTIO automatically enters in “Video Recorder” mode when more than 8 image sources are activated simultaneously. The tracking is only available for up to 8 image sources at the same time. In “Video Recorder” mode only recording and analyzing acquired trials will be possible.

In “Video Recorder “mode, the only buttons enabled are Devices, Analysis, Path Settings, Recording Settings, Experiment Info, Player, User Manual and About; in the Player, all buttons will be enabled, including those that allow recording videos for all cameras. Additionally, a banner indicating if the Experiment is in Read Only Mode or in Video Recorder Mode will be added in the main screen.



If an Experiment with more than 8 active live cameras is also in Read Only mode, the Video Recorder Mode will not be applied, and it will not be possible record videos.

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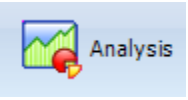
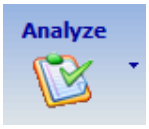
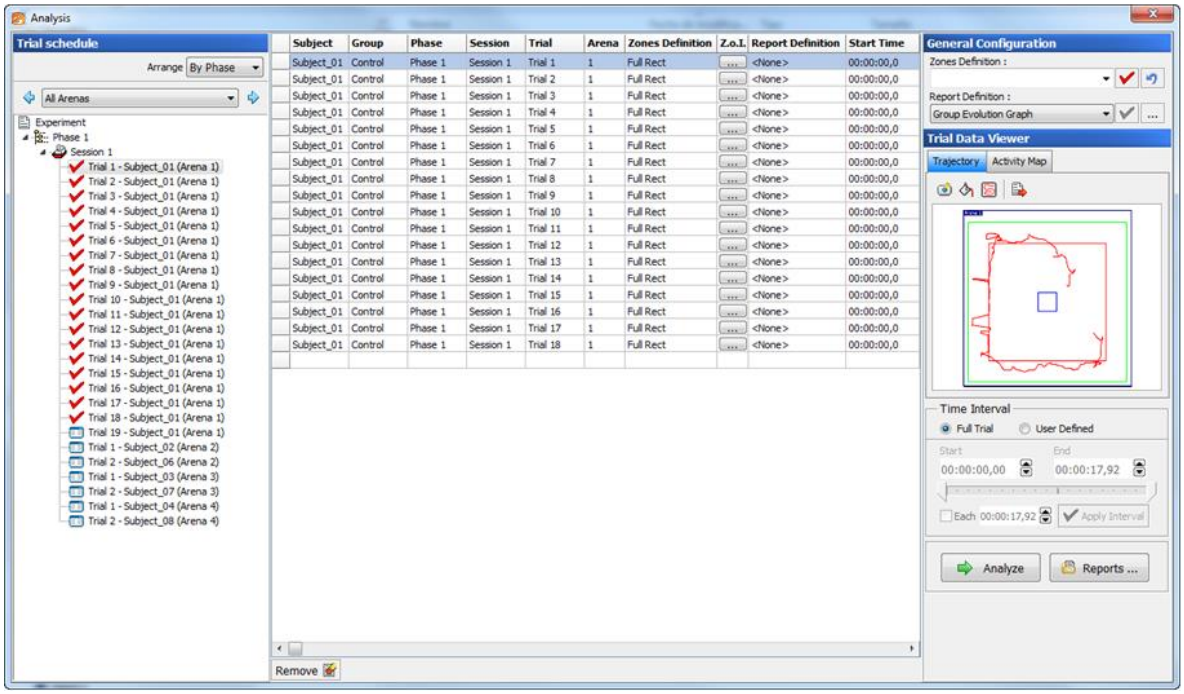
20. DATA ANALYSIS



Analysis is the process of obtaining numeric and graphic reports from a list of finished trials and a specific configuration. To access the Analysis tool, click on the **Analysis** button in the **Experimentation Assistant bar**.

	The Analysis tool is not available when a data acquisition process is running. Wait until all the trials finish before accessing the Analysis tool.
	The **Starting Assistant** launched when SMARTIO is executed also provides a specific **Analyze** button to open an experimental file and access the analysis process immediately. 

This is the main window of the analysis process:



The general procedure to generate analysis reports is the following

1. Select the trials to analyze.
2. For each trial or block of trials:
    - Select the zone definition and zones/associations of interest.
    - Configure and select the analysis report.
    - Set the time intervals to be analyzed.
3. Generate and review the reports.
4. Export the results to Excel or to image formats.

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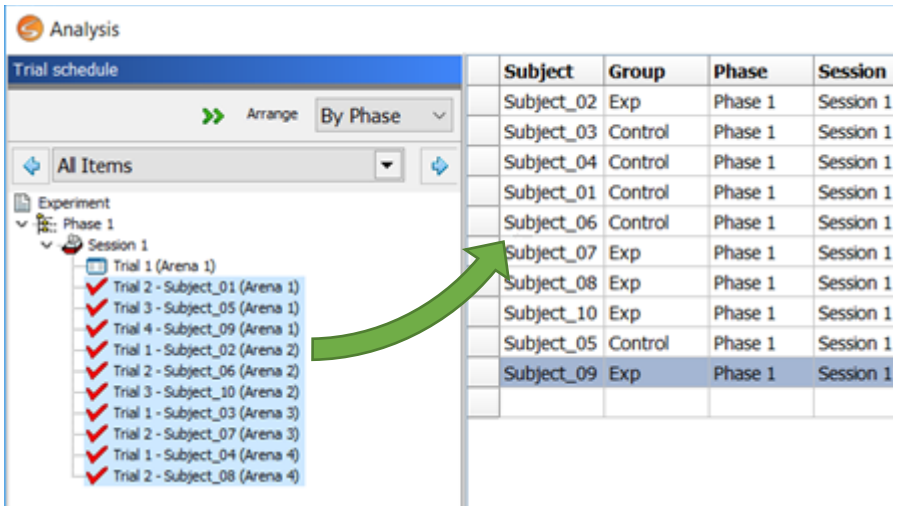
	The license key is not needed for analysis.
	In order to adapt the analysis capabilities to the extensions licensed, the SMARTIO analysis module combines two different elements: • The SMARTIO USB protection key: if plugged (remember that it is not needed having a protection key plugged to analyze data), SMARTIO will consider the extensions licensed in the key. • The selected trials: SMARTIO will also consider as active every extension that was active when the selected trials were executed, independently on whether the extensions are or not active in the present protection key (if connected).

20.1. SELECTING THE TRIALS TO BE ANALYZED

The trials to be analyzed must be dragged from the **Trial schedule** section of the **Analysis** panel to the main list located in the center of the window.



A unique trial, a group of selected trials, a session (all trials of the session will be included) or a phase (all trials in all sessions of the phase will be included) can be dragged.

SMARTIO MA	An "Arena selector" control is provided for SMARTIO-MA users within the **Trial schedule** section in the **Analysis** panel. Select the arena with the selector to view the list of trials belonging specifically to that arena.  The "Arena selector" control in the **Analysis** panel provides an exclusive option called "All Arenas" to facilitate selecting trials acquired in different arenas.

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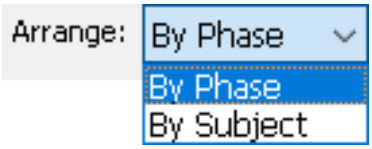
⚠	Even if an image source is deactivated (see chapter 7.1 - Live image source), the trials already recorded in its arenas are still visible in this panel.

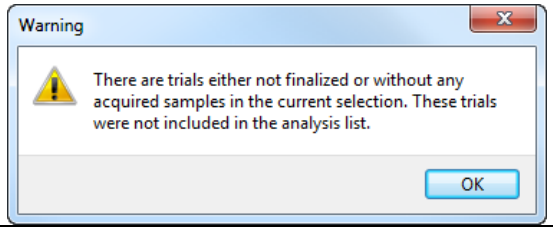
The trials in the **Trial schedule** section can be arranged in two different ways:



- By Phase: the trials' tree is arranged in an experiment-phase-session scheme. This option facilitates selecting multiple trials acquired in the same session and thus analyzing the evolution of a group of subjects.
- By Subject: the trials' tree is arranged in an experiment-subject-phase-session scheme. This option facilitates selecting the trials in which a specific subject participated and thus analyzing the evolution of this subject in particular.

The trials selected to analyze are automatically included into the main table of the **Analysis** panel showing the following information:

- **Subject**: code that the subject had when the trial was acquired.
- **Group**: group to which the subject belonged when the trial was acquired.
- **Phase**: name of the phase in which the trial has been acquired.
- **Session**: name of the session in which the trial has been acquired.
- **Trial**: name of the trial defined in the Scheduler.
- **Arena**: name of the arena in which the trial was acquired.
- **Zones Definition**: name of the zones definition that will be used for the analysis. The zones definition used during data acquisition is selected by default.
- **Report Definition**: name of the report that will be used for the analysis.
- **Start Time**: start time of the analysis. By default, this is set to zero.
- **End Time**: end time of the analysis. By default, this matches with the trial total duration.
- **Interval Time**: duration of the subintervals that will be considered during the trial analysis. By default, it matches with the trial total duration so that a single subinterval is evaluated.

⚠	A trial without any information acquired cannot be dragged to the main table. In that case, a warning message is shown: 
	A trial dragged in the queue for the analysis cannot be discarded nor deleted (see chapter 15.2 - Deleting a trial and chapter 15.2 - Discarding a trial)

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To remove a group of trials selected to analyze:

1. Select the trials in the table using the combination of the mouse clicks and the [SHIFT] / [CTRL] keys.
2. Press the **Remove** button located at the bottom left corner of the table.

Analyzing the same trial with different conditions

Each trial can be analyzed multiple times using different conditions (zones definition, report definition, time settings). To do that:

1. Drag and drop the trial (or selected trials) several times to the analysis grid.
2. Apply the different conditions to every trial independently. Please refer to the next chapters in this section for more details on how to apply a specific analysis configuration to a trial.
   In this example, the same trial (Subject_01, Phase 1, Session 1, Trial 1) is analyzed with different reports, time settings and zone definitions.

	Subject	Group	Phase	Session	Trial	Arena	Zones Definition	Z.o.I.	Report Definition	Start Time	End Time	Interval Time
	Subject_01		Phase 1	Session 1	Trial 1	1	Smart - OpenField_RS4	...	Summary Report	00:00:00,00	00:00:15,20	00:00:15,20
	Subject_01		Phase 1	Session 1	Trial 1	1	Smart - OpenField_RS4	...	Track Coordinates Report	00:00:00,00	00:00:15,20	00:00:15,20
	Subject_01		Phase 1	Session 1	Trial 1	1	Smart - OpenField_RS4	...	Zone Transition List	00:00:00,00	00:00:15,20	00:00:15,20
	Subject_01		Phase 1	Session 1	Trial 1	1	Smart - OpenField_RS4	...	Summary Report	00:00:00,00	00:00:10,00	00:00:10,00
	Subject_01		Phase 1	Session 1	Trial 1	1	Smart - OpenField_RS - Arena 1	...	Summary Report	00:00:00,00	00:00:15,20	00:00:15,20

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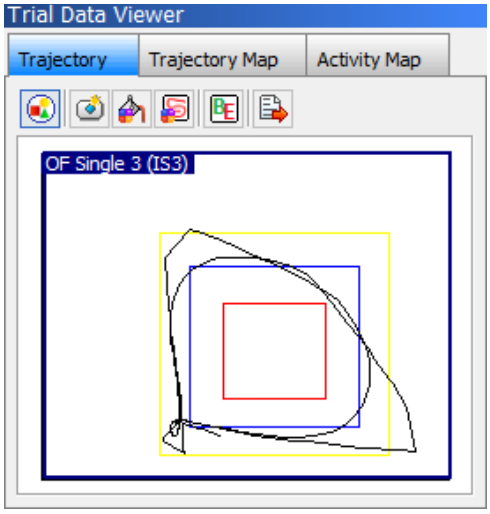
20.2. THE TRIAL DATA VIEWER

Whenever a trial is selected in the table, the **Trial Data Viewer** section is automatically updated with a summary of the information acquired, that is, the trajectory and/or the activity map (depending on the detection mode selected before executing the trial). When several trials are selected, the Trail Data Viewer shows the view corresponding to the first trial selected.

Trajectory

The **Trajectory** viewer is only available when one of the Tracking detection modes (center of mass, TriWise, color, or manual) was used for acquiring the data of the selected trial.



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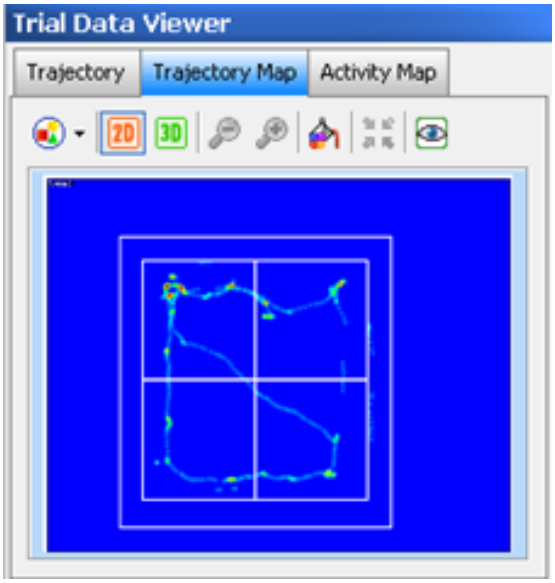
The following tools are provided:

	Shows or hides the zone definition applied on the track image.
	Shows or hides the background image captured before acquiring the track.
	Sets the color of the background (click on this button to choose the color from a pallet of colors).
	Sets the color of the trajectory (click on this button to choose the color from a pallet of colors).
	Shows or hide the B (Beginning) and E (End) points of the track.
	Exports the current track view to a *.bmp file.

Trajectory map

The **Trajectory map** viewer is only available when one of the Tracking detection modes (center of mass, TriWise, color, or manual) was used for acquiring the data of the selected trial.



The **Trajectory Map** tool is a basic tool showing a visual resume of the zones of maximal permanence of the subject. This tool does not generate any numeric data that could be used for calculation or statistics. It is strictly for illustration purposes only. The trajectory map is based on the detection of the track coordinates changes and time spent in specific zones of the experimental area. The redder the zone, the more time spent there by the subject; a yellow zone indicates a low amount of time while.

The Trajectory Map may be visualized in 2D or 3D by selecting the respective buttons. The 3D map may also be rotated to visualize it from different angles.

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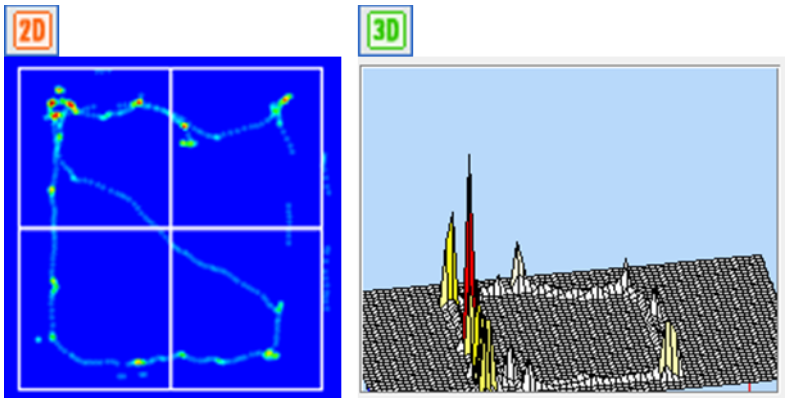



Change the background color


Zoom in / Zoom out (only 3D map)

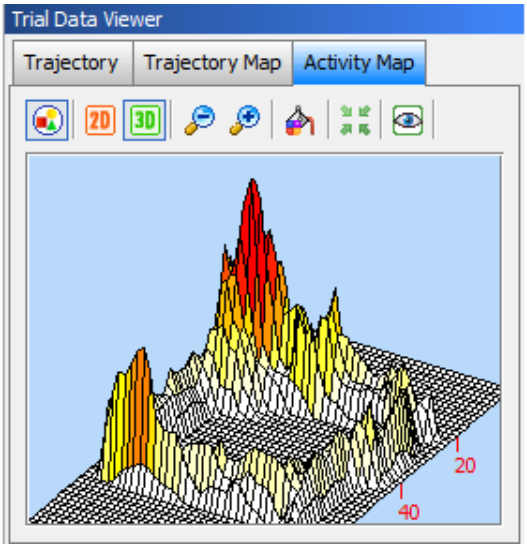

Restore the 3D map to its original position


Launches the **Trajectory Map Viewer** tool to analyze the activity map in detail and export the image


Export the map as a .bmp file

Activity map

The **Activity Map** viewer is only available when the Activity detection mode was used for registering the data of the selected trial.



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The activity map shown in the Trial Data Viewer provides the same functionalities as the runtime panel available during the data acquisition process. Please refer to chapter 17.6 - Global activity details for more details on the tools provided within the Activity Map panel to publish the results.

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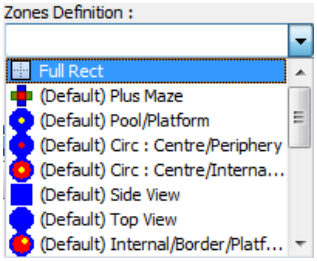
20.3. ZONES DEFINITION

Although the data acquisition process was done using a particular zone definition, the data can be analyzed again using a different one for each trial. To change the zones definition, follow these steps:

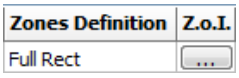
1. Select the trials in the grid to which the zones definition will be reassigned. Use the [CTRL]+Click combination to select multiple individual trials, [SHIFT] + Click to select a block of contiguous trials, or [CTRL] + [A] to select all of them.
2. Select the desired zone from the list **Zone definition** in the right top side of the analysis window. When a zone definition is selected, it is shown in the **Trial Data Viewer** area which also shows the trajectory followed by the subject during the first selected trial and its activity map.
3. Click on the  button to assign the selected zone to the selected trials. The Zone Definition column of the selected trials in the grid is updated with the selected list zone.



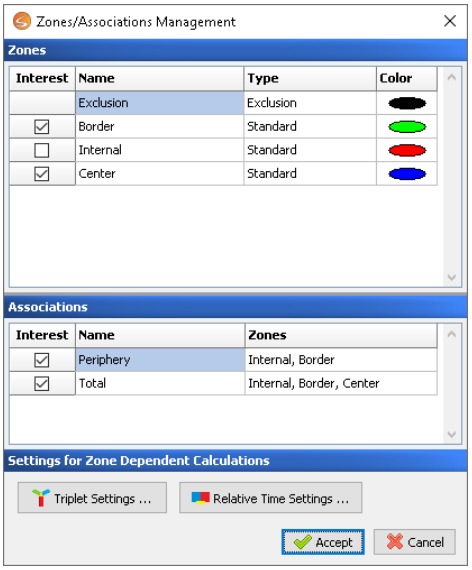
If the global activity information was acquired and the zone definition to be applied is different than the one used during the data acquisition, the global activity per zone cannot be calculated.

4. The original acquisition zone of the selected trials may be restored at any time by clicking the  button.

Zones and associations of interest



Additionally, the included zones and associations of interest that will be considered during the analysis process can be also selected. To do that, a specific tool is provided through the ... button located in the **Z.o.I.** (Zones Of Interest) column.



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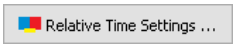
The list of zones and associations defined within the selected zones definition is shown in the **Zones** and **Associations** sections of the panel. Except the exclusion zone (which is always considered during the analysis), the rest of zones and associations are automatically selected. Uncheck the box of any zone or association to discard the partial calculations done for that zone/association during the analysis process.

	Even if the analysis stop condition is based on the entries or permanence time on a particular zone and this zone is not selected as of-interest, the stop condition will be evaluated as usual.

Some specific calculations are closely related to the zones defined and depend on the names given to them. One example of this case is the triplet calculation, which is a common measure within the T-Y Maze paradigm.

- The **Triplet Settings** button provides a tool to select the zones of your particular definition that must be considered to assess the alternations triplet calculation. Please refer to the chapter 21.3 - Alternation Triplet for more details on the alternation's triplet calculation and its application within the T-Y Maze paradigm.

- Another example is the calculation of the **Relative Time** in the compartment in a conditioned place preference experiment. Please refer to the chapter 21.3 - Relative Time in Zone (%) for more details.



If multiple trials were selected in the analysis grid before showing the **Zones or Associations of Interest** panel, check the **Apply to all trials…** box to apply your new settings regarding the zones or associations of interest to all the trials selected that have the same zone definition applied.

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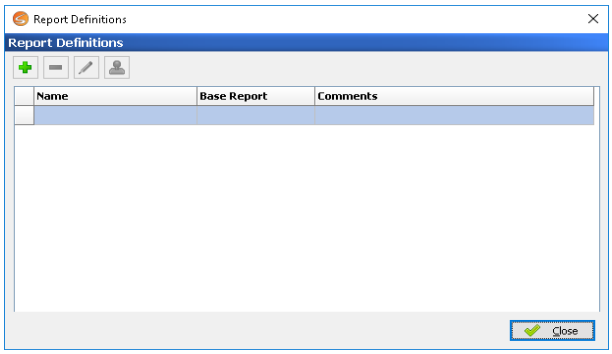
20.4. REPORT DEFINITION

SMARTIO can generate a wide variety of analysis reports depending on the protocol and particular requirements desired. Each report definition implies a different content and arrangement of the analysis results and a specific analysis configuration also.

SMARTIO users are provided with a **Report Definitions Manager** tool that allows one to freely design analysis reports and configure the analysis process independently. Moreover, each trial can be analyzed using a different report definition.

Managing analysis report definitions

Click on the ... button located at the right side of the Report Definition list to open the **Report Definition Manager** tool.



This panel shows the list of the analysis reports already defined in the experimental file. The following information is provided for each definition:

- **Name**: the user defined name of the report.
- **Base report**: the name of the standard base report definition used to create the new definition. Every new report definition must be based in the structure of an already existing base report. Each base report determines the tabular structure of the information and the calculations provided to generate the analysis reports.
- **Comments**: additional information regarding the report definition.

SMARTIO provides the following base reports to cover a variety of reporting needs.

Summary report

When data will be exported to a 3rd party statistical software package (SPSS, StatView, SigmaStat, Statistica, or similar), using the Summary Report will save time. The resulting report is arranged with one row per trial (meaning one row per subject). When different zones and intervals of time are configured, the parameters calculated for each zone and interval time are represented in different columns (see next figures as example).

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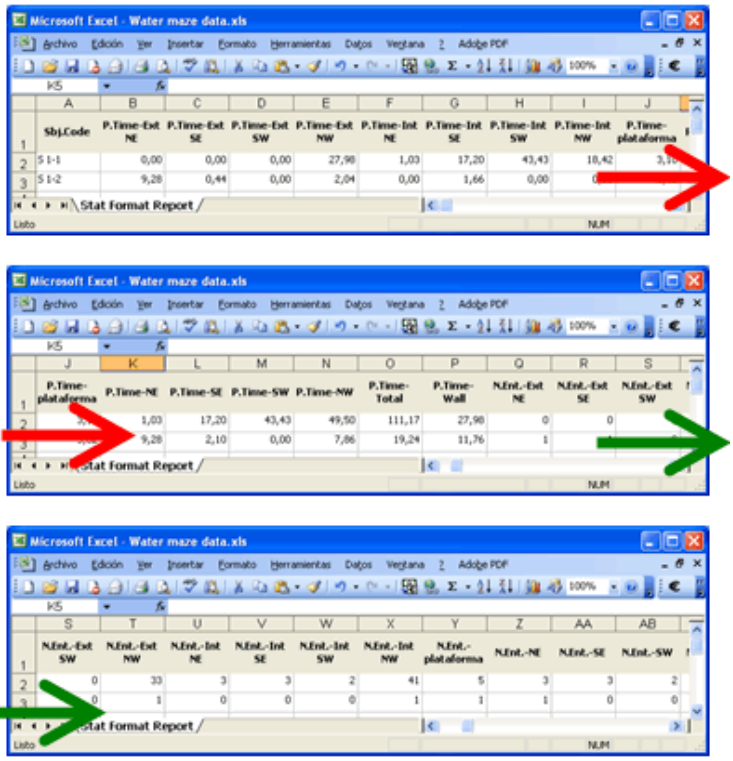
Microsoft Excel - Water maze data.xls
Archivo Edición Ver Insertar Formato Herramientas Datos Ventana ? Adobe PDF
K5
A B C D E F G H I J
SbjCode P.Time-Ext NE P.Time-Ext SE P.Time-Ext SW P.Time-Ext NW P.Time-Int NE P.Time-Int SE P.Time-Int SW P.Time-Int NW P.Time-plataforma
S 1-1 0,00 0,00 0,00 27,98 1,03 17,20 43,43 18,42
S 1-2 9,28 0,44 0,00 2,04 0,00 1,66 0,00
Stat Format Report /
Listo
NUM

Microsoft Excel - Water maze data.xls
Archivo Edición Ver Insertar Formato Herramientas Datos Ventana ? Adobe PDF
K5
J K L M N O P Q R S
P.Time-plataforma P.Time-NE P.Time-SE P.Time-SW P.Time-NW P.Time-Total P.Time-Wall N.Ent.-Ext NE N.Ent.-Ext SE N.Ent.-Ext SW
1,03 17,20 43,43 49,50 111,17 27,98 0 0
9,28 2,10 0,00 7,86 19,24 11,76 1
Stat Format Report /
Listo
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Microsoft Excel - Water maze data.xls
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S T U V W X Y Z AA AB
N.Ent.-Ext SW N.Ent.-Ext NW N.Ent.-Int NE N.Ent.-Int SE N.Ent.-Int SW N.Ent.-Int NW N.Ent.-plataforma N.Ent.-NE N.Ent.-SE N.Ent.-SW
0 33 3 3 2 41 5 3 3 2
0 1 0 0 0 1 1 1 0 0
Stat Format Report /
Listo
NUM

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Event list

Provides a list and time distribution of the events registered manually via the **Event Marker** tool. Please refer to chapter 17.8 - RECORDING MANUAL EVENTS for more details on how to record events. The following calculations are included within the Event List report:

- Event Index Nr.
- Event Rel. Time (Seconds)
- Event Rel. Time (HH:MM:SS,00)
- Event System Time.
- Event Name.
- Event Duration (Seconds).
- Event Zone START.
- Event Zone END.

Please refer to chapter 0 -

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DATA – EVENT LIST REPORT for more details on the meaning of these calculations.

Event trigger list

Provides a list and time distribution of the Trigger events sent to a third-party system through the Panlab LINKBOX interface. The following calculations are included within the Event List report:

- Action Description
- Action Index Nr.
- Action Time (HH:MM:SS,00)
- Action Time (Seconds)

Please refer to the chapter 21.5 - DATA – EVENT TRIGGER LIST REPORT for more details on the meaning of these calculations.

Track coordinates report

Provides a list of all the recorded coordinates of each subject for every sample. The following calculations are included within the Track Coordinates Report:

- Sample Index Nr.
- Sample Time (Seconds)
- Sample Time (HH:MM:SS,00)
- X Coordinate
- Y Coordinate
- Sample Zone
- Vector Angle (Degrees)
- Vector Angular Speed (Degrees/Second)
- Parallel Index
- Turning Tendency
- Segment Angle (Degrees)
- Segment Length
- Speed
- Speed Status
- Sync. Timestamp (Seconds).

Please refer to the chapter 21.6 - DATA – TRACK COORDINATES REPORT for more details on the meaning of these calculations.

Speed episodes list

Provides a list of the speed status episodes automatically detected and registered by SMARTIO and sorted chronologically. The following calculations are included within the Speed Episode List Report:

- Episode Index Nr.
- Episode Rel. Time (Seconds)
- Episode Rel. Time (HH:MM:SS,00)

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- Event Name.
- Episode Duration (Seconds).
- Episode Zone START.
- Episode Zone END.

Please refer to the chapter 21.7 - DATA – SPEED EPISODES LIST REPORT for more details on the meaning of these calculations.

Activity episodes list

Only if Global Activity detection has been used during data acquisition. This report would generate a list of the activity episodes automatically detected and registered by SMARTIO sorted chronologically. The following calculations are included within the Activity Episode List report:

- Episode Index Nr.
- Episode Rel. Time (Seconds)
- Episode Rel. Time (HH:MM:SS,00)
- Event Name.
- Episode Duration (Seconds).
- Episode Zone START.
- Episode Zone END.
- Sync. Timestamp (Seconds).

Please refer to the chapter 21.8 - DATA – ACTIVITY EPISODES LIST REPORT for more details on the meaning of these calculations.

TriWise rotation list

Only if TriWise detection has been used during data acquisition. This base report will generate a list and time distribution of the rotation events automatically detected and registered by SMARTIO. The following calculations are included within the Rotation List report:

- Rotation Index Nr.
- Rotation Rel. Time (Seconds)
- Rotation Rel. Time (HH:MM:SS,00)
- Event Name.
- Rotation Duration (Seconds).
- Rotation Zone START.
- Rotation Zone END.
- Sync. Timestamp (Seconds).

Please refer to the chapter 21.9 - DATA – TRIWISE ROTATION LIST REPORT for more details on the meaning of these calculations.

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TriWise rearing list

Only if TriWise detection has been used during data acquisition. This base report generates a list and time distribution of the rearing events automatically detected and registered by SMARTIO. The following calculations are included within the Rearing List report:

- Rearing Index Nr.
- Rearing Rel. Time (Seconds)
- Rearing Rel. Time (HH:MM:SS,00)
- Event Name.
- Rearing Duration (Seconds).
- Rearing Zone START.
- Rearing Zone END.
- Sync. Timestamp (Seconds).

Please refer to the chapter 21.10 - DATA – TRIWISE REARING LIST REPORT for more details on the meaning of these calculations.

TriWise stretching list

Only if TriWise detection has been used during data acquisition. This base report will generate a list of the stretching events automatically detected and registered by SMARTIO sorted chronologically. The following calculations are included within the Stretching List report:

- Stretching Index Nr.
- Stretching Rel. Time (Seconds)
- Stretching Rel. Time (HH:MM:SS,00)
- Event Name.
- Stretching Duration (Seconds).
- Stretching Zone START.
- Stretching Zone END.
- Sync. Timestamp (Seconds).

Please refer to the chapter 21.11 - DATA – TRIWISE STRETCHING REPORT for more details on the meaning of these calculations.

Zone transition list

The software provides a list and time distribution of the zone transitions automatically detected and registered by SMARTIO. The following calculations are included within the Zone Transition List report:

- Transition Index Nr.
- Transition Rel. Time (Seconds)
- Transition Rel. Time (HH:MM:SS,00)
- Transition Current Zone.
- Transition Time In Zone.
- Accumulated Time.
- Entries Nr.

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- Distance in Zone
- Sync. Timestamp (Seconds).

Please refer to the chapter 21.12 - ZONE TRANSITIONS LIST REPORT for more details on the meaning of these calculations.

Association transition list

The software provides a list and time distribution of the zone association transitions automatically detected and registered by SMARTIO. The following calculations are included within the Zone Association Transition List report:

- Transition Index Nr.
- Transition Rel. Time (Seconds)
- Transition Rel. Time (HH:MM:SS,00)
- Transition Current Zone.
- Transition Time in Zone.
- Accumulated Time.
- Entries Nr.
- Distance in Zone.
- Sync. Timestamp (Seconds).

Please refer to the chapter 21.13 - DATA – ASSOCIATION TRANSITION LIST REPORT for more details on the meaning of these calculations.

Global activity raw data report

Provides a list of all the global activity for each subject for every sample. The following calculations are included within the Global Activity Raw Data Report:

- Sample Index Nr.
- Sample Time (Seconds)
- Sample Time (HH:MM:SS,00)
- Current Global Activity in zone
- Current Global Activity

Please refer to the chapter 21.14-- DATA – GLOBAL ACTIVITY RAW DATA REPORT for more details on the meaning of these calculations.

Creating and editing a report definition

To create a new report definition:

1. Click on the  button located in the toolbar of the panel.
2. Select a base report definition to make the new report. Then click on the Accept button.

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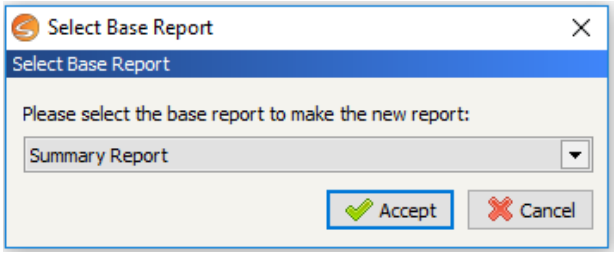
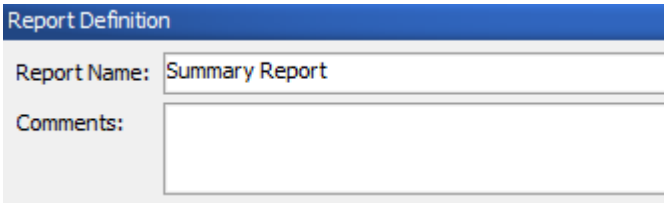


3. Enter the report name and comments of the new report definition.



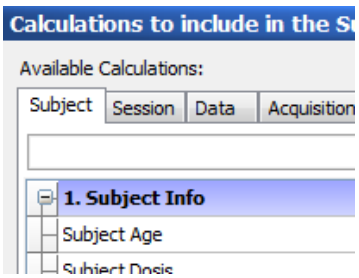
To edit an already existing report definition:

1. Select the report definition to edit in the table and click on the button located in the toolbar of the panel.
2. Alternatively, a double click on the report definition's row in the table exerts the same effect.
3. Enter the new report name and comments.

Selecting the calculations

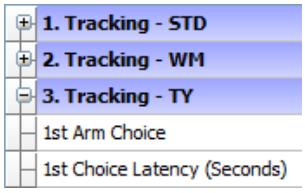
Select the calculations to be included in the report from the Available Calculations section:

1. Select the tab (section) to which the calculations belong:



- **Subject**: contains all the subject's properties configurable within the Subject Database tool.
- **Session**: contains useful information regarding experiments and trials.
- **Data**: contains the main tracking, event and global activity calculations arranged.
- **Acquisition Config.**: contains the information related to the calibration, detection settings and time settings used during the data acquisition process.
- **Analysis Config.**: contains the information related to the thresholds, zone definition, timing configuration and filters applied to generate the analysis reports.

2. Navigate through the calculation groups belonging to the selected section. Sections can be stretched and expanded by using the [-] and [+] buttons located in the first column of the table.



Alternatively, enter a portion of the name of the desired calculations in the **Search calculation** text box to filter all the calculations matching that portion of text. Clean the filter again using the button.

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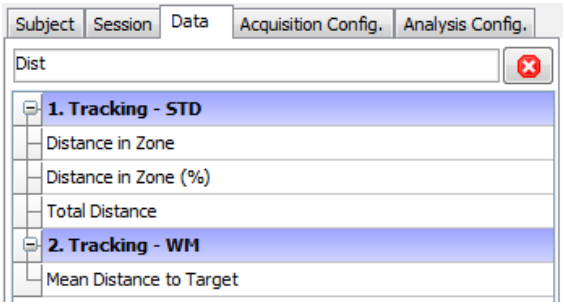

3. Select all the calculations that will be included in the analysis report by using the combination of [CTRL] / [SHIFT] keys and the mouse's left button.
4. Use the right-arrow buttons to move the selected calculations/all calculations to the **Included Calculations** list.

Use the left-arrow buttons to remove calculations that have been included in the list.

Use the top/down-arrows to change the order of the calculation within the list.

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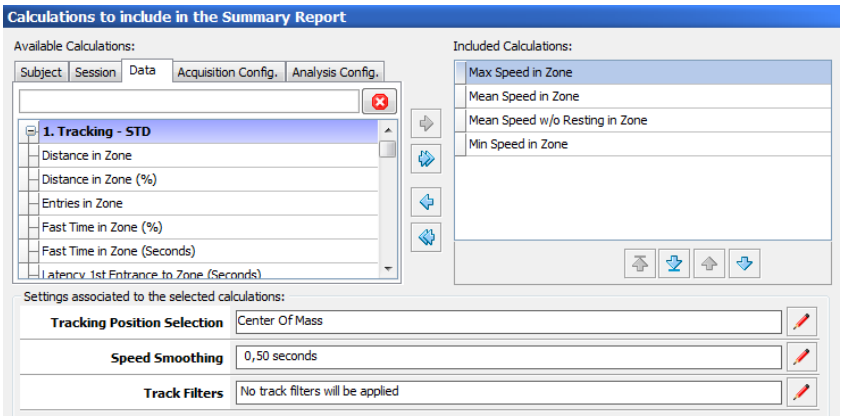
Configuring the calculations

Configure the included calculations with their associated settings:



5. Select a calculation within the **Included Calculations** list. The settings associated with the calculation are shown just below the **Available Calculations** list.
6. The settings associated to the selected calculations may be edited clicking on the button.

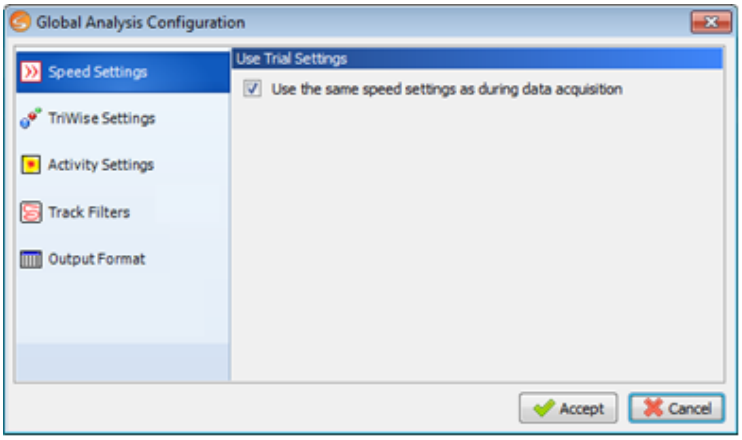
⚠	When a global setting is adjusted, all the calculations that use it are automatically updated with the new setting.

Individual settings associated are accompanied by a button. Edit the value directly in the textbox and press the button to accept the new setting.

⚠	Individual settings only apply to the selected calculation and do not affect any other calculation.

Global analysis configuration

Some calculations included in the list during the analysis process can be adjusted through the Analysis configuration panel. Use the **Edit global analysis configuration** button to access this panel.



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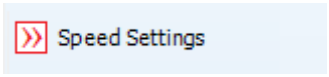
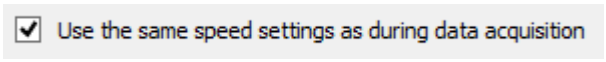
The menu on the left shows the available sections of the analysis settings that can be edited. The following settings can be changed for analysis (depending on the modules and extensions enabled during the trial's data acquisition process):

- Speed Settings
- TriWise Settings
- Activity Settings
- Track Filtering and smoothing
- Format of the output reports

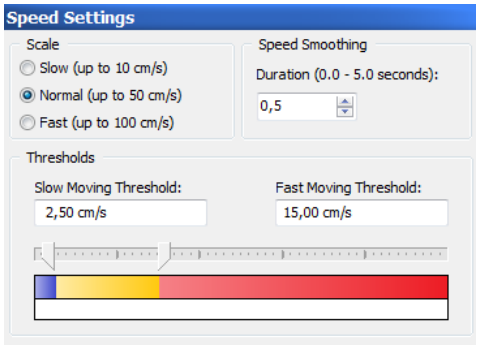
Speed settings



If the option **Use the same speed settings as during data acquisition** is checked, the analysis process will use the settings used during the data acquisition process of every trial. To specify different speed settings for analysis, uncheck this box.

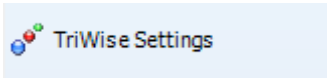


The speed thresholds for all the selected trials can be adjusted after tracking data is acquired.

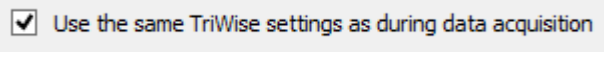


Please refer to chapter 20.4 - Creating and editing a report definition for more details on how to set the new speed settings for the analysis process. Note that the "Arena Selector" tool is not available in this case as every trial is analyzed independently.

TriWise settings



If the box **Use the same TriWise settings as during data acquisition** is checked, the analysis process will use the settings used during the data acquisition process of every trial. To specify different TriWise settings for analysis, uncheck this box.



The TriWise thresholds for all the selected trials can be adjusted after TriWise data was acquired.

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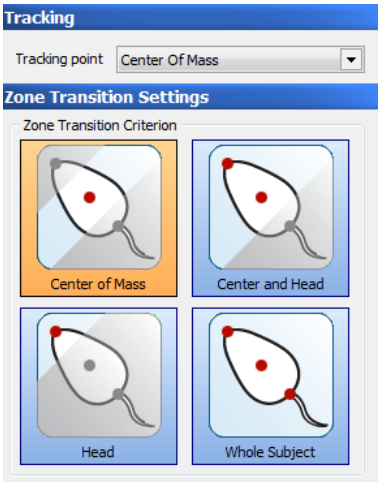
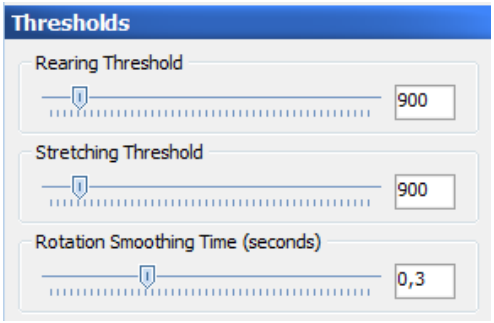


Please refer to chapter 12.3 - TriWise mode additional settings for more information about how to configure the **Tracking Point** and the **Zone Transition Criterion**.

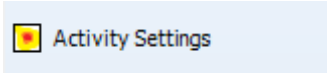
The panel is provided with a threshold selector for Rearing, Stretching and Rotation smoothing time. These configurations are the same that were provided during the data acquisition process using the TriWise detection.



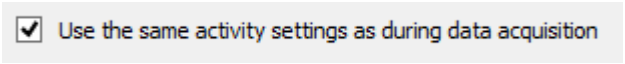
Please refer to chapter 20.4 - Creating and editing a report definition for more details on how to set the new zone transition, rearing, rotation and stretching settings for the analysis process.

Note that the "Arena Selector" tool is not available specifically in this case as every trial is analyzed independently.

Activity settings



If the option **Use the same activity settings as during data acquisition** is checked, the analysis process will use the settings used during the data acquisition process of every trial.
To specify different activity settings for analysis, uncheck this box.



The activity thresholds for all the selected trials can be adjusted after global activity data is acquired.

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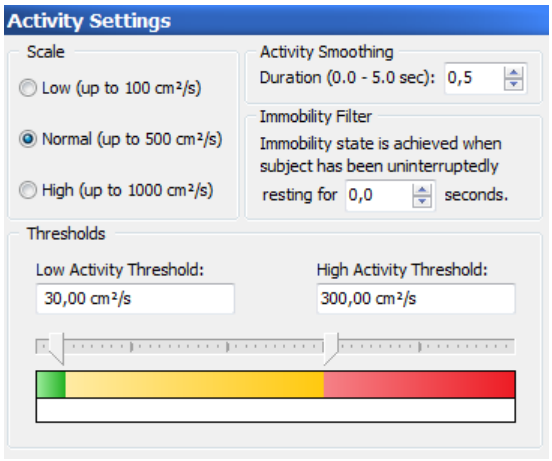
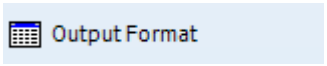
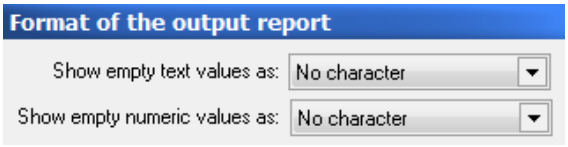


Please refer to chapter 20.4 - Creating and editing a report definition for more details on how to set the new speed settings for the analysis process. Note that the "Arena Selector" tool is not available specifically in this case as every trial is analyzed independently.

Format of the output reports

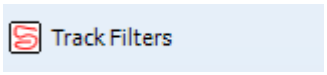


The following options are provided to adjust the content of the reports when the values cannot be evaluated or calculated correctly (in some cases, this is not applicable) during the analysis process.

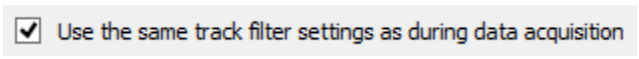


Different options are provided for text and numeric values.

Filtering and smoothing techniques in SMARTIO



If the box **Use the same track filter settings as during data acquisition** is checked, the analysis process will use the settings used during the data acquisition process of every trial.
To specify different track filter settings for analysis, uncheck this box.



Due to the nature of video-tracking systems, some image noise could appear and is typical:

- **Outliers**: momentary observations that are away from other neighboring (in time), increasing dramatically the distance travelled and the mean speed.
- **Body wobble**: small movements such as head scanning or shifts of body weight might affect the position of its center and, consequently, the measured distance and speed, even if it is not displacing.

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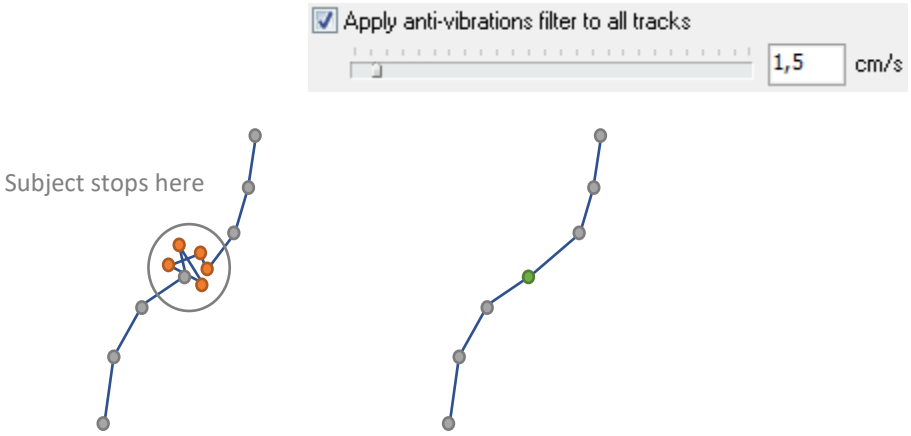
- **Accumulated error**: little variations of the center of mass could imply a considerable error in the total distance travelled.
- **Mislead stop detection**: oscillations of the measured speed producing a poor detection of a stop and the stop's duration.

SMARTIO provides a variety of filtering and smoothing algorithms to substantially improve the precision of the analysis calculations avoiding all these effects.

	Applying filters to the acquired trajectory may drastically impact the final results if they are compared with the results shown in the Runtime Viewer panel.
	Filters and smoothing lead to a different set of position samples (trajectory) and thus a different list of zone entries, permanence time, speed ranges, immobility episodes and, ultimately, stop conditions.

- **Anti-vibrations filter**: During the intervals of time in which the subject's displacement is slower than the configured speed will be automatically ignored so that the animal is considered to be "static" and thus the total distance travelled is not accumulated.



This is an example of how several erratic samples are generated by a poor image's quality during the data acquisition process when the subject stops. The anti-vibrations filter automatically corrects them with a long duration stop without displacement (zero speed).
Drag and drop the marker to set your speed value for the anti-vibrations filter. Click on the marker and use the [LEFT] and [RIGHT] arrow keys to refine the value.

	Setting the anti-vibrations filter too high can cause the system to ignore a real movement of the subject between consecutive frames, resulting in speed values that are not accurate.

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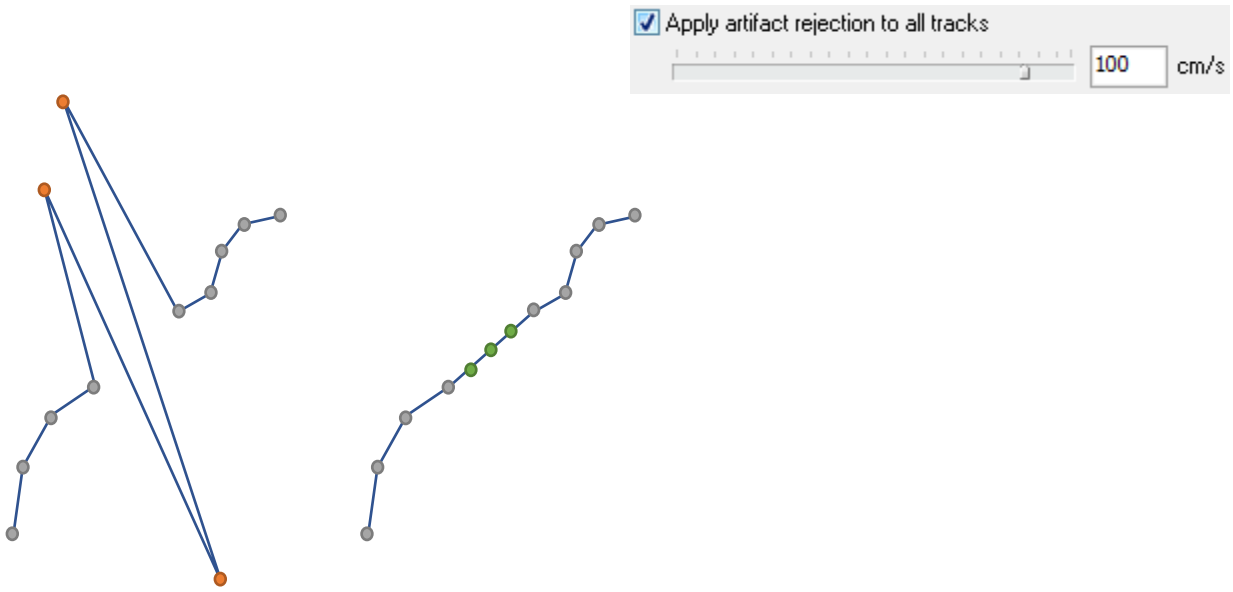
	Rats and mice are usually displaced at 5-10 cm/sec (2-4 inches/s) so an anti-vibration filter lower than 1 cm/s (0.5 inches/s) would be suitable. The maximum intensity of the filter is 25 cm/s (25 inches/s) so that a wider range of experimental conditions are covered.
	This filter is adjusted as a speed value; therefore, the calibration process must be correctly completed before the filter can be utilized. Please refer to chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration for more details on how to calibrate the image source.

- **Artifact rejection filter**: During the intervals of time in which the subject's displacement is faster than the configured speed will be automatically corrected by a linear interpolation of the subject's positions.



This is an example of how several erratic samples generated by an artifact during the data acquisition process are automatically corrected by linear interpolation of the anti-artifacts filter.
Drag and drop the marker to set the particular speed value for the anti-artifacts filter. Click on the marker and use the [LEFT] and [RIGHT] arrow keys to refine the value.

	Setting the anti-artifacts filter too low can cause the system to artificially interpolate a real smooth movement of the subject between consecutive frames, resulting in speed values that are not accurate.
	Rats and mice are usually displaced at 5-10 cm/sec (2-4 inches/s) so an anti-vibration filter lower than 1 cm/s (0.5 inches/s) would be suitable. The maximum intensity of the filter is 25 cm/s (25 inches/s) so that a wider range of experimental conditions are covered.
	This filter is adjusted as a speed value; therefore, the calibration process must be correctly completed before the filter can be utilized. Please refer to chapter 7.1 - Live image source calibration and chapter 7.1 - Video file image calibration for more details on how to calibrate the image source.

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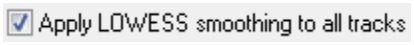
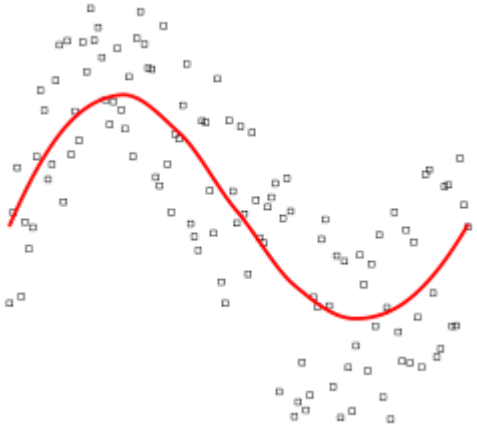
- **LOWESS smoothing algorithm**: LOWESS (LOcally WEighted Scatterplot Smoothing) is a modern regression algorithm designed to generate a more smoothed and thus realistic trajectory of the subjects tracked with SMARTIO.



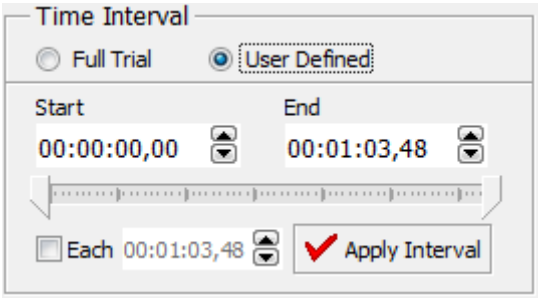
LOWESS combines much of the simplicity of linear least squares regression with the flexibility of nonlinear regression. It does this by fitting simple models to the subject's trajectory to build up a function that describes the deterministic part of the variation in the data, point by point. One of the main attractions of this method is that no specific user-configuration is required. Because it is so computationally demanding, LOWESS cannot be executed during the data acquisition setting but only during the analysis process.



This is an example of how several erratic samples are generated by an artifact during the data acquisition process are automatically corrected by the LOWESS filter during the analysis process.

20.5. SETTING THE TIME INTERVALS

When the trials are inserted in the analysis grid, SMARTIO automatically selects the full intervals to be analyzed. However, it is possible to change this for each trial or for a group of them at the same time.



1. Select the trial or a group of trials in the list (using the [CTRL] and [SHIFT] keys combined with mouse clicks).
2. In the **Time Interval** section (located at the right bottom side of the analysis window), select the **User Defined** option.

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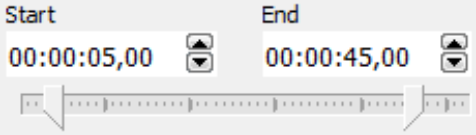
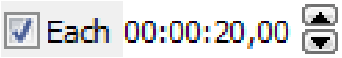
3. Select the portion of the trial to be analyzed by selecting a START and END time by changing the text into Start/End boxes or by moving the markers along the available bar.



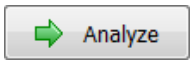
4. To split the calculation into subintervals of time, check the **Each box** and enter the duration of the subintervals to be considered.



5. Click on the **Apply Interval** button to apply the specified time interval settings to the selected trials.

Each subinterval will generate a new group of columns in the numerical analysis report. One additional column for each calculation evaluated by time intervals will be included. If the calculation depends on the zones / associations of interest, additional columns will be also included for each subinterval.

20.6. GENERATING THE REPORTS



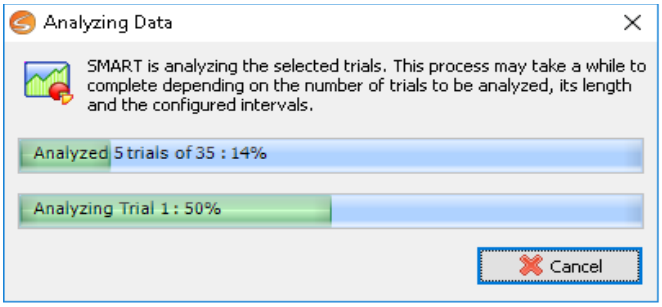
Once the analysis process has been configured, the analysis reports can be generated in two different formats:

- **Numeric Reports**: for which a Microsoft® Excel® spreadsheet is generated including numerical results of the calculations.
- **Graphic Reports**: for which a visual plot is generated. This plot can be later exported to a file in BMP format.

In any case, analysis report generation will start when the Analyze button (located in the bottom right-hand side of the analysis window) is pressed.

Numeric reports

When a numeric report is generated, a progress bar is shown indicating that the data is being analyzed:



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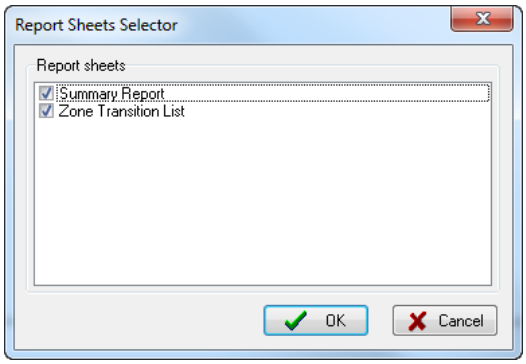
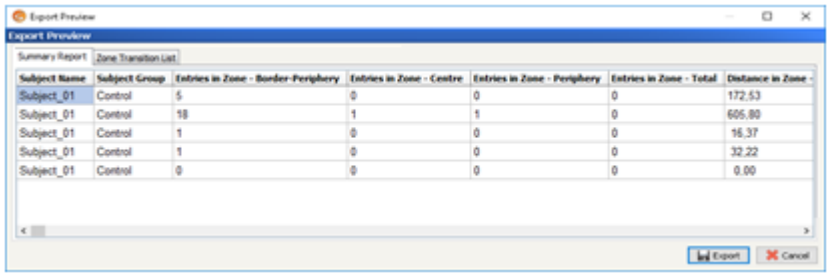
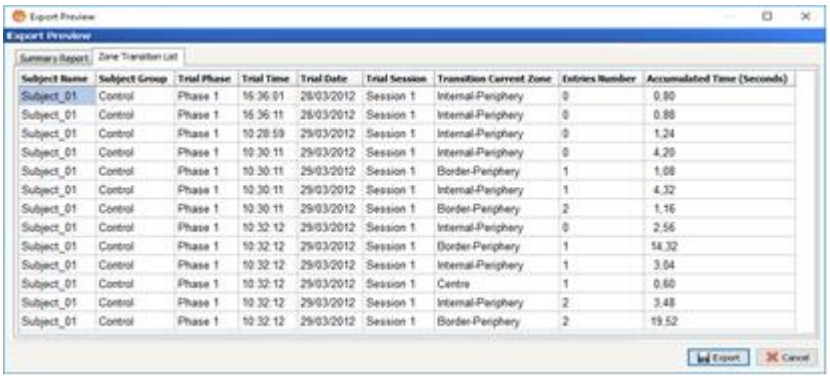
If more than one report definition was applied to the trials, a **Report Sheets Selector** tool is shown providing a way to select which of them needs to be considered.



Check the boxes corresponding to the sheets that might be included in the report and click on the **OK** button. An **Export Preview** window will be shown with all the information calculated during the analysis process and arranged through the selected sheets.





The report can now be exported to Microsoft® Excel® by clicking on the **Export** button. During the export, the user defines the file name.

	At least one Excel sheet is generated per sheet selected to export. Additional sheets may be required if the number of columns exceed the limitations of Microsoft® Excel®.
	SMARTIO suggest saving the Excel® file under the “Report files” path configured in the system. Please refer to chapter 16.1 - PATH SETTINGS for more details on how to configure the default paths.

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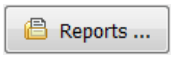
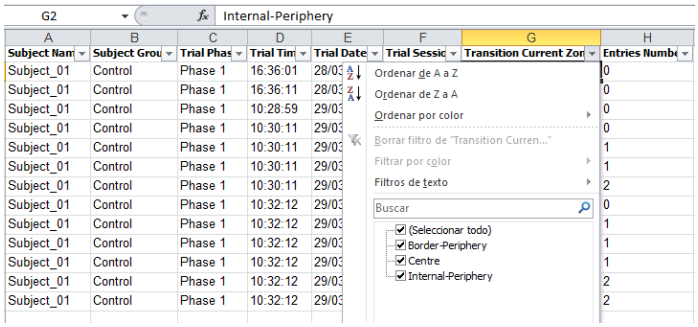


The **Reports** button in the **Analysis** window gives quick access to the folder in which the file was saved.

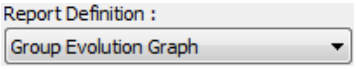
The data arranged in the file can be filtered using the auto filter option within Microsoft® Excel® to select what phase, session, subject, zone, etc. must be reviewed.



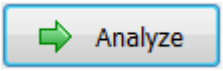
Graphic reports: Group Evolution Graph

SMARTIO group evolution graph is a useful tool to study the evolution of a parameter for a group of subjects and comparing with another groups. The Group Evolution Graph only applies to the calculation available in the SMARTIO summary reports.

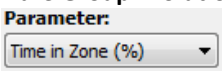
1. Select a set of trials carried out with subjects of different groups in different sessions within the experiment.
2. Apply the zones definition and intervals to analyze each trial.
3. Select the **Group Evolution Graph** option in the **Report definition** list of the analysis window.



4. Start the analysis process.

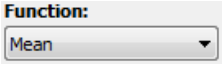


5. In the **Group Evolution Graph** window, select a parameter to study its evolution.

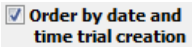


6. Select a specific zone or association in which the parameter will be analyzed.

7. Select the statistical function to apply. This function will be applied to the values of the selected parameter calculated for all the subjects of the same group in a particular session.



8. Check **Order by date and time trial creation** option to sort the sessions from their date and time of creation. If the option is not checked, it will be considered the order in which the trials were selected.



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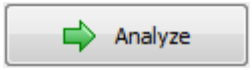
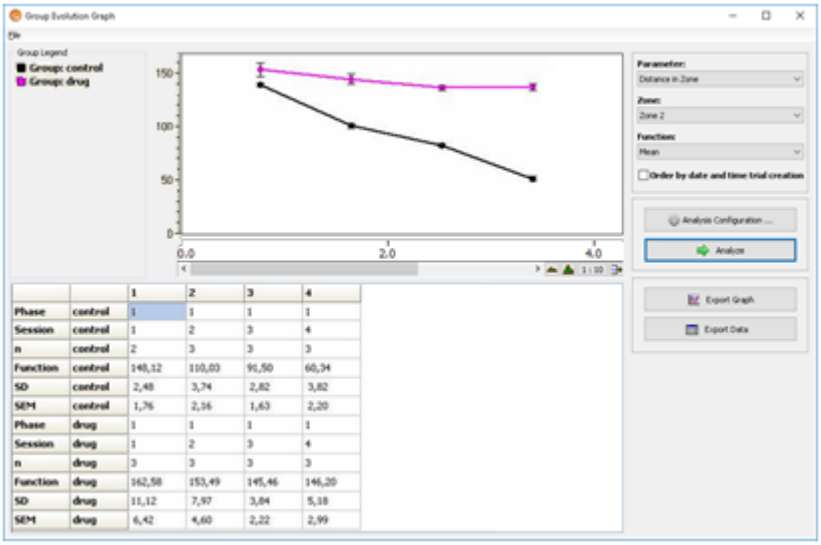
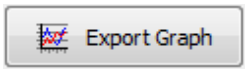
9. Click on the **Analyze** button again to generate and preview the graph.



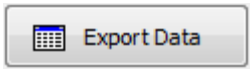


Each data series in the group evolution graph represents a different subject group within the selected trials. Each point represents the value of the statistical function for each session and subject group. Standard deviations are also shown. The numeric information is also calculated to facilitate the understanding of the plot. Both numeric and graphic data can be exported by means of the buttons located at the right side of the window:

- **Export Graph**: To export the plot to a BMP file.



- **Export Data**: To export the statistical results to an Excel® file.



20.7. ANALYZING DATA. SMARTIO FOR TRIGGERING THIRD-PARTY SYSTEM

This chapter provides some information about the analyses specificities related to the use of SMARTIO in a third-party stimulation system (such as laser optogenetics).

- The SMARTIO software can trigger a third-party system through the activation of 2 output channels of the Panlab LINKBOX interface.
- A data report of the timing of these trigger instructions can be exported by SMARTIO through the Even Trigger report.
- The data of the Even Trigger report and all the other SMARTIO behavioral event reports can be then exported to another analysis/statistical software such as MATLAB for further analysis and correlations.

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21. DATA MEANING

21.1. SUBJECT

SUBJECTS INFO

Subject Age

Reports	All reports
Description	Age of the subject used in the experiment.
Units	User-defined units
Origin of the data	Experimentation Assistant/Subject Data base
Created/Defined by	User

Subject Dose

Reports	All reports
Description	Dose of the treatment given by the subject used in the experiment (if present).
Units	User-defined unit
Origin of the data	Experimentation Assistant/Subject Data base
Created/Defined by	User

Subject Extra-Field

Reports	All reports
Description	Additional information/comment about the subject used in the experiment.
Origin of the data	Experimentation Assistant/Subject Data base
Created/Defined by	User

Subject Gender

Reports	All reports
Description	Gender of the subject used in the experiment.
Origin of the data	Experimentation Assistant/Subject Data base
Created/Defined by	User

Subject Genotype

Reports	All reports
Description	Genotype of the subject used in the experiment.
Scientific meaning	The genotype is the genetic background of the subject used in the experiment. Example: Wild-type, DAT-KO etc.
Origin of the data	Experimentation Assistant/Subject Data base
Created/Defined by	User

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Subject Group

Reports	All reports
Description	Name of the group of the subject used in the experiment.
Origin of the data	Experimentation Assistant/Subject Data base
Created/Defined by	User

Subject Name

Reports	All reports
Description	Name of the subject used in the experiment.
Origin of the data	Experimentation Assistant/Subject Data base
Created/Defined by	User

Subject Phenotype

Reports	All reports
Description	Phenotype of the subject used in the experiment.
Scientific meaning	The phenotype is the observable characteristics or traits of the subject, resulting from the expression of its genotype in a given environment.
Origin of the data	Experimentation Assistant/Subject Data base
Created/Defined by	User

Subject Treatment

Reports	All reports
Description	Treatment given to the subject used in experiment (if present).
Origin of the data	Experimentation Assistant/Subject Data base
Created/Defined by	User

21.2. SESSION

EXPERIMENT INFO

Exp. Code

Reports	All reports
Description	Code provided to the experiment
Origin of the data	View/Experiment Info panel
Created/Defined by	User

Exp. Comments

Reports	All reports
Description	Additional information/comment about the subject
Origin of the data	View/Experiment Info panel
Created/Defined by	User

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Exp. File Date

Reports	All reports
Data Subsection	Experiment Info
Description	Date of creation of the SMARTIO experimental file containing the analyzed trial (synchronized with the date of the computer).
Units	The format of the date depends on the regional configuration of the computer used.
Origin of the data	View/Experiment Info panel
Created/Defined by	SMARTIO (automated field)

Exp. File Date Last Modif.

Reports	All reports
Description	Date of the last modification of the SMARTIO experimental file containing the analyzed trial (synchronized with the date of the computer).
Units	The format of the date depends on the regional configuration of the computer used.
Origin of the data	Saving process
Created/Defined by	SMARTIO (automated field)

Exp. File Name

Reports	All reports
Description	Name of the experimental file given by the User when the file has been saved for the first time.
Origin of the data	Saving process
Created/Defined by	User

Exp. File Time

Reports	All reports
Description	Time of creation of the SMARTIO experimental file containing the analyzed trial (synchronized with the time of the computer).
Units	The format of the time depends on the regional configuration of the computer used. Commonly expressed in HH:MM:SS
Origin of the data	View/Experiment Info panel
Created/Defined by	SMARTIO (automated field)

Exp. File Time Last Modif.

Reports	All reports
Description	Time of the last modification of the SMARTIO experimental file containing the analyzed trial (synchronized with the time of the computer).
Units	The format of the time depends on the regional configuration of the computer used. Commonly expressed in HH:MM:SS
Origin of the data	Saving process
Created/Defined by	SMARTIO (automated field)

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Exp. Module Type

Reports	All reports
Description	Name of the SMARTIO module used for creating experimental file containing the analyzed trial.
Origin of the data	Starting assistant choice or File/new choice
Created/Defined by	SMARTIO (automated field)

Experimenter

Reports	All reports
Description	Name of the Experimenter who registered the analyzed trial
Origin of the data	View/Experiment Info panel
Created/Defined by	User

TRIAL INFO

Trial Arena

Reports	All reports
Description	Name/number of the Zone definition Arena in which the trial has been registered.
Origin of the data	Experimentation Assistant/Arenas, Zones Definition
Created/Defined by	User

Trial Comments

Reports	All reports
Description	Comments associated to the trial and entered during the data acquisition process.
Origin of the data	Experimentation Assistant/Scheduler
Created/Defined by	User

Trial Date

Reports	All reports
Description	Date of registration of the analyzed trial (synchronized with the date of the computer).
Units	The format of the date depends on the regional configuration of the computer used.
Origin of the data	Saving process (computer date)
Created/Defined by	SMARTIO (automated parameter)

Trial Duration (HH:MM:SS,00)

Reports	All reports
Description	Total duration of the registered trial.
Units	HH:MM:SS,00.
Origin of the data	Trial registration process
Created/Defined by	SMARTIO (automated parameter)

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Trial Image Source

Reports	All reports
Description	Name of the Image Source used during the trial.
Origin of the data	Saving process (computer date)
Created/Defined by	User

Trial Name

Reports	All reports
Description	Name/number of the trial.
Origin of the data	Experimentation Assistant/Scheduler
Created/Defined by	User

Trial Phase

Reports	All reports
Description	Name of the experimental phase in which the Trial has been registered.
Origin of the data	Experimentation Assistant/Scheduler
Created/Defined by	User

Trial Sample Number

Reports	All reports
Description	Total number of data samples registered during the Trial. The sample number depends on the sampling time and on the duration of the trial
Formula	Sample number = Trial duration / Sampling Time.
Units	Seconds.
Scientific meaning	This information reflects the number of trajectory sample points registered per each unit of time.
Created/Defined by	SMARTIO (automated parameter)

Trial Sampling Time (Seconds)

Reports	All reports
Description	The interval of time between two consecutive registered data samples in analyzed trial. The sampling time depends on the image source used during the experiment.
Units	Seconds.
Scientific meaning	This information reflects the number of trajectory sample points registered per each unit of time.
Origin of the data	Experimentation Assistant/Image source/Cameras considerations
Created/Defined by	SMARTIO (automated parameter)

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Trial Session

Reports	All reports
Description	Name of the experimental session in which the Trial has been registered.
Origin of the data	Experimentation Assistant/Scheduler
Created/Defined by	User

Trial Time

Reports	All reports
Description	Time of registration of the analyzed trial (synchronized with the time of the computer).
Units	The format of the time depends on the regional configuration of the computer used. Commonly expressed as HH:MM:SS.
Origin of the data	Saving process (computer time)
Created/Defined by	SMARTIO (automated parameter)

21.3. DATA – SUMMARY REPORT

TRACKING – STD

Distance in Zone

Description	Distance covered by the subject in the zones defined in the analyzed trial.
Units	Calibration units (cm or inches)
Scientific meaning	Quantitative evaluation of animal locomotor activity.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest. Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Distance in Zone (%)

Description	Percentage of distance covered by the subject in the zones of the zone definition associated to the analyzed trial.
Formula & Special cases	% Distance in Zone = (Time in Zone x100)/Total Distance
Units	%
Scientific meaning	Quantitative evaluation of animal locomotor activity.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Track Filters Tracking point selection (only if TW extension is used)

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	Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Entries in zone

Description	Number of subject entries into the zones of the zone definition associated to the analyzed trial.
Formula & Special cases	The zone in which the subject begins the trial is not counted as an entry.
Units	Number of entries.
Scientific meaning	This parameter can be used for the interpretation of data in some experimental paradigms. Here some examples: • Evaluation of animal locomotor activity. • Evaluation of animal preference respect to a given zone in a maze used as anxiety, memory, rewarding, exploration (etc.) index.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Fast Speed Time in Zone (%)

Description	Percentage of time spent in Fast speed state and calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	% Time Fast in Zone = (Time Fast in Zone x 100)/(Time Resting + Time Slow + Time Fast)
Units	%
Scientific meaning	Qualitative evaluation of animal locomotor activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Track Filters Speed smoothing time Speed threshold Resting/Slow Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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Fast Speed Time in Zone (Seconds)

Description	Time spent in Fast speed and calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	The subject is considered in Fast state when its speed is ≥ to the user-defined Slow/Fast speed threshold setting.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal locomotor activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Speed smoothing time Speed threshold Resting/Slow Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Fast Speed Total Duration (Seconds)

Description	Total time spent in Fast speed during the analyzed trial
Formula & Special cases	The subject is considered in Fast state when its speed is ≥ to the user-defined Slow/Fast speed threshold setting.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal locomotor activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Speed smoothing time Speed threshold Resting/Slow Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Latency 1st Entrance in Zone (Seconds)

Description	Time elapsed until the first entry of the subject into the zones of the zone definition associated to the analyzed trial.
Formula & Special cases	The zone in which the subject begins the trial is not counted as an entry.
Units	Seconds
Scientific meaning	This parameter can be used for the interpretation of data in some experimental paradigms. Here some examples:

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	• Evaluation of animal anxiety state. • Evaluation of animal response time (arm choice…)
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest

Max Speed in Zone

Description	Max speed reached by the subject and calculated for each zone of the zone definition associated to the analyzed trial.
Units	Units of calibration (cm or inches)/seconds
Scientific meaning	Qualitative evaluation of animal locomotor activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Speed smoothing time Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Mean Speed in Zone

Description	Mean speed of subject calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	Mean Speed in Zone = Distance covered in Zone / Time in Zone
Units	Units of calibration (cm or inches)/seconds
Scientific meaning	Qualitative evaluation of animal locomotor activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Speed smoothing time Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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Mean Speed w/o Resting in Zone

Description	Mean speed of subject displacement (excluding periods in which the subject is immobile) calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	Mean Speed w/o Resting in Zone = (Distance covered in Zone – Distance covered when the subject is Resting state in Zone) / (Time in Zone – Time spent in Resting state)
Units	Units of calibration (cm or inches)/seconds
Scientific meaning	Qualitative evaluation of animal locomotor activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Speed smoothing time Speed threshold Resting/Slow Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Min Speed in Zone

Description	Minimal speed reached by the subject and calculated for each zone of the zone definition associated to the analyzed trial.
Units	Units of calibration (cm or inches)/seconds
Scientific meaning	Qualitative evaluation of animal locomotor activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Speed smoothing time Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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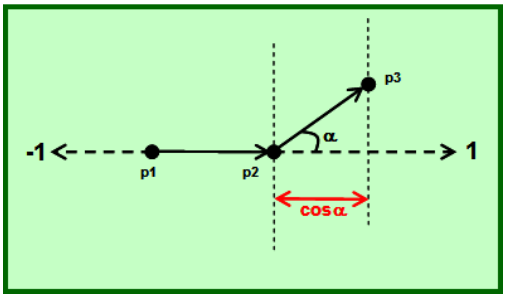
Parallel Index in Zone

Description	The parallel index indicates how parallel is a given direction of the animal's single movement in comparison to the direction of its previous single movement.
Formula & Special cases	The angle of the path between the current direction of movement (p2 to p3, see illustration) and the previous direction of movement (p1 to p2) is considered for calculation the parallel index. If this angle is < 20°, the value of the cosine of this angle is considered for this sample in the parallel index calculation.  If this angle is < 90°, the value 0 is considered for this sample in the parallel index calculation. If this angle is > 90°, the value -1 is considered for this sample in the parallel index calculation. The samples corresponding to track displacement < 0.5 cm are not taken into consideration in the calculation. The parallel index is the average of all the values of the considered samples. The possible values of parallel index are then between -1 and 1. The more the index is closed to 1, the more the animal follows a straight line. The more the index is -1, the more the animal changes direction in its displacements.
Scientific meaning	The parallel index has been proposed to reflect the overall tendency to turn and the angular magnitude of turns. The parallel index is not directly dependent on the distance covered by the animal and seems to significantly reflect subtle changes in the pattern of locomotor activity that is characteristic of the exploration of an unfamiliar environment compared to the locomotor movement in frequently visited areas. It is postulated that the parallel index decreases with the familiarity of the area being explored. The parallel index can also be used for characterizing the effect of a drug on the pattern of animal locomotor displacements (as an example, its value is increased by amphetamine).
Created/Defined by	SMARTIO (automated calculation
Related analysis settings	Zones Definition Zone of interest Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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Resting Time in Zone (%)

Description	Percentage of time spent in Resting state (immobile) and calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	% Time Resting in Zone = (Time Resting in Zone x 100)/(Time Resting + Time Slow + Time Fast)
Units	%
Scientific meaning	Qualitative evaluation of animal locomotor activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Speed smoothing time Speed threshold Resting/Slow Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Resting Time in Zone (Seconds)

Description	Time spent in Resting state and calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	The subject is considered in Resting state when its speed is < to the user-defined Resting/Slow speed threshold setting.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal locomotor activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Speed smoothing time Speed threshold Resting/Slow Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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Resting Total Duration (Seconds)

Description	Total time spent in resting state during the analyzed trial
Formula & Special cases	The subject is considered in Resting state when its speed is < to the user-defined Resting/Slow speed threshold setting.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal locomotor activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Speed smoothing time Speed threshold Resting/Slow Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Sample Number in Zone

Description	Total number of data samples registered in each zone during the Trial. The sample number depends on the sampling time and of the time spent in the zone.
Formula & Special cases	Sample number = Time spent in the zone / Sampling Time.
Origin of the data	Image source
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Slow Speed Time in Zone (%)

Description	Percentage of time spent in Slow speed state and calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	% Time Slow in Zone = (Time Slow in Zone x 100)/(Time Resting + Time Slow + Time Fast) Units %
Units	%
Scientific meaning	Qualitative evaluation of animal locomotor activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Speed smoothing time Speed threshold Resting/Slow

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	Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Slow Speed Time in Zone (Seconds)

Description	Time spent in Slow speed and calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	The subject is considered in Slow state when its speed is ≥ to the user-defined Resting/Slow speed threshold setting and < to the user-defined Slow/Fast speed threshold setting.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal locomotor activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Speed smoothing time Speed threshold Resting/Slow Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Slow Speed Total Duration (Seconds)

Description	Total time spent in Slow speed during the analyzed trial
Formula & Special cases	The subject is considered in Slow state when its speed is ≥ to the user-defined Resting/Slow speed threshold setting and < to the user-defined Slow/Fast speed threshold setting.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal locomotor activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Speed smoothing time Speed threshold Resting/Slow Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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Time in Zone (%)

Description	Percentage of time spent by the subject for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	% Time in Zone = (Time in Zone x100)/Total Time
Units	%
Scientific meaning	The evaluation of animal position or preference respect to a given zone in a maze is a key aspect in paradigms to measure anxiety, memory, rewarding, exploration.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Time in Zone (Seconds)

Description	Time spent by the subject in the zones of the zone definition associated to the analyzed trial.
Units	Seconds
Scientific meaning	The evaluation of animal position or preference respect to a given zone in a maze is a key aspect in paradigms to measure anxiety, memory, rewarding, exploration.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Time out of the Zone (Seconds)

Description	Time spent out of a user-defined zone.
Units	Seconds
Scientific meaning	In case of the use of a zone definition with more than 2 zones, this calculation allows getting a fast evaluation of the time spent by the animal out of one of these zones (avoidance behavior).
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest

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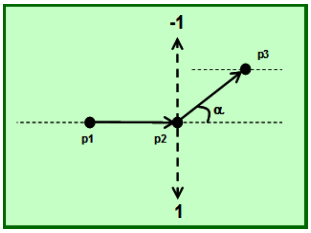
	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Total Distance

Description	Total distance covered by the subject during the analyzed trial.
Units	Calibration units (cm or inches)
Scientific meaning	Quantitative evaluation of animal locomotor activity.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Turning Tendency in Zone

Description	The turning tendency gives an indication of the pattern of rotation of the animal trajectory.
Formula & Special cases	The turning tendency is calculated for each sample of time considering the angle between the current direction of movement (p2 to p3, see illustration) and the previous direction of movement (p1 to p2).  A positive value of 1 is given to each sample which angle corresponds to a rotation to the right direction. A negative value of -1 is given to each sample which angle corresponds to a rotation to the right direction. The samples corresponding to track displacement < 0.5 cm are not taken into consideration in the calculation. The turning tendency index is the average of all the value of the considered samples.
Scientific meaning	The turning tendency calculation is of particular interest in any experiment in which the animal is expected to have a turning tendency, as an example, in studies on animal models of Parkinson disease with unilateral lesions in the dopaminergic nigrostriatal system. In that case, the amount of rotation is correlated with the volume of the lesion.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Track Filters Zone Transition Criterion (only if TW extension is used)

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	START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Zone Transition Number

Description	Total number of zone transitions made by the subject in the analyzed trial.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

TRACKING WATER-MAZE (WM)

Distance to Target

Description	Distance covered by the subject until reaching the Target zone.
Formula & Special cases	In the zone definition of the trial to analyze, at least one zone must be defined as Target (Zone definition, Zones and Associations management). If several zones are defined as “Target”, the value of this calculation is the distance covered until the subject is detected in the first of those zones (and fulfilling the timing STOP condition). When the Target zone is not reached by the subject during the trial, the value given is the distance covered until the trial ends.
Units	Unit of calibration (cm or inches)
Scientific meaning	The evolution of the value of this parameter is used as an index of spatial memory in the Morris water maze test.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (a zone must be defined as “Target” zone). Timings STOP conditions Track Filters Tracking point Criterion (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings
Distribution mode	Calculated only for the Target zone

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Latency 1st Entrance to Target (Seconds)

Description	Time elapsed until the subject enters the Target zone for the first time.
Formula & Special cases	In the zone definition of the trial to analyze, at least one zone must be defined as Target (Zone definition, Zones and Associations management). If several zones are defined as “Target”, the latency is the time until the subject is detected in the first of those zones. When the Target zone is not reached by the subject during the trial, the system returns an empty field.
Units	Seconds
Scientific meaning	This calculation helps in the interpretation relative to the Latency to Target calculation in the Morris water maze test.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (a zone must be defined as “Target” zone). Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings
Distribution mode	Only calculated for the Target zone

Latency to Target (Seconds)

Description	Time elapsed until the subject enters the Target zone for the first time.
Formula & Special cases	In the zone definition of the trial to analyze, at least one zone must be defined as Target (Zone definition, Zones and Associations management). If several zones are defined as “Target”, the latency is the time until the subject is detected in the first of those zones. When the Target zone is not reached by the subject during the trial, the system returns an empty field.
Units	Seconds
Scientific meaning	This calculation helps in the interpretation relative to the Latency to Target calculation in the Morris water maze test.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (a zone must be defined as “Target” zone). Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings
Distribution mode	Only calculated for the Target zone

Mean Directionality to Target

Description	The mean directionality is the numerical average of all absolute values of directionality expressed in degrees defined as a measure of the extent to which the path heads directly towards the Target zone (platform) as against veering in different directions.
Formula & Special cases	In the zone definition of the trial to analyze, at least one zone must be defined as Target (Zone definition, Zones and Associations management).

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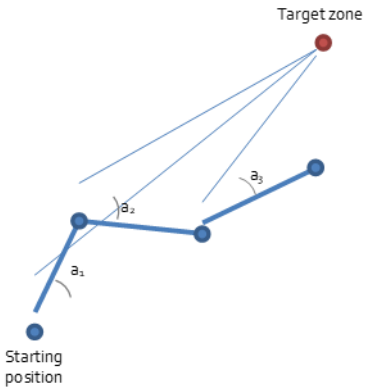
	Formula = ArcTan(Avg(Sin(a)) / Avg(Cos(a))). Where ArcTan = Tangential Arc (inverse of tangent) Avg = Average Sin = Sine Cos = Cosine a =angle between the vector of the current direction of the subject path respect to the vector representing the direct path heading to center of the target zone.  The portions of the track with displacement < 0.5 cm are not taken into consideration in the calculation.
Units	Unit of calibration (cm or inches)
Scientific meaning	When MDT is close to 0º, the subject's trajectory tends to head directly to the target zone. When MDT is close to 90º, the subject's trajectory tends to take an "east" direction perpendicular to the target zone direction. When MDT is close to 180º, the subject's trajectory tends to take a direction opposite to the target zone direction. When MDT is close to 270º, the subject's trajectory tends to take a "west" direction perpendicular to the target zone direction
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (a zone must be defined as "Target" zone). Timings STOP conditions Track Filters Tracking point Criterion (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings
Distribution mode	Only calculated for the Target zone

Mean Distance to Target

Description	The mean distance to target calculates the shortest distance between the tracked point of the subject and the target zone and gives the average of this value by unit of time.
Formula & Special cases	In the zone definition of the trial to analyze, at least one zone must be defined as Target (Zone definition, Zones and Associations management).

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	If several zones are defined as “Target”, the value of this calculation is the distance covered until the subject is detected in the first of those zones (and fulfilling the timing STOP condition).
Units	Unit of calibration (cm or inches)
Scientific meaning	In the Morris water maze test, this parameter gives an index of how much a subject has been close to the platform during the trial.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (a zone must be defined as “Target” zone). Timings STOP conditions Track Filters Tracking point Criterion (only if TW extension is used) Zone Transition Criterion (only if TW extension is used)
Distribution mode	Only calculated for the Target zone

Target Crossings

Description	Number of times the subject has crossed the target zone (platform) during the trial.
Formula & Special cases	In the zone definition of the trial to analyze, at least one zone must be defined as Target (Zone definition, Zones and Associations management). If several zones are defined as “Target”, the number crossing will be the total number of target crossings of all the zones together.
Scientific meaning	The former platform crossings made during the probe phase of the Morris water maze test can be used as an index of memory.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (a zone must be defined as “Target” zone). Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings
Distribution mode	Only calculated for the Target zone

Whishaw’s Error

Description	The Whishaw’s error is the percentage time the path of the subject is outside the beeline corridor defined by the user between the starting point of the track and the center of mass of the Target zone (platform).
Formula & Special cases	In the zone definition of the trial to analyze, at least one zone must be defined as Target (Zone definition, Zones and Associations management). The Whishaw’s error corridor Width is defined by the user when selecting the calculation to be included in the report (in the section “Settings associated to the selected calculation”)

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	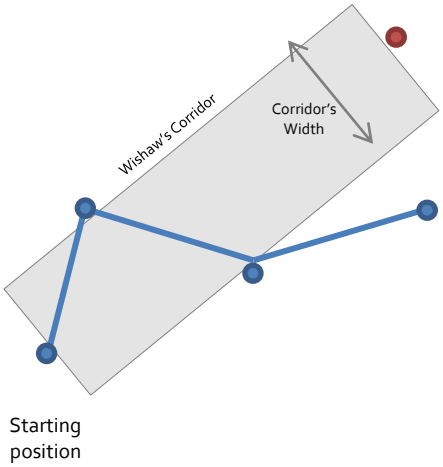 Whishaw's Error = ((Tt – TC) x 100)/Tt Tt = Total duration of the trial TC = Time spent in the Whishaw's corridor If the entire subject's track is contained in the Whishaw's corridor the value of the Whishaw's error is 0%. If the entire subject's track is out of the Whishaw's corridor the value of the Whishaw's error is 100%.
Units	The unit considered for both the corridor width and Whishaw's error calculation is the unit of calibration used during the acquisition of each trial (cm or inches)
Scientific meaning	This parameter has been defined as a measure of average directionality to the target zone.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (a zone must be defined as "Target" zone). Corridor Width Timings STOP conditions Track Filters Tracking point Criterion (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings

TRACKING T/Y-MAZE (TY)

1st Arm Choice

Description	Name of the first arm chosen/visited by the subject.
Formula & Special cases	The zones corresponding to the 3 arms of the T/Y maze and a Middle zone must be selected in the zone definition associated to the trials to analyze (section "Settings for Zone Dependent Calculations/Triplet Settings)
Scientific meaning	This calculation is used in T- and Y-maze procedures (two-trial task) as an index of spatial working memory.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (the arms must be defined in the Triplet settings).

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	Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings

1st Choice Latency (Seconds)

Description	Time elapsed until the subject makes its first arm choice.
Formula & Special cases	The zones corresponding to the 3 arms of the T/Y maze and a Middle zone must be selected in the zone definition associated to the trials to analyze (section "Settings for Zone Dependent Calculations/Triplet Settings)
Units	Seconds
Scientific meaning	This calculation is used in T- and Y-maze procedures (two-trial task) as an index of spatial working memory.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (the arms must be defined in the Triplet settings). Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings

Alternation Triplet

Description	Number of 3 consecutive entries in different arms.
Formula & Special cases	The zones corresponding to the 3 arms of the T/Y maze and a Middle zone must be selected in the zone definition associated to the trials to analyze (section "Settings for Zone Dependent Calculations/Triplet Settings)
Scientific meaning	This calculation is used in T- and Y-maze procedures (two-trial task) as an index of spatial working memory.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (the arms must be defined in the Triplet settings). Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings

Alternation Triplet (%)

Description	Percentage number of alternations triplet respect to the maximal number of possible alternations.
Formula & Special cases	Alternation Triplet % = (Number of alternation triplet x 100)/(Max Alternation Triplet) See details of Alternation Triplet calculation in the corresponding section
Units	%
Scientific meaning	This calculation is used in T- and Y-maze procedures (two-trial task) as an index of spatial working memory.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (the arms must be defined in the Triplet settings). Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings

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Max Alternation Triplet

Description	Maximal number of possible alternations.
Formula & Special cases	Max Alternation Triplet = Total Arm Entries – 2 See details about the calculation of Total Arm Entries in the corresponding section.
Scientific meaning	This calculation is used in T- and Y-maze procedures (two-trial task) as an index of spatial working memory.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (the arms must be defined in the Triplet settings). Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings

Total Arm Entries

Description	Total number of entries into each arm.
Formula & Special cases	The zones corresponding to the 3 arms of the T/Y maze and a Middle zone must be selected in the zone definition associated to the trials to analyze (section "Settings for Zone Dependent Calculations/Triplet Settings)
Scientific meaning	This calculation is used in T- and Y-maze procedures (two-trial task) as an index of spatial working memory.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (the arms must be defined in the Triplet settings). Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings

TRACKING CONDITIONED PLACE PLATFORM (CPP)

Relative Time in Zone (%)

Description	% expression of the Relative Time in Zone (for more details see definition of this calculation in this chapter) respect to the total time spent into the zones considered in the calculation.
Formula & Special cases	A zone which time will be distributed to all the other zones must be selected in the zone definition associated to the trials to analyze (section "Settings for Zone Dependent Calculations/Relative Time Settings) Relative time in zone (n) % = (Relative time in zone (n) x 100)/Total time. n = name of the zone. See details of the calculation of the Relative time in zone in the related section.
Units	%
Scientific meaning	This calculation is commonly used in the conditioned place preference or aversion experimental paradigm in which a box with 3 zones is used (2 compartments and 1 corridor). The relative time into the compartments is used to proportionally distribute the time spent into the corridor into the value of the time spent into the 2 other compartments of the box. This operation normalizes the data between each subject and allows comparing the % of time spent in each of the compartments associated to the Drug or Placebo treatments.

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Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (a “Distributed” zone must be selected) Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings
Distribution mode	Calculated by considering all the zones included in the zone definition file but the data is shown only for the zones defined by the user as zone of interest.

Relative Time in Zone (Seconds)

Description	The relative time in a zone can be calculated in a zone definition containing more than 1 zone. In that case, the time spent in one user-defined zone is distributed to all the other zones of the zone definition associated to the analyzed trial, in a way that maintains constant the total duration of the trial. The recalculated time spent into the “receiving zone” is then named “relative time”.
Formula & Special cases	A zone which time will be distributed to all the other zones must be selected in the zone definition associated to the trials to analyze (section “Settings for Zone Dependent Calculations/Relative Time Settings) Example of a zone definition with 3 zones: A, B, C. The C zone is the “distributed” one, so its time will be distributed to all the other zones of the zone definition. TrA = (TA x (TA +TB+TC))/(TA + TB). and TrB = (TB x (TA +TB+TC))/(TA + TB). Where, TrA = Relative Time in Zone A TA = time spent in Zone A TB = time spent in Zone B TC = time spent in Zone C This calculation is independent of the selection of “zone of Interest”.
Units	Seconds
Scientific meaning	This calculation is commonly used in the conditioned place preference or aversion experimental paradigm in which a box with 3 zones is used (2 compartments and 1 corridor). The relative time into the compartments is used to proportionally distribute the time spent into the corridor into the value of the time spent into the 2 other compartments of the box. This operation normalizes the data between each subject and allows comparing the % of time spent in each of the compartments associated to the Drug or Placebo treatments.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition (a “Distributed” zone must be selected) Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings
Distribution mode	Calculated by considering all the zones included in the zone definition file but the data is shown only for the zones defined by the user as zone of interest.

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CCW Rotation Duration Total (Seconds)

Description	Total time spent doing counterclockwise rotations in the analyzed trial.
Units	Seconds
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

CCW Rotation Duration in Zone (Seconds)

Description	Time spent doing counterclockwise rotations calculated for each zone of the zone definition associated to the analyzed trial.
Units	Seconds
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

CCW Rotation Latency (Seconds)

Description	Time elapsed until the first counterclockwise Rotation event is detected with the TriWise extension.
Units	Seconds
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that

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	case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

CCW Rotation Mean Duration (Seconds)

Description	Mean duration of the counterclockwise rotation events detected in the analyzed trial with the TriWise extension.
Formula & Special cases	CCW Rotation Mean Duration = CCW Rotation Duration Total / CCW Rotation Number Total
Units	Seconds
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

CCW Rotation Number Total

Description	Total number of counterclockwise rotation events detected in the analyzed trial using the TriWise extension.
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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CCW Rotation Number in Zone

Description	Total number of counterclockwise rotation events detected using the TriWise extension and calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	The number of counterclockwise rotation events detected in each zone is considered as the number of counterclockwise rotation events that have been initiated in this zone.
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

CCW Rotation Rate (ev./min)

Description	Number of counterclockwise rotation events detected in the analyzed by unit of time.
Units	Events/minute
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

CW Rotation Duration Total (Seconds)

Description	Total time spent doing clockwise rotations in the analyzed trial.
Units	Seconds
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that

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	case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

CW Rotation Duration in Zone (Seconds)

Description	Time spent doing clockwise rotations calculated for each zone of the zone definition associated to the analyzed trial.
Units	Seconds
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

CW Rotation Latency (Seconds)

Description	Time elapsed until the first clockwise Rotation event is detected with the TriWise extension.
Units	Seconds
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings

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Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

CW Rotation Mean Duration (Seconds)

Description	Mean duration of the clockwise rotation events detected in the analyzed trial with the TriWise extension.
Formula & Special cases	CW Rotation Mean Duration = CW Rotation Duration Total / CW Rotation Number Total
Units	Seconds
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

CW Rotation Number Total

Description	Total number of clockwise rotation events detected in the analyzed trial using the TriWise extension.
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

CW Rotation Number in Zone

Description	Total number of clockwise rotation events detected using the TriWise extension and calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	The number of clockwise rotation events detected in each zone is considered as the number of clockwise rotation events that have been initiated in this zone.
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model

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	of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

CW Rotation Rate (ev./min)

Description	Number of clockwise rotation events detected in the analyzed by unit of time.
Units	Events/minute
Scientific meaning	This calculation helps with the evaluation of the animal global motor pattern. In a more specific manner, the measurement of rotation behavior is commonly used in animal model of Parkinson with a lateral lesion of the dopaminergic innervation of the striatum. In that case, the extent of rotation in a given direction is correlated with the extent of the lesion performed.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Rearing Duration Total (Seconds)

Description	Total time spent doing rearing in the analyzed trial.
Units	Seconds
Scientific meaning	The measurement of rearing behavior is commonly used as an index of animal exploration behavior.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rearing Threshold Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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Rearing Duration in Zone (Seconds)

Description	Time spent doing rearing calculated for each zone of the zone definition associated to the analyzed trial.
Units	Seconds
Scientific meaning	The measurement of rearing behavior is commonly used as an index of animal exploration behavior.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Rearing Threshold Track Filters Tracking Point Selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Rearing Latency (Seconds)

Description	Time elapsed until the first rearing event is detected with the TriWise extension.
Units	Seconds
Scientific meaning	The measurement of rearing behavior is commonly used as an index of animal exploration behavior.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rearing Threshold Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Rearing Mean Duration (Seconds)

Description	Mean duration of the rearing events detected in the analyzed trial with the TriWise extension.
Formula & Special cases	Rearing Mean Duration = Rearing Duration Total / Rearing Number Total
Units	Seconds
Scientific meaning	The measurement of rearing behavior is commonly used as an index of animal exploration behavior.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rearing Threshold Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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Rearing Number Total

Description	Total number of rearing events detected in the analyzed trial using the TriWise extension.
Scientific meaning	The measurement of rearing behavior is commonly used as an index of animal exploration behavior.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rearing Threshold Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Rearing Number in Zone

Description	Total number of rearing events detected using the TriWise extension and calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	The number of rearing events detected in each zone is considered as the number of rearing events that have been initiated in this zone.
Scientific meaning	The measurement of rearing behavior is commonly used as an index of animal exploration behavior.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Rearing Threshold Track Filters Tracking Point Selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Rearing Rate (ev./min)

Description	Number of rearing events detected in the analyzed by unit of time.
Units	Events/minute
Scientific meaning	The measurement of rearing behavior is commonly used as an index of animal exploration behavior.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rearing Threshold Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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Stretching Duration Total (Seconds)

Description	Total time spent doing stretching events (or animal body elongations) in the analyzed trial.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Stretching Threshold Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Stretching Duration in Zone (Seconds)

Description	Time spent doing stretching events (or animal body elongations) calculated for each zone of the zone definition associated to the analyzed trial.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Stretching Threshold Track Filters Tracking Point Selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest. Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Stretching Latency (Seconds)

Description	Time elapsed until the detection of the first stretching event (or animal body elongations) with the TriWise extension in the analyzed trial.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Stretching Threshold Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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Stretching Mean Duration (Seconds)

Description	Mean duration of the stretching events (or animal body elongations) detected in the analyzed trial with the TriWise extension.
Formula & Special cases	Stretching Mean Duration = Stretching Duration Total / Stretching Number Total
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Stretching Threshold Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Stretching Number Total

Description	Total number of stretching events (or animal body elongations) detected in the analyzed trial using the TriWise extension.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Stretching Threshold Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Stretching Number in Zone

Description	Total number of stretching events (or animal body elongations) detected using the TriWise extension and calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	The number of stretching events detected in each zone is considered as the number of stretching events that have been initiated in this zone.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Stretching Threshold Track Filters Tracking Point Selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest. Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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Stretching Rate (ev./min)

Description	Number of stretching events (or animal body elongations) detected in the analyzed by unit of time.
Units	Events/minute
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Stretching Threshold Track Filters Tracking Point Selection (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

EVENT SCORING

Event (n) Duration Total (Seconds)

Description	Total duration of events registered manually by the user (using the Event Marker) during the data acquisition process of the analyzed trial.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Event (n) Duration in Zone (Seconds)

Description	Total duration of events registered manually by the user (using the Event Marker) during the data acquisition process of the analyzed trial and calculated for each zone of the associated zone definition.
Formula & Special cases	The presence of the animal in a given zone is determined by the position of the tracking points registered concomitantly to the manual event scoring process, so the reliability of this data will depend on the reliability of the registered track.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest. Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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Event (n) Latency Time (Seconds)

Description	Time elapsed until the detection of the first events registered manually by the user (using the Event Marker) during the data acquisition process of the analyzed trial.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Event (n) Mean Duration (Seconds)

Description	Mean duration of events registered manually by the user (using the Event Marker) during the data acquisition process of the analyzed trial.
Formula & Special cases	Event Mean Duration = Event Duration Total / Event Number Total
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Event (n) Name

Description	Name of events registered manually by the user (using the Event Marker) during the data acquisition process of the analyzed trial.
Origin of the data	View/Event Marker used during the Data Acquisition process
Created/Defined by	User-defined

Event (n) Number Total

Description	Total number of event (n) manually scored by the user (using the Event Marker) during the data acquisition process of the analyzed trial.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Event (n) Number in Zone

Description	Total number of events registered manually by the user (using the Event Marker) during the data acquisition process of the analyzed trial and calculated for each zone of the associated zone definition
Formula & Special cases	The presence of the animal in a given zone is determined by the position of the tracking points registered concomitantly to the manual event scoring process, so the reliability of this data will depend on the reliability of the registered track.

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Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Track Filters Zone Transition Criterion (only if TW extension is used) START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest. Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Event (n) Rate (ev./min)

Description	Number of events registered manually by the user (using the Event Marker) during the data acquisition process of the analyzed trial and expressed by unit of time.
Units	Events/minute
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

GLOBAL ACTIVITY

Global Activity Total

Description	Total global activity registered in the analyzed trial.
Formula & Special cases	The value of global activity is the sum of the differences between each 2 consecutive frames of the image source processed during the acquisition of the data and expressed in surface of calibration units. This accumulated difference is then divided by two as a punctual movement of the animal generates a change in both the new place and in the space left in the image.
Units	Surface of calibration unit (cm^2 or $inches^2$)
Scientific meaning	Quantitative evaluation of animal global activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Activity Smoothing START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Global Activity in Zone

Description	Global activity registered and calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	The value of global activity is the sum of the differences between each 2 consecutive frames of the image source processed during the acquisition of the data and expressed in surface of calibration units. This accumulated difference is then divided by two as a

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	punctual movement of the animal generates a change in both the new place and in the space left in the image.
Units	Surface of calibration unit (cm^2 or $inches^2$)
Scientific meaning	Quantitative evaluation of animal global activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Activity Smoothing START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Global Activity in Zone (%)

Description	Percentage of global activity registered in the zone with respect to the total global activity registered in the analyzed trial.
Formula & Special cases	% GA in Zone = (GA in Zone x 100)/GA Total
Units	%
Scientific meaning	Quantitative evaluation of animal global activity and movement pattern.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Activity Smoothing START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

High Act. Duration Total (%)

Description	Percentage of time spent in high activity state in the analyzed trial respect to the total duration of the trial.
Formula & Special cases	% High Act. Duration Total = (High Act. Duration Total x 100)/(Immobility Duration + Low Act. Duration + High Act. Duration).
Units	%
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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High Act. Duration Total (Seconds)

Description	Time spent in high activity state in the analyzed trial.
Formula & Special cases	The subject is considered in high activity state when its global activity is ≥ to the user-defined High Activity threshold setting.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes. Index for antidepressant-like effect of therapeutic drugs in the Forced-swimming (Porsolt) and Tail-suspension tests. Index related to emotional memory in the Fear conditioning test.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

High Act. Duration in Zone (%)

Description	Percentage of time spent in high activity state calculated for each zone of the zone definition associated to the analyzed trial
Formula & Special cases	% High Act. Duration in Zone = (High Act. Duration in Zone x 100)/ (Total Immobility Duration + Total Low Act. Duration + Total High Act. Duration).
Units	%
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Immobility Filter Activity Smoothing High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

High Act. Duration in Zone (Seconds)

Description	Time spent in high activity state calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	The subject is considered in high activity state when its global activity is ≥ to the user-defined High Activity threshold setting.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)

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Related analysis settings	Zones Definition Zone of interest Immobility Filter Activity Smoothing High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

High Act. Latency (Seconds)

Description	Time elapsed until the detection of the first high activity state in the analyzed trial.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

High Act. Mean Duration (Seconds)

Description	Mean duration of the high activity events detected in the analyzed trial.
Formula & Special cases	High Act. Mean Duration = High Act. Duration Total / High Act. Number Total
Units	Seconds
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

High Act. Number Total

Description	Number of high activity episodes detected in the analyzed trial.
Formula & Special cases	The subject is considered in high activity state when its global activity is ≥ to the user-defined High Activity threshold setting.
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)

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Related analysis settings	Immobility Filter Activity Smoothing High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

High Act. Rate (ev./min)

Description	Number of high activity events detected in the analyzed trial by unit of time.
Units	Events/minute
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings

Immobility Duration Total (%)

Description	Percentage of time spent in immobility state in the analyzed trial respect to the total duration of the trial.
Formula & Special cases	%Immobility Duration Total = (Immobility Duration Total x 100)/(Immobility Duration + Low Act. Duration + High Act. Duration).
Units	%
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes. Index for antidepressant-like effect of therapeutic drugs in the Forced-swimming (Porsolt) and Tail-suspension tests. Index related to emotional memory in the Fear conditioning test.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Low Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Immobility Duration Total (Seconds)

Description	Time spent in immobility state in the analyzed trial.
Formula & Special cases	The subject is considered in immobility state when its global activity is < to the user-defined Low Activity threshold setting.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.

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	Index for antidepressant-like effect of therapeutic drugs in the Forced-swimming (Porsolt) and Tail-suspension tests. Index related to emotional memory in the Fear conditioning test.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Low Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Immobility Duration in Zone (%)

Description	Percentage of time spent in immobile state calculated for each zone of the zone definition associated to the analyzed trial
Formula & Special cases	% Immobility Duration in Zone = (Immobility Duration in Zone x 100)/ (Total Immobility Duration + Total Low Act. Duration + Total High Act. Duration).
Units	%
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes. Index for antidepressant-like effect of therapeutic drugs in the Forced-swimming (Porsolt) and Tail-suspension tests. Index related to emotional memory in the Fear conditioning test. Etc.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Immobility Filter Activity Smoothing Low Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Immobility Duration in Zone (Seconds)

Description	Time spent in immobility state calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	The subject is considered in immobility state when its global activity is < to the user-defined Low Activity threshold setting.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes. Index for antidepressant-like effect of therapeutic drugs in the Forced-swimming (Porsolt) and Tail-suspension tests. Index related to emotional memory in the Fear conditioning test. Etc.

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Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Immobility Filter Activity Smoothing Low Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Immobility Latency (Seconds)

Description	Time elapsed until the detection of the first immobility state in the analyzed trial.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes. Index for antidepressant-like effect of therapeutic drugs in the Forced-swimming (Porsolt) and Tail-suspension tests. Index related to emotional memory in the Fear conditioning test. Etc.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Low Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Immobility Mean Duration (Seconds)

Description	Mean duration of the immobility events detected in the analyzed trial.
Formula & Special cases	High Act. Mean Duration = Immobility Duration Total / Immobility Number Total
Units	Seconds
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes. Index for antidepressant-like effect of therapeutic drugs in the Forced-swimming (Porsolt) and Tail-suspension tests. Index related to emotional memory in the Fear conditioning test. Etc.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Low Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings

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Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Immobility Number total

Description	Number of low activity episodes detected in the analyzed trial.
Formula & Special cases	The subject is considered in Immobile state when its global activity is < to the user-defined Low Activity threshold setting.
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes. Index for antidepressant-like effect of therapeutic drugs in the Forced-swimming (Porsolt) and Tail-suspension tests. Index related to emotional memory in the Fear conditioning test. Etc.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Low Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Immobility Rate (ev./min)

Description	Number of immobility events detected in the analyzed by unit of time.
Units	Events/minute
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes. Index for antidepressant-like effect of therapeutic drugs in the Forced-swimming (Porsolt) and Tail-suspension tests. Index related to emotional memory in the Fear conditioning test. Etc.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Low Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings

Low Act. Duration Total (%)

Description	Percentage of time spent in low activity state in the analyzed trial respect to the total duration of the trial.
Formula & Special cases	% Low Act. Duration Total = (Low Act. Duration Total x 100)/(Immobility Duration + Low Act. Duration + High Act. Duration).
Units	%
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)

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Related analysis settings	Immobility Filter Activity Smoothing Low and High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Low Act. Duration Total (Seconds)

Description	Time spent in low activity state in the analyzed trial.
Formula & Special cases	The subject is considered in low activity state when its global activity is ≥ to the user-defined Low Activity threshold setting and < to the user-defined High Activity threshold setting.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes. Index for antidepressant-like effect of therapeutic drugs in the Forced-swimming (Porsolt) and Tail-suspension tests. Index related to emotional memory in the Fear conditioning test.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Low and High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Low Act. Duration in Zone (%)

Description	Percentage of time spent in low activity state calculated for each zone of the zone definition associated to the analyzed trial
Formula & Special cases	% Low Act. Duration in Zone = (Low Act. Duration in Zone x 100)/ (Total Immobility Duration + Total Low Act. Duration + Total High Act. Duration).
Units	%
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Immobility Filter Activity Smoothing Low and High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

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Low Act. Duration in Zone (Seconds)

Description	Time spent in low activity state calculated for each zone of the zone definition associated to the analyzed trial.
Formula & Special cases	The subject is considered in low activity state when its global activity is ≥ to the user-defined Low Activity threshold setting and < to the user-defined High Activity threshold setting.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zones Definition Zone of interest Immobility Filter Activity Smoothing Low and High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each zone defined by the user as zone of interest Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Low Act. Latency (Seconds)

Description	Time elapsed until the detection of the first low activity state in the analyzed trial.
Units	Seconds
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Low and High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Low Act. Mean Duration (Seconds)

Description	Mean duration of the low activity events detected in the analyzed trial.
Formula & Special cases	Low Act. Mean Duration = Low Act. Duration Total / Low Act. Number Total
Units	Seconds
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Low and High Activity threshold START/END Time interval settings

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	EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Low Act. Number Total

Description	Number of low activity episodes recorded in the analyzed trial.
Formula & Special cases	The subject is considered in low activity state when its global activity is ≥ to the user-defined Low Activity threshold setting and < to the user-defined High Activity threshold setting.
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Low and High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings
Distribution mode	Calculated for each subinterval of time defined by the user in the Analysis Time Interval section (Each…)

Low Act. Rate (ev./min)

Description	Number of low activity events detected in the analyzed trial by unit of time.
Units	Events/minute
Scientific meaning	Qualitative evaluation of animal global activity and movement pattern in standard open-field test or in any mazes.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Low and High Activity threshold START/END Time interval settings EACH/SPLIT Time interval settings

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21.4. DATA – EVENT LIST REPORT

EVENTS

Event Duration (Seconds)

Description	Total duration of each event manually scored by the user.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	START/END Time interval settings

Event Index Nr.

Description	Index number of the each event manually scored by the user. The Event Index Number begins at 1 and increases each time a new event is acquired.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	START/END Time interval settings

Event Name

Description	Name of each event manually scored by the user.
Origin of the data	View/Event Marker/Config…
Created/Defined by	User-Defined
Related analysis settings	Event Marker configuration

Event Rel. Time (HH:MM:SS,00)

Description	Time elapsed from the beginning of the trial until the beginning of each event manually scored by the user.
Units	HH:MM:SS,00
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Event Rel. Time (Seconds)

Description	Time elapsed from the beginning of the trial until the beginning of each event manually scored by the user.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	START/END Time interval settings

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Event System Time

Description	Date and Clock time corresponding in which each event manually scored by the user started.
Units	The format of the time depends of the regional configuration of the computer used. Commonly expressed in mm/dd/yy HH:MM:SS
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	START/END Time interval settings

Event Zone END

Description	Zone in which the manual scoring of the event ended.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone definition Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Event Zone START

Description	Zone in which the manual scoring of the event started.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone definition Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

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21.5. DATA – EVENT TRIGGER LIST REPORT

SAMPLE DATA

Action Description

Description	Condition to be fullfilled to trigger an action
Origin of the data	Protocols
Created/Defined by	User-Defined
Related analysis settings	Event Marker configuration

Action Index Nr.

Description	Index number of the each action. The Action Index Number begins at 1 and increases each time a new event is acquired.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	START/END Time interval settings

Action Time (HH:MM:SS,00)

Description	Time elapsed from the beginning of the trial until the beginning of each action.
Units	HH:MM:SS,00
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Action Time (Seconds)

Description	Time elapsed from the beginning of the trial until the beginning of each action.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	START/END Time interval settings

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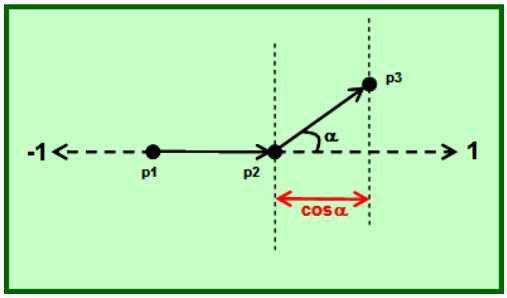
21.6. DATA – TRACK COORDINATES REPORT

SAMPLE DATA

Parallel Index

Description	The parallel index indicates how parallel is a given direction of the animal single movement in comparison to the direction of its previous single movement.
Formula & Special cases	The angle of the path between the current direction of movement (p2 to p3, see illustration) and the previous direction of movement (p1 to p2) is considered for calculation the parallel index. If this angle is < 20°, the value of the cosine of this angle is considered for this sample in the parallel index calculation.  If this angle is < 90°, the value 0 is considered for this sample in the parallel index calculation. If this angle is > 90°, the value -1 is considered for this sample in the parallel index calculation. The samples corresponding to track displacement < 0.5 cm are not taken into consideration in the calculation. The possible values of parallel index are then between -1 and 1. The more the index is closed to 1, the more the animal's displacement follows a straight line. The more the index is -1, the more the animal experiences changes of direction in its displacements.
Scientific meaning	The parallel index has been proposed to reflect the overall tendency to turn and the angular magnitude of turns. The parallel index is not directly dependent on the distance covered by the animal, and seems to significantly reflect subtle changes in the pattern of locomotor activity that is characteristic of the exploration of an unfamiliar environment compared to the locomotor movement in frequently visited areas. It is postulated that the parallel index decreases with the familiarity of the area being explored. The parallel index can also be used for characterizing the effect of a drug on the pattern of animal locomotor displacements (as an example, its value is increased by amphetamine).
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Track Filters Tracking point selection (only if TW extension is used) Subject of Interest START/END Time interval settings
Distribution mode	Calculated for each subject participating in the current trial.

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Sample Index Nr.

Description	Index number of each sample. The Sample Index Number begins at 1 and increases with each stored sample.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Subject of Interest START/END Time interval settings

Sample Time (HH:MM:SS,00)

Description	Time elapsed from the beginning of the trial until the beginning of the current sample.
Units	HH:MM:SS,00
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Subject of Interest START/END Time interval settings

Sample Time (Seconds)

Description	Time elapsed from the beginning of the trial until the beginning of the current sample.
Units	Seconds
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Subject of Interest START/END Time interval settings

Sample Zone

Description	Zone name where the subject is located in each sample.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Track Filters Tracking point selection (only if TW extension is used) Zone Transition Criterion (only if TW extension is used) Subject of Interest START/END Time interval settings
Distribution mode	Calculated for each subject participating in the current trial.

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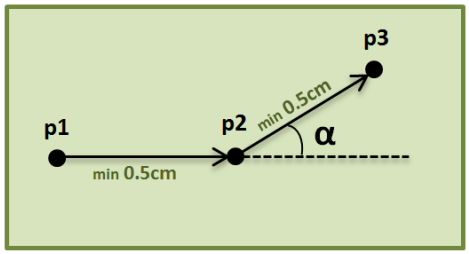
Segment Angle (Degrees)

Description	Angle formed by the last three consecutive valid coordinates of the subject that are at least 0.5cm and 2pixels apart.
Formula & Special cases	The angle of the path between the current direction of movement (p2 to p3, see illustration) and the previous direction of movement (p1 to p2) according to the last three consecutive valid coordinates that are at least 0.5cm and 2pixels appart. 
Units	Degrees
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Track Filters Tracking point selection (only if TW extension is used) Subject of Interest START/END Time interval settings
Distribution mode	Calculated for each subject participating in the current trial.

Segment Length

Description	Length of each segment to be considered to calculate the segment angle.
Units	Units as configured in calibration
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Track Filters Tracking point selection (only if TW extension is used) Subject of Interest START/END Time interval settings
Distribution mode	Calculated for each subject participating in the current trial.

Speed

Description	The value of speed of the subject in each sample. A filter that is averaged according the duration configured by the user is applied.
Units	Units of calibration (cm or inches)/seconds
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Track Filters Speed smoothing Tracking point selection (only if TW extension is used) Subject of Interest START/END Time interval settings
Distribution mode	Calculated for each subject participating in the current trial.

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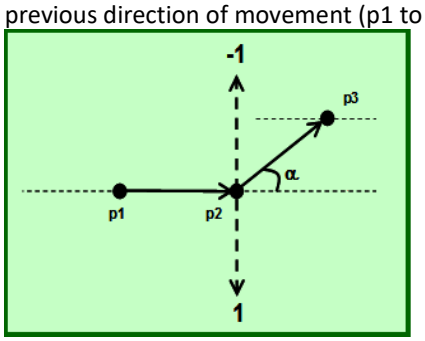
Speed Status

Description	Name of the status of each speed of the subject: Resting, Slow, or Fast.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Track Filters Speed Thresholds Tracking point selection (only if TW extension is used) Subject of Interest START/END Time interval settings
Distribution mode	Calculated for each subject participating in the current trial.

Turning Tendency

Description	The turning tendency gives an indication of the pattern of rotation of the animal trajectory.
Formula & Special cases	The turning tendency is calculated for each sample of time taking into account the angle between the current direction of movement (p2 to p3, see illustration) and the previous direction of movement (p1 to p2).  A positive value of 1 is given to each sample which angle corresponds to a rotation to the right direction. A negative value of -1 is given to each sample which angle corresponds to a rotation to the right direction. The samples corresponding to track displacement < 0.5 cm are not taken into consideration in the calculation.
Scientific meaning	The turning tendency calculation is of particular interest in any experiment in which the animal is expected to have a turning tendency, as an example, in studies on animal models of Parkinson disease with unilateral lesions in the dopaminergic nigrostriatal system. In that case, the amount of rotation is correlated with the volume of the lesion.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Track Filters Tracking point selection (only if TW extension is used) Subject of Interest START/END Time interval settings
Distribution mode	Calculated for each subject participating in the current trial.

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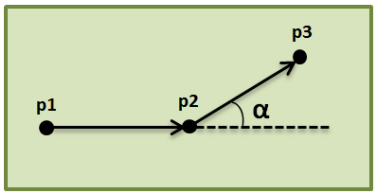
Vector Angle (Degrees)

Description	Angle formed by the last three consecutive valid coordinates of the subject.
Formula & Special cases	The angle of the path between the current direction of movement (p2 to p3, see illustration) and the previous direction of movement (p1 to p2). 
Units	Degrees
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Track Filters Tracking point selection (only if TW extension is used) Subject of Interest START/END Time interval settings
Distribution mode	Calculated for each subject participating in the current trial.

Vector Angular Speed (Degrees/Second)

Description	Speed in degrees/second between each vector angle and the previous vector angle.
Units	Degrees/Second
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Track Filters Tracking point selection (only if TW extension is used) Subject of Interest START/END Time interval settings
Distribution mode	Calculated for each subject participating in the current trial.

X Coordinate

Description	Calibrated X coordinate of the position of the subject in each sample.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Track Filters Tracking point selection (only if TW extension is used) Subject of Interest START/END Time interval settings
Distribution mode	Calculated for each subject participating in the current trial.

Y Coordinate

Description	Calibrated Y coordinate of the position of the subject in each sample.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Track Filters Tracking point selection (only if TW extension is used) Subject of Interest START/END Time interval settings
Distribution mode	Calculated for each subject participating in the current trial.

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21.7. DATA – SPEED EPISODES LIST REPORT

TRACKING – STD

Episode Duration (Seconds)

Description	Total duration of each speed episode.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Speed Scale Speed Smoothing Speed Thresholds START/END Time interval settings

Episode Index Nr.

Description	Index number of each speed episode. The Episode Index Number begins at 1 and increases each time a new episode is acquired.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Speed Smoothing Speed Thresholds START/END Time interval settings

Episode Rel. Time (HH:MM:SS,00)

Description	Time elapsed from the beginning of the trial until the beginning of the current speed episode.
Units	HH:MM:SS,00
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Speed Scale Speed Smoothing Speed Thresholds START/END Time interval settings

Episode Rel. Time (Seconds)

Description	Time elapsed from the beginning of the trial until the beginning of the current speed episode.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Speed Scale Speed Smoothing Speed Thresholds START/END Time interval settings

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Episode Zone END

Description	Zone in which each speed episode ended.
Formula & Special cases	The presence of the animal in a given zone is determined by the position of the tracking points registered concomitantly to the manual event scoring process, so the reliability of this data will depend on the reliability of the registered track.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone definition Speed Scale Speed Smoothing Speed Thresholds Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Episode Zone START

Description	Zone in which each speed episode started.
Formula & Special cases	The presence of the animal in a given zone is determined by the position of the tracking points registered concomitantly to the manual event scoring process, so the reliability of this data will depend on the reliability of the registered track.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone definition Speed Scale Speed Smoothing Speed Thresholds Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Event Name

Description	Name of each speed episode: Resting, Slow, or Fast.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Speed Scale Speed Smoothing Speed Thresholds START/END Time interval settings

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21.8. DATA – ACTIVITY EPISODES LIST REPORT

ACTIVITY EPISODES

Episode Duration (Seconds)

Description	Total duration of each activity episode.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Low Activity threshold START/END Time interval settings

Episode Index Nr.

Description	Index number of each activity episode. The Episode Index Number begins at 1 and increases each time a new episode is acquired.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Immobility Filter Activity Smoothing Activity Thresholds START/END Time interval settings

Episode Rel. Time (HH:MM:SS,00)

Description	Time elapsed from the beginning of the trial until the beginning of each activity episode.
Units	HH:MM:SS,00
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Activity Thresholds START/END Time interval settings

Episode Rel. Time (Seconds)

Description	Time elapsed from the beginning of the trial until the beginning of each activity episode.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Activity Thresholds START/END Time interval settings

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Episode Zone END

Description	Zone in which each activity episode ended.
Formula & Special cases	The presence of the animal in a given zone is determined by the position of the tracking points registered concomitantly to the manual event scoring process, so the reliability of this data will depend on the reliability of the registered track.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone definition Immobility Filter Activity Smoothing Low Activity threshold Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Episode Zone START

Description	Zone in which each activity episode started.
Formula & Special cases	The presence of the animal in a given zone is determined by the position of the tracking points registered concomitantly to the manual event scoring process, so the reliability of this data will depend on the reliability of the registered track.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone definition Immobility Filter Activity Smoothing Low Activity threshold Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Event Name

Description	Name of each activity episode: Immobile, Low Activity or High Activity.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Immobility Filter Activity Smoothing Low Activity threshold

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21.9. DATA – TRIWISE ROTATION LIST REPORT

ROTATIONS

Event Name

Description	Name of the detected event: CW rotation or CCW rotation.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing START/END Time interval settings

Rotation Duration (Seconds)

Description	Total duration of each rotation event.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing START/END Time interval settings

Rotation Index Nr.

Description	Index number of each rotation event. The rotation Index Number begins at 1 and increases each time a new episode is acquired.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Rotation Smoothing START/END Time interval settings

Rotation Rel. Time (HH:MM:SS,00)

Description	Time elapsed from the beginning of the trial until the beginning of the rotation event.
Units	HH:MM:SS,00
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing START/END Time interval settings

Rotation Rel. Time (Seconds)

Description	Time elapsed from the beginning of the trial until the beginning of the current rotation event.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rotation Smoothing START/END Time interval settings

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Rotation Zone END

Description	Zone in which each rotation event ended.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone definition Rotation Smoothing Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Rotation Zone START

Description	Zone in which of each rotation event started.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone definition Rotation Smoothing Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

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21.10. DATA – TRIWISE REARING LIST REPORT

REARINGS

Event Name

Description	Name of the detected event: rearing.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rearing threshold START/END Time interval settings

Rearing Duration (Seconds)

Description	Total duration of each rearing event.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rearing threshold START/END Time interval settings

Rearing Index Nr.

Description	Index number of each rearing event. The Rearing Index Number begins at 1 and increases each time a new episode is acquired.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Rearing threshold START/END Time interval settings

Rearing Rel. Time (HH:MM:SS,00)

Description	Time elapsed from the beginning of the trial until the beginning of the rearing event.
Units	HH:MM:SS,00
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rearing threshold START/END Time interval settings

Rearing Rel. Time (Seconds)

Description	Time elapsed from the beginning of the trial until the beginning of the current rearing event.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Rearing threshold START/END Time interval settings

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Rearing Zone END

Description	Zone in which each rearing event ended.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone definition Rearing threshold Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Rearing Zone START

Description	Zone in which of each rearing event started.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone definition Rearing threshold Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

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21.11. DATA – TRIWISE STRETCHING REPORT

STRETCHINGS

Event Name

Description	Name of the detected event: stretching.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Stretching threshold START/END Time interval settings

Stretching Duration (Seconds)

Description	Total duration of each stretching event.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Stretching threshold START/END Time interval settings

Stretching Index Nr.

Description	Index number of each stretching event. The stretching Index Number begins at 1 and increases each time a new episode is acquired.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Stretching threshold START/END Time interval settings

Stretching Rel. Time (HH:MM:SS,00)

Description	Time elapsed from the beginning of the trial until the beginning of the stretching event.
Units	HH:MM:SS,00
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Stretching threshold START/END Time interval settings

Stretching Rel. Time (Seconds)

Description	Time elapsed from the beginning of the trial until the beginning of the current stretching event.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Stretching threshold START/END Time interval settings

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Stretching Zone END

Description	Zone in which each stretching event ended.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone definition Stretching threshold Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Stretching Zone START

Description	Zone in which each stretching event started.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone definition Stretching threshold Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

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21.12. ZONE TRANSITIONS LIST REPORT

ZONE TRANSITIONS

Accumulated Time (Seconds)

Description	Total time spent in each zone from the beginning of the trial.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Entries Number

Description	Total number of times the subject entered each zone from the beginning of the trial.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Transition Current Zone

Description	Name of each zone.
Origin of the data	Experimentation Assistant/Zone Definition/ZonesAssoc. Management
Created/Defined by	User-Defined
Related analysis settings	Zone Definition (user-defined zone name) Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Transition Index Nr.

Description	Index number of the zone transitions. The transition Index Number begins at 1 and increases each time the animal enters in a new zone.
Formula & Special cases	The zone in which the subject begins the trial is not counted as an entry, so nor as a transition.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

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Transition Rel. Time (HH:MM:SS,00)

Description	Time elapsed from the beginning of the trial until the subject enters into a new zone (transition). Expressed in HH:MM:SS,00.
Units	HH:MM:SS,00
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	START/END Time interval settings

Transition Rel. Time (Seconds)

Description	Time elapsed from the beginning of the trial until the subject enters into a new zone (transition). Expressed in Seconds
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Transition Time In Zone (Seconds)

Description	Time spent in each zone. This time is reset when the subject enters again in the same zone.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Traveled Distance

Description	Distance traveled by the subject in each zone. This value is reset when the subject enters again in the same zone.
Units	Calibration units (cm or inches)
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone Transition Criterion (only for TW extension). Track Filters. START/END Time interval settings.

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21.13. DATA – ASSOCIATION TRANSITION LIST REPORT

ZONE TRANSITIONS

Accumulated Time (Seconds)

Description	Total time spent in each zone association from the beginning of the trial.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Entries Number

Description	Total number of times the subject entered into each zone association from the beginning of the trial.
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Transition Current Zone

Description	Name of each zone association.
Origin of the data	Experimentation Assistant / Zone Definition / Zones Assoc. Management
Created/Defined by	User-Defined
Related analysis settings	Zone Definition (user-defined zone name) Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Transition Index Nr.

Description	Index number of the zone association transitions. The transition Index Number begins at 1 and increases each time the animal enters in a new zone association.
Formula & Special cases	The zone association in which the subject begins the trial is not counted as an entry, so nor as a transition.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Transition Rel. Time (HH:MM:SS,00)

Description	Time elapsed from the beginning of the trial until the subject enters into a new zone association (transition). Expressed in HH:MM:SS,00.
Units	HH:MM:SS,00

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Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	START/END Time interval settings

Transition Rel. Time (Seconds)

Description	Time elapsed from the beginning of the trial until the subject enters into a new zone association (transition). Expressed in Seconds
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Transition Time In Zone (Seconds)

Description	Time spent in each zone association. This time is reset when the subject enters again in the same zone association.
Units	Seconds
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone Transition Criterion (only for TW extension) Track Filters START/END Time interval settings

Traveled Distance

Description	Distance traveled by the subject in each zone association. This value is reset when the subject enters again in the same zone association.
Units	Calibration units (cm or inches)
Created/Defined by	SMARTIO (automated calculation)
Related analysis settings	Zone Transition Criterion (only for TW extension). Track Filters. START/END Time interval settings.

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21.14. DATA – GLOBAL ACTIVITY RAW DATA REPORT

SAMPLE DATA

Current Global Activity

Description	Accumulate of the global activity in all zones.
Units	Units of calibration (cm or inches)^2/seconds
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Zone of Interest Immobility Filter Activity Smoothing Activity Thresholds START/END Time interval settings

Current Global Activity In Zone

Description	Global activity in each zone.
Units	Units of calibration (cm or inches)^2/seconds
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	Zone of Interest Immobility Filter Activity Smoothing Activity Thresholds START/END Time interval settings
Distributed mode	Calculated for each zone defined by the user as zone of interest

Sample Index Nr.

Description	Index number of each sample. The Sample Index Number begins at 1 and increases with each stored sample.
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	START/END Time interval settings

Sample Time (HH:MM:SS,00)

Description	Time elapsed from the beginning of the trial until the beginning of the current sample.
Units	HH:MM:SS,00
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	START/END Time interval settings

Sample Time (Seconds)

Description	Time elapsed from the beginning of the trial until the beginning of the current sample.
Units	Seconds
Created/Defined by	SMARTIO (automated parameter)
Related analysis settings	START/END Time interval settings

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21.15. ACQUISITION CONFIGURATION

CALIBRATION

ACQ. Calibration Horizontal

Reports	Summary report, Zone transition list, Activity episode list, TriWise rearing list, TriWise rotation list, TriWise stretching list.
Description	Horizontal calibration used during trial acquisition.
Formula & Specific cases	This is the horizontal dimension of 1 pixel expressed in unit of calibration.
Origin of the data	Experimentation Assistant/Calibration
Units	Calibration unit: cm or inches
Scientific meaning	The calibration is needed to make SMARTIO able to provide related data directly in cm or inches (distance, speed…).
Created/Defined by	User-defined

ACQ. Calibration Units

Reports	Summary report, Zone transition list, Activity episode list, TriWise rearing list, TriWise rotation list, TriWise stretching list.
Description	Unit of length used during trial acquisition.
Origin of the data	Experimentation Assistant/Calibration
Units	Calibration unit: cm or inches
Scientific meaning	The calibration is needed to make SMARTIO able to provide related data directly in cm or inches (distance, speed…).
Created/Defined by	User-defined

ACQ. Calibration Vertical

Reports	Summary report, Zone transition list, Activity episode list, TriWise rearing list, TriWise rotation list, TriWise stretching list.
Description	Vertical calibration used during trial acquisition.
Formula & Specific cases	This is the vertical dimension of 1 pixel expressed in unit of calibration.
Origin of the data	Experimentation Assistant/Calibration
Units	Calibration unit: cm or inches
Scientific meaning	The calibration is needed to make SMARTIO able to provide related data directly in cm or inches (distance, speed…).
Created/Defined by	User-defined

ACQ. Image Height (Pixels)

Reports	Summary report, Zone transition list, Activity episode list, TriWise rearing list, TriWise rotation list, TriWise stretching list.
Description	Height of the image received from the image source.
Units	Pixels.
Created/Defined by	SMARTIO (automated parameter)

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ACQ. Image Width (Pixels)

Reports	Summary report, Zone transition list, Activity episode list, TriWise rearing list, TriWise rotation list, TriWise stretching list.
Description	Width of the image received from the image source.
Units	Pixels.
Created/Defined by	SMARTIO (automated parameter)

DETECTION

ACQ. Activity Detection Threshold

Reports	Summary report, Zone transition list, Activity episode list, TriWise rearing list, TriWise rotation list, TriWise stretching list, Event list.
Description	Value of the Track Detection Threshold configuration used during trial acquisition.
Origin of the data	Experimentation Assistant/Detection settings/Track detection
Units	N/A (value from 0 to 255)
Created/Defined by	User-defined

ACQ. Detection Brightness

Reports	Summary report, Zone transition list, Activity episode list, TriWise rearing list, TriWise rotation list, TriWise stretching list, Event list.
Description	Value of the General Brightness configuration used during trial acquisition.
Origin of the data	Experimentation Assistant/Detection settings/Brightness & Contrast
Created/Defined by	User-defined

ACQ. Detection Contrast

Reports	Summary report, Zone transition list, Activity episode list, TriWise rearing list, TriWise rotation list, TriWise stretching list, Event list.
Description	Value of the General Contrast configuration used during trial acquisition.
Origin of the data	Experimentation Assistant/Detection settings/Brightness & Contrast
Created/Defined by	User-defined

ACQ. Track Detection Anti-Vibration Filter

Reports	Summary report, Zone transition list, Activity episode list, TriWise rearing list, TriWise rotation list, TriWise stretching list, Event list.
Description	Value of the option “Use anti-vibrations filter” during trial acquisition.
Origin of the data	Experimentation Assistant/Detection settings/Track detection
Units	Cm/sec or inch/sec.
Created/Defined by	User-defined

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ACQ. Track Detection Erosion

Reports	Summary report, Zone transition list, Activity episode list, TriWise rearing list, TriWise rotation list, TriWise stretching list, Event list.
Description	Value of the Track Detection Erosion configuration used during trial acquisition.
Origin of the data	Experimentation Assistant/Detection settings/Track detection
Units	Pixels.
Created/Defined by	User-defined

ACQ. Track Detection Mode

Reports	Summary report, Zone transition list, TriWise rearing list, TriWise rotation list, TriWise stretching list, Event list.
Description	Track detection mode used for data acquisition and selected in the Detection settings panel
Origin of the data	Experimentation Assistant/Detection settings/Track detection
Created/Defined by	User-defined

ACQ. Track Detection Threshold

Reports	Summary report, Zone transition list, Activity episode list, TriWise rearing list, TriWise rotation list, TriWise stretching list, Event list.
Description	Value of the Track Detection Threshold configuration used during trial acquisition.
Origin of the data	Experimentation Assistant/Detection settings/Track detection
Units	N/A (value from 0 to 255)
Created/Defined by	User-defined

ACQ. Tracking Point Selection (TW)

Reports	Summary report, Zone transition list, TriWise rearing list, TriWise rotation list, TriWise stretching list, Event list.
Description	Tracking point used during trial acquisition: Head, Center of mass or Tail.
Origin of the data	Experimentation Assistant/Detection settings/Track detection/TriWise Settings
Created/Defined by	User-defined

ACQ. Zone Specific BC

Reports	Summary report, Zone transition list, Activity episode list, TriWise rearing list, TriWise rotation list, TriWise stretching list, Event list.
Description	Acquisition zone specific Brightness/Contrast: state of activation of the option “Use special lighting conditions for this zone” set by the User in the SMARTIO Detection panel.
Origin of the data	Experimentation Assistant/Detection settings/Brightness & Contrast
Created/Defined by	User-defined

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ACQ. Zone Transition Criterion (TW)

Reports	Summary report, Zone transition list, TriWise rearing list, TriWise rotation list, TriWise stretching list, Event list.
Description	Zone transition criterion used during trial acquisition: Center of mass, Center and head, head or Whole subject.
Origin of the data	Experimentation Assistant/Detection settings/Track detection/TriWise Settings
Created/Defined by	User-defined

TIMING SETTINGS

ACQ. Timing Acquisition Time (HH:MM:SS,00)

Reports	All reports
Description	Data acquisition time (duration) set by the user.
Origin of the data	Experimentation Assistant/Timing settings/Trial Execution
Units	HH:MM:SS,00
Created/Defined by	User-defined

ACQ. Timing Latency Time (HH:MM:SS,00)

Reports	All reports
Description	Time latency set until data acquisition starts.
Origin of the data	Experimentation Assistant/Timing settings/Trial Execution
Units	HH:MM:SS,00
Created/Defined by	User-defined

ACQ. Timing Mode

Reports	All reports
Description	Timing Mode set by the User in the SMARTIO Time Settings panel: free running or Pre-set time.
Origin of the data	Experimentation Assistant/Timing settings/Trial Execution
Created/Defined by	User-defined

ACQ. Timing Stop Mode

Reports	All reports
Description	Option and configuration set by the User for stopping data acquisition.
Origin of the data	Experimentation Assistant/Timing settings/Finishing Conditions
Created/Defined by	User-defined

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21.16. ANALYSIS CONFIGURATION

TRACKING – STD

AN. Speed Smoothing

Reports	Summary report, Zone transition list, TriWise rearing list, TriWise rotation list, TriWise stretching list.
Description	Value of the Speed Smoothing used for analysis.
Origin of the data	Experimentation Assistant/Analysis/Report Definition/ Report (select one)/Edit Global analysis configuration/Speed Settings
Units	Seconds
Created/Defined by	User-defined

AN. Speed Threshold Fast

Reports	Summary report, Zone transition list, TriWise rearing list, TriWise rotation list, TriWise stretching list.
Description	Speed Fast Moving Threshold used in analysis for defining the subject Fast moving state.
Origin of the data	Experimentation Assistant/Analysis/Report Definition/ Report (select one)/Edit Global analysis configuration/Speed Settings
Units	Cm or inches per seconds (depending on the calibration settings)
Created/Defined by	User-defined

AN. Speed Threshold Resting

Reports	Summary report, Zone transition list, TriWise rearing list, TriWise rotation list, TriWise stretching list.
Description	Speed Slow Moving Threshold used in analysis for defining the subject resting state.
Origin of the data	Experimentation Assistant/Analysis/Report Definition/ Report (select one)/Edit Global analysis configuration/Speed Settings
Units	Cm or inches per seconds (depending on the calibration settings)
Created/Defined by	User-defined

TRACKING –-TW

AN. Rearing Threshold (TW)

Reports	Summary report, TriWise Rearing list.
Description	TriWise threshold used in analysis for the automated detection of rearing events.
Origin of the data	Experimentation Assistant/Analysis/Report Definition/ Report (select one)/Edit Global analysis configuration/TriWise Settings
Created/Defined by	User-defined

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AN. Rotation Smoothing (TW)

Reports	Summary report, TriWise rotation list.
Description	TriWise smoothing time used in analysis for the automated detection of rotation events.
Origin of the data	Experimentation Assistant/Analysis/Report Definition/ Report (select one)/Edit Global analysis configuration/TriWise Settings
Units	Seconds
Created/Defined by	User-defined

AN. Stretching Threshold (TW)

Reports	Summary report, TriWise stretching list.
Description	TriWise theshold used in analysis for the automated detection of stretching events.
Origin of the data	Experimentation Assistant/Analysis/Report Definition/ Report (select one)/Edit Global analysis configuration/TriWise Settings
Created/Defined by	User-defined

AN. Track Point Selection (TW)

Reports	Summary report, Zone transition list, TriWise rearing list, TriWise rotation list, TriWise stretching list, Event list.
Description	Tracking point used in Analysis: Head, Center of mass or Tail.
Origin of the data	Experimentation Assistant/Analysis/Report Definition/ Report (select one)/Edit Global analysis configuration/TriWise Settings
Created/Defined by	User-defined

AN. Zone Transition Criteria (TW)

Reports	Zone transition criterion used in Analysis: Center of mass, Center and head, head or Whole subject.
Description	Tracking point used in Analysis: Head, Center of mass or Tail.
Origin of the data	Experimentation Assistant/Analysis/Report Definition/ Report (select one)/Edit Global analysis configuration/TriWise Settings
Created/Defined by	User-defined

GLOBAL ACTIVITY

AN. Activity Smoothing

Reports	Summary report, Activity episode list.
Description	Value of the Activity Smoothing used for analysis.
Origin of the data	Experimentation Assistant/Analysis/Report Definition/ Report (select one)/Edit Global analysis configuration/Activity Settings
Units	Seconds
Created/Defined by	User-defined

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AN. Activity Threshold High

Reports	Summary report, Activity episode list.
Description	Low Activity Threshold used in analysis for defining the subject low and high activity episodes.
Origin of the data	Experimentation Assistant/Analysis/Report Definition/ Report (select one)/Edit Global analysis configuration/Activity Settings
Units	cm2/s or inch2/s (depending of the calibration settings)
Created/Defined by	User-defined

AN. Activity Threshold Low

Reports	Summary report, Activity episode list.
Description	Low Activity Threshold used in analysis for defining the subject immobility episodes.
Origin of the data	Experimentation Assistant/Analysis/Report Definition/ Report (select one)/Edit Global analysis configuration/Activity Settings
Units	cm2/s or inch2/s (depending of the calibration settings)
Created/Defined by	User-defined

AN. Immobility Filter

Reports	Summary report, Activity episode list.
Description	Value of Immobility filter used in analysis for filtering the immobility episodes by their duration.
Origin of the data	Experimentation Assistant/Analysis/Report Definition/ Report (select one)/Edit Global analysis configuration/Activity Settings
Units	Seconds
Created/Defined by	User-defined

ZONE DEFINITION

AN. Whishaw's Error Corridor Width

Reports	Summary report
Description	Width of the Whishaw's Error corridor used in analysis.
Formula & Specific cases	See details about the Whishaw's error corridor in chapter 21.3 - Whishaw's Error
Origin of the data	Experimentation Assistant/Analysis/Summary report/Whishaw's error/Settings associated to the selected calculation
Units	Calibration unit: cm or inches
Created/Defined by	User-defined

AN. Zone Associations Number

Reports	All reports
Description	Number of associations contained in the Zone Definition used for the analysis of the trial.
Created/Defined by	User-defined

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AN. Zone Definition Name

Reports	All reports
Description	Name of the Zone definition used for the analysis of the trial.
Origin of the data	Experimentation Assistant/Analysis/General Configuration/Zones Definition
Created/Defined by	User-defined

AN. Zone Relative Time Settings

Reports	Summary report
Description	Name of the "distributed zone" used for calculating the relative time in zones.
Formula & Specific cases	See details about the Relative time in zones in chapter 21.3 - Relative Time in Zone (%)
Origin of the data	Experimentation Assistant/Zones Definition/Zones - Assoc. Management/Show Zones-Assoc. Management dialog…/Settings for Zone Dependent Calculations/Relative Time Settings
Created/Defined by	User-defined

AN. Zone Triplet Settings

Reports	Summary report
Description	Name of the zones Center zone and the 3 Arms zones (A, B and C) respectively used for calculating the data related to T- maze and Y-maze experiments (alternation triples, max. number of arm visits etc.).
Formula & Specific cases	See details about the related calculation in chapter 21.3 - Alternation Triplet
Origin of the data	Experimentation Assistant/Zones Definition/Zones - Assoc. Management/Show Zones-Assoc. Management dialog…/Settings for Zone Dependent Calculations/Triplet Settings
Created/Defined by	User-defined

AN. Zones Number

Reports	All reports
Description	Number of zones contained in the Zone Definition used for the analysis of the trial.
Created/Defined by	User-defined

TIMING

AN. Time Interval SPLIT

Reports	Summary report, Zone transition list, Activity episode list, TriWise rearing list, TriWise rotation list, TriWise stretching list.
Description	Subinterval of time of trial analysis set by the user for analysis.
Origin of the data	Experimentation Assistant/Analysis/General Configuration/Time Interval/User Defined/Each
Units	HH:MM:SS,00
Created/Defined by	User-defined

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AN. Time Interval START

Reports	All reports
Description	Starting time of trial analysis (respect to the starting time of the full trial) set by the user for analysis.
Origin of the data	Experimentation Assistant/Analysis/General Configuration/Time Interval/User Defined/Start
Units	HH:MM:SS,00
Created/Defined by	User-defined

AN. Time Interval STOP

Reports	All reports
Description	Ending time of trial analysis (respect to the starting time of the full trial) set by the user for analysis.
Origin of the data	Experimentation Assistant/Analysis/General Configuration/Time Interval/User Defined/End
Units	HH:MM:SS,00
Created/Defined by	User-defined

AN. Timing Stop Mode

Reports	All reports
Description	Option and configuration set by the User for setting the stopping conditions to be taken into account for analysis.
Origin of the data	Experimentation Assistant/Analysis/report definitions (select one)/Edit global analysis configuration…/General data/Stop Conditions
Created/Defined by	User-defined

DATA FILTERING

AN. Anti-Artifact Filter

Reports	Summary report, Zone transition list, Event list, TriWise rearing list, TriWise rotation list, TriWise stretching list.
Description	Threshold of speed set for the artifact rejection filter and used for analysis.
Origin of the data	Experimentation Assistant/Analysis/report definitions (select one)/Edit global analysis configuration…/Trajectory filtering and smoothing/Apply artifact rejection to all tracks
Units	cm/s or inch/s (depending of the calibration unit settings)
Created/Defined by	User-defined

AN. Anti-Vibration Filter

Reports	Summary report, Zone transition list, Event list, TriWise rearing list, TriWise rotation list, TriWise stretching list.
Description	Threshold of speed set for the anti-vibration filter and used for analysis.
Origin of the data	Experimentation Assistant/Analysis/report definitions (select one)/Edit global analysis configuration…/Trajectory filtering and smoothing/Apply anti-vibrations filter to all tracks

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Units	cm/s or inch/s (depending of the calibration unit settings)
Created/Defined by	User-defined

AN. Smoothing LOWESS

Reports	Summary report, Zone transition list, Event list, TriWise rearing list, TriWise rotation list, TriWise stretching list.
Description	Status of activation of the Smoothing LOWESS option.
Origin of the data	Experimentation Assistant/Analysis/report definitions (select one)/Edit global analysis configuration…/Trajectory filtering and smoothing/Apply artifact rejection to all tracks
Created/Defined by	User-defined

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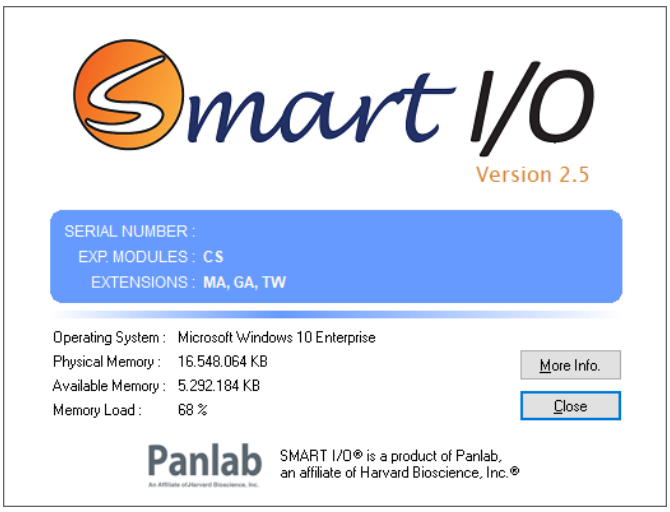
22. CONTACT INFORMATION

We are available to help you with your questions and concerns. Should you hit a roadblock or need some additional training, please feel free to visit the **HBIO Behavior Support Center** at **https://hbiobehavior.zendesk.com** to find articles and helpful information in our knowledge base, Chat with an agent, or setup time to receive one-on-one consultation. You can also visit our webpage on www.panlab.com and send an email to our technical support at support@panlab.com .
We are happy to help!

When contacting us, please remember to share the screenshot of the information about your license. You will find it clicking on **Help** in the Menu bar and then selecting **About:**



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