PLEXON
Neurotechnology Research Systems

User Guide

Version 1 - August 2021

Plexon®
Multi-Wavelength Photometry System

Plexon Inc
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USA

WARNING
Never look directly into any optical fibers!

Depending on the wavelength and intensity of light emitted, you risk serious damage to your vision if you look directly into any optical fiber.

For example, UV light is not visible to the human eye, but it can damage the eye. The Plexon system emits relatively weak UV light, but it is still wise to avoid looking directly into the fibers. Even if colored light is being emitted, it is possible that other, damaging light, such as UV light could be present.

Do not risk eye damage!

CAUTION
Avoid damage to the Digital Input/Output interface pins

Voltages exceeding ±28V will permanently damage the digital inputs and digital outputs of the Trigger Box.

Voltages exceeding –0.5V to +6.5V will permanently damage the Status Indicator.

For details, see Appendix C, Trigger Box and Digital Input/Output.

CAUTION
Avoid damage to the camera

Do not connect or disconnect the behavioral camera while the computer power is on, as doing this might damage the camera.

CAUTION
Avoid damage to the USB Security Key

Before installing SafeNet® Sentinel™ security key drivers remove *all* Sentinel USB keys from the PC. If a system driver is installed with a USB key in the port, the key may become unusable.

CAUTION
Electrostatic Discharge

Some devices can be damaged by improper handling. Use appropriate electrostatic discharge (ESD) procedures when handling these devices. See http://www.esda.org/ for additional information on ESD procedures.

Plexon®
Multi-Wavelength Photometry System

User Guide

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Publication History

May 2021

This is the first issue of the user guide for the Plexon® Multi-Wavelength Photometry System. It is based on version 1 of the software.

When hardware or software is revised or updated in the future, you will be able to see a summary of the changes by accessing the Change Log for this product on the Plexon website, www.plexon.com.

August 2021

Minor updates.

Contents

Chapter 1
System Applications and Features

1.1 Scope of this User Guide

The scope of this user guide is the Plexon® Multi-Wavelength Photometry System, referred to in this document as “the system.” It is a research tool for multi-fiber, multi-wavelength photometry imaging combined with laboratory animal tracking and behavioral analysis.

The hardware and software for this system are fully integrated and tested in the Plexon factory prior to shipment.

See the Plexon website to learn about Plexon systems that allow you to combine photometry and behavioral studies with other research options such as electrophysiology procedures and optical or electrical stimulation.

The system features and options described in this chapter meet the requirements of a wide range of experiment designs. If you would like assistance in determining whether the system is applicable to a particular experimental design, please contact Plexon at +1 214-369-4957 or support@plexon.com.

1.2 System Overview

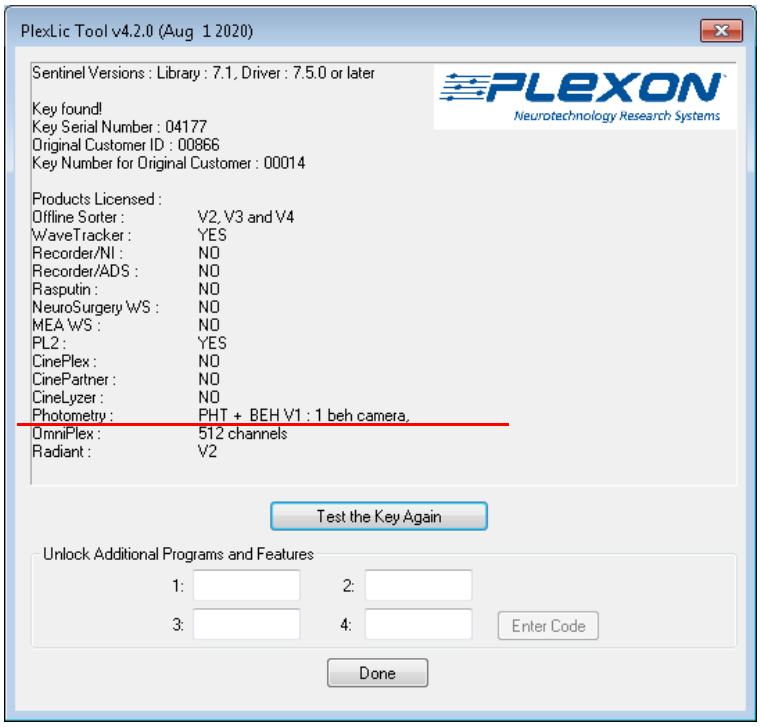
The system provides these features:

- Multi-wavelength Photometry—Detection and recording of fluorescence emitted in the brain or nervous system in response to applied excitation wavelengths, and event definitions based on fluorescence power levels emitted by genetically encoded calcium indicators (GECIs) and genetically encoded voltage indicators (GEVIs) in the brain.
- Recording—Labeling for experiments and individual sessions (descriptors for experiments and variables for sessions), video recording, digital inputs, video playback, timestamp and experiment database management features.
- Input/Output—The system can receive digital input signals from external equipment and transmit digital output signals to external equipment.
- Behavioral video capture and analysis—Video recording of freely behaving animals, with capability for arena definition and dimensional calibration, digital tracking, zone and event definitions, tracking in specific zones and zone sequences, data output capabilities and behavioral analysis tools.

The software runs on the Windows® 7 and Windows® 10 operating systems. The Windows 10 operating system is installed on the computer supplied by Plexon as part of the system. It can also run on a standalone customer-supplied computer that meets certain minimum requirements. The standalone computer must have minimum memory, processor and instruction set to run the system software efficiently. The recommendations for the computer are summarized in Section 1.8, “Computer to Run Photometry Software” on page 11. The appropriate Plexon license key must be plugged into a USB port on the computer.

1.3 Licensing

You can view the list of licenses installed on your system by selecting **About** from the **Help** dropdown list then clicking on the **Licensing** button. The image below shows typical licensing information.



1.4 Features

This section provides high-level details about features provided by the system:

- Photometry Features
- Recording, Playback and Re-recording Features
- Input/Output Features
- Tracking Features
- Arena, Zone and Calibration Features
- Behavioral Analysis Features
- Data Export Features

1.4.1 Photometry Features

The photometry hardware includes LEDs that emit excitation wavelengths centered at 560nm, 465nm, and 410nm, and video cameras that detect and measure fluorescence emitted by GECIs and GEVIs. A full functional description of the photometry excitation and fluorescence wavelengths is provided in Chapter 4, Photometry Features and Procedures. With the photometry functions you can

- Utilize up to four fibers to deliver excitation wavelengths and record fluorescence from multiple brain regions within a single subject, or the same region across separate subjects
- Measure changes in fluorescence emission levels in the brain of a freely moving animal
- Collect and process fluorescence data synchronized with video tracking images, data and behavioral events
- Define photometry events for each fiber based on the power level of the detected light emitted by the brain cells
- Define combination events within each individual fiber
- Define combination events across multiple fibers and the behavioral camera
- Utilize an Overlay feature, which provides a convenient means of loading photometry-related settings.
- Utilize input/output lines that allow you to detect up to 12 digital events from external sources and output up to 12 digital signals when specific photometric events occur**.**
- Export data in comma-separated values (CSV) format for further analyses

1.4.2 Recording, Playback and Re-recording Features

The following features are provided:

- Recording (online) function—Records digital video to disk in **Cameras** mode
- Playback and re-recording (offline) functions—Analyzes previously recorded video in **Files** mode

The video functions are as follows:

- Provides a digital video capture and recording capability.
- Simultaneously records four video streams, three from the photometry devices and one from the behavioral camera.
- Compresses the raw video using MPEG-4 compression and stores the processed videos as Audio Video Interleaved (AVI) files.

Note: AVI files recorded by the system are compatible with Windows Media Player® and many other media players.

- Provides the capability to start and stop recording by means of external signals.
- Records directly to an internal hard drive.
- Optionally supports Near IR (infrared) behavioral video recording. See Section 1.5, “Hardware and Software” on page 8 regarding IR imaging.

The experiments and sessions database allows you to:

- Define experiments and sessions (multiple sessions per experiment) to meet your research objectives. For details, see Chapter 3, Preparing Your Experiment Database.
- Add descriptors that help identify key aspects of each experiment, and session variables that assist with analysis of data from all sessions within the experiment.
- Load an existing experiment, including all of the current parameter values associated with that experiment.

1.4.3 Input/Output Features

The digital input and output interfaces in the Trigger Box provide lines for 12 input events and 12 output events. The standard system is configured for six high true and six low true lines for both input and output. However, when a system is ordered from Plexon, the number of high true and low true lines can be adjusted according to customer request. Consult with Plexon Support or your Plexon sales representative regarding these adjustments. You can see how your existing system is configured by looking at the label on the bottom of the Trigger Box. You can also see how your system is set by looking at the Input or Output line dropdown lists in any of the **Event** tabs in the GUI. See Appendix C, Trigger Box and Digital Input/Output for additional details about the Trigger Box and the high/low true logic.

1.4.4 Tracking Features

The system provides these tracking features in the behavioral video:

- Automatically tracks an animal’s position, speed and direction.
- Provides options for LED tracking (up to three LEDs), object contour tracking, and color marker tracking (up to 12 color markers) in the arena.
- Analyzes each frame of video data to determine the positions of the objects being tracked as a function of time, timestamps each frame, and saves the data to an AVI file.
- Allows tracking data to be analyzed and results to be exported to comma separated values (CSV) files on the host PC.

1.4.5 Arena, Zone and Calibration Features

The system provides these spatial features in the behavioral video:

- Allows you to define an arena with simple or complex geometries for tracking in particular zones of interest (for example, open versus closed arms in an elevated “plus” maze).
- Supports creation of both static and dynamic zones.
- Allows you to define zone sequences useful for learning tasks such as the water maze, radial maze and T-maze.
- Provides the option of calibrating the behavioral video dimensions and tracking data in inches or centimeters.
- Allows you to adjust the arena geometry and calibration on a per-session basis. You can change the position, dimensions and dimensional calibration of arenas on a per-session basis as you record and analyze sessions. This feature is useful when a camera is accidentally moved during an experiment or when you want to focus on a subject’s behavior in a certain location. (However, if you add or delete a shape, that addition or deletion affects all sessions—past, present and future—for the current experiment.)
- Includes an Overlay feature, which provides a convenient means of loading photometry settings and behavioral settings (arena, zones and calibrations) from a previously-recorded experiment onto a new experiment. Overlays can also be loaded onto an existing experiment as long as the behavioral tracking mode of the overlay matches that of the existing experiment (if the behavioral features are used).

Note: For more information about flexible geometry and overlays, see Section 11.13, “Using the Overlay Feature during Analysis” on page 292.

If your experiment involves movements that might be difficult to track, see Section 1.7, “Behavioral Video—Special Considerations” on page 10.

1.4.6 Behavioral Analysis Features

The behavioral analysis feature set includes zone and event definitions, data input/output signals and behavioral analysis tools. This capability includes the following features and functions.

- Allows you to define events based on a zone, a sequence of zones, angles and speed.
- Monitors animals entering and exiting zones and sequences of zone that you have defined within the experimental arena, and generates events that you have defined.
- Allows you to define combination events on the behavioral camera video, and combination events between the behavioral video and the photometry videos.

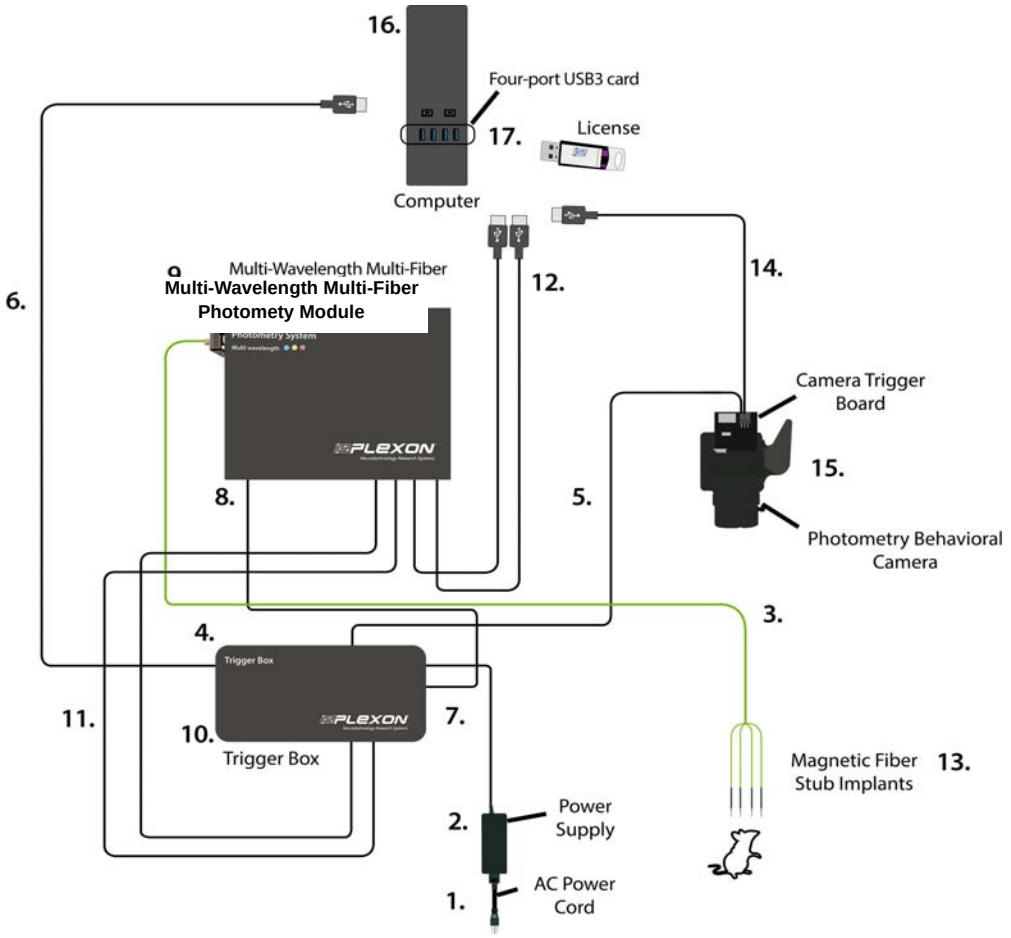
- Provides real-time and offline data about behavioral events and tracked objects, including such attributes as speed, direction (vector), limb angles, head direction, presence in particular zones in the arena, proximity to other objects, sequence of zones visited, and many similar metrics.
- Facilitates data analysis by sorting and grouping experimental data based on user-defined variables.
- Accumulates and displays behavioral event statistics
- Allows you to output digital signals for behavioral events
- Allows you to specify time intervals for analysis, so portions of acquired recordings (sessions) can be ignored during analysis
- Includes input/output lines that allow you to detect up to 12 digital events from external sources and output up to 12 digital signals when specific behavioral events occur in the arena**.**
- Allows you to export data in comma-separated values (CSV) format for further analyses by other programs

1.4.7 Data Export Features

The data export feature allows you to export photometry data, tracking coordinates and recorded events on a per-session basis. All exported files have CSV (comma separated values) format.

1.5 Hardware and Software

This section describes the items included in the standard system.



The standard system includes the following hardware:

- A host PC, specially prepared by Plexon, including the Windows 10 operating system, the system software, and four-port USB3 card
- One monitor (second monitor optional and recommended)
- A keyboard and mouse
- The Multi-Wavelength Multi-Fiber Photometry Module (referred to in this document as the **Photometry Module**), which transmits excitation light from the excitation LEDs and receives fluorescent light emitted by the test subject
- The Trigger Box, which processes and coordinates the start and stop signals for the LED excitation lights, and provides digital input and digital output ports

- One photometry behavioral camera kit for video capture of the subject's movements, consisting of the camera, lens, quick release mounting plate, and Camera Trigger Board
- All required interconnecting cables
- License key

Optical patch cable and fiber implants (optional)

Optionally, Plexon can provide an optical patch cable and a set of four magnetic fiber stub implants. Please speak with your Plexon representative regarding these items.

Status Indicator and Input Generator boards

The system includes two circuit boards that plug into the Trigger Box— a Status Indicator and an Input Generator. The functions of these boards are described in Appendix C, Trigger Box and Digital Input/Output.

Software

The system software is provided on a flash drive. In addition, the latest software updates can be downloaded from the Plexon website.

When a complete system is purchased, the software is already installed on the PC and tested by Plexon prior to shipment. The flash drive with a copy of the software is included in the shipment. The appropriate USB license key is also included.

Behavioral Camera Kit supplied with standard system

The standard system can be ordered with one of the camera models listed below. The camera is used to record the movement and behaviors of the subject(s) within the experimental arena.

Note: The use of cameras other than those listed here might not produce acceptable results in your experiments, and is not supported.

- USB 2.0 camera, 640 (H) x 480 (V) pixels, 1/3" CMOS; CS-Mount:
  - — Model FMVU-03MTC-CS (for color imaging), or
  - — Model FMVU-03MTM-CS (for black and white or infrared imaging)
- 1/3" High-Resolution varifocal lens (3 to 8mm), installed by Plexon
- Camera heat sink and RC2 Rapid Connect Adaptor with 200PL-14 Plate
- Universal 2" - 6" adjustable pan tilt
- USB 2.0 A to Mini-B 5-pin cable, 2m long
- Camera Trigger Board and cable that provide power and signaling to the behavioral camera

1.6 Camera Repositioning

The system allows rapid repositioning of the behavioral camera to suit the specific needs of each experiment. After mounting the camera, you can enter new arena geometries and video parameter values for the repositioned camera, calibrate the new arena (if necessary) and proceed with the experiment. In many cases, this repositioning process and resetting of parameters can be accomplished in a few minutes.

1.7 Behavioral Video—Special Considerations

Some experiments might involve challenging conditions that make it difficult to track an animal, for example:

- Multiple animals of the same color must be tracked.
- The experiment involves objects that move at very high speeds so they cannot be reliably tracked at the maximum frame rate (30 frames per second).
- Tracking multiple spots on an animal is desired, but there is no way to apply colors (as with a fish, for example).
- The camera cannot be positioned to record the animal in the region of interest (in a tunnel maze, for example).
- The ratio of the size of the trackable arena to the size of the animal is such that the animal's image is too small to be tracked or too large to be meaningful.
- If the experiment requires tracking an animal in the dark (without LEDs), use the infrared (IR) capable camera. See Section 1.5, "Hardware and Software" on page 8 regarding IR requirements.

If your experiment involves any of the conditions listed above, please contact Plexon for assistance at +1 214-369-4957 or support@plexon.com.

1.8 Computer to Run Photometry Software

After recording a session, you can perform data analysis and export in **Files** mode (also referred to as offline mode) on the host PC that was supplied with your system.

Alternatively, you can transfer the files to a different computer for analysis and data export. To run the software in Files mode, the computer requirements are as follows.

- Processor: Intel® i7 or Intel® i9 or equivalent Intel® Xeon® (at least Quad core), 4 GHz
- RAM: 16 GB
- Hard drive: 1 TB
- At least two USB3 and three USB2 ports
- Display adaptor: NVIDIA® GeForce® GT730 or better; recommended resolution is 1920x1200
- Windows® 10 operating system

Note: To run the software, the Plexon Multi-Wavelength Photometry System license must be plugged into the computer.

Requirements are subject to change over time, therefore, we recommend that you contact Plexon Support (support@plexon.com or +1 214-369-4957) for the most current information.

Chapter 2
Installing and Starting the System

2.1 Installing the Software

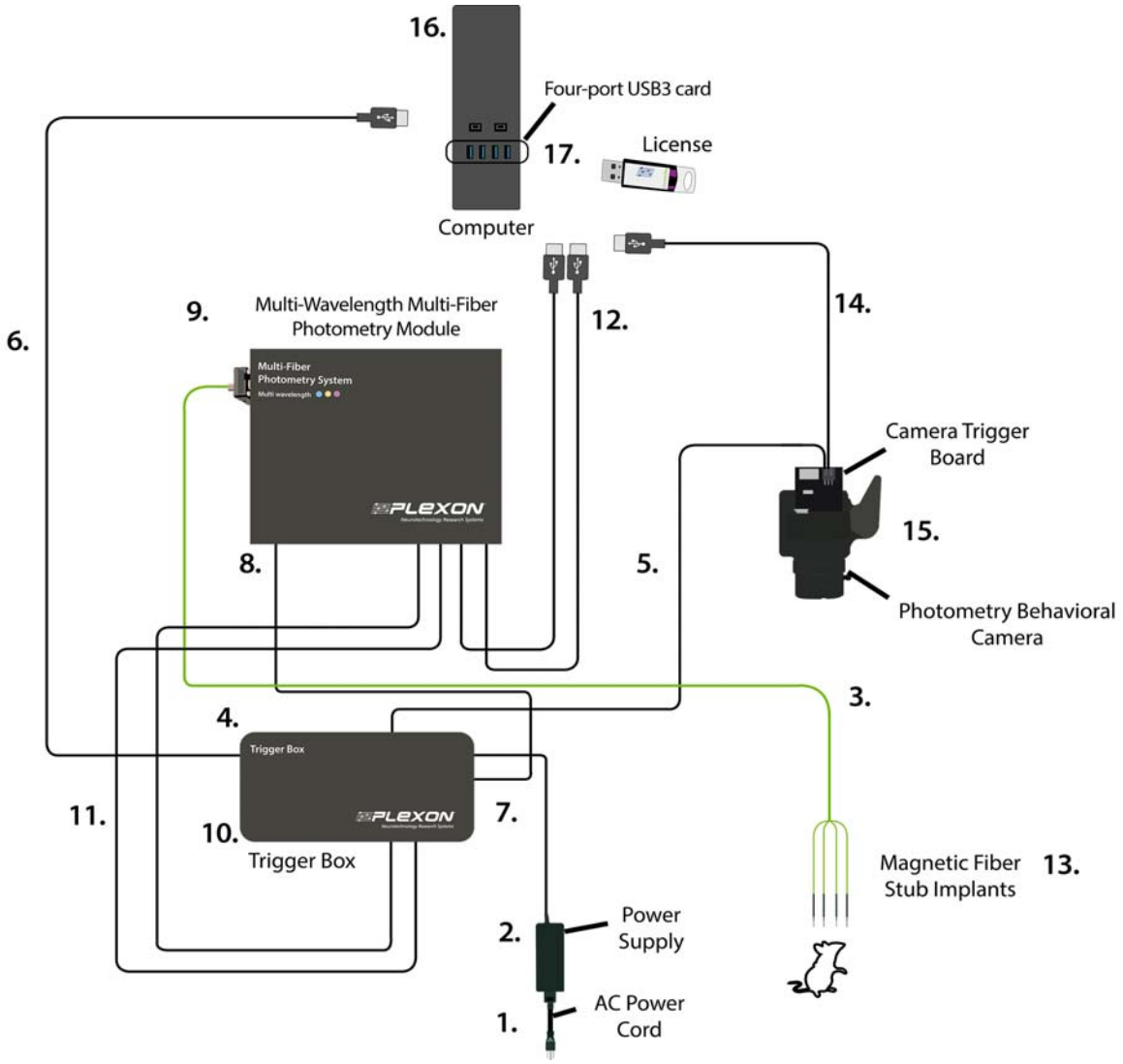
The software is already installed on the computer by Plexon®, and a copy of the software is included on a flash drive. If assistance is needed, contact Plexon Support at +1 214-369-4957 or support@plexon.com.

2.2 Assembling the Hardware

This section identifies the hardware and connecting cables and explains how to assemble the system.

Equipment and Cables

The Photometry System components and connections are shown below.



The Photometry System includes the following components:

1. AC Power Cord
2. Power Supply
3. Multi-fiber patch cable (optional item)
4. Digtial IO Status Indicator
5. Trigger Box to Behavioral Camera Cable
6. USB cable type A to type B
7. Trigger Box
8. Trigger Box to Photometry Module cable
9. Multi-Wavelength Multi-Fiber Photometry Module
10. Digital Input Generator
11. Trigger Box to Photometry Camera Cable (x2)
12. USB3 cable type A to type B with screwlocks (x2)
13. Fiber stub implants
14. USB cable type A to type mini-B
15. Photometry Behavioral Camera & Trigger Board
16. Computer
17. Four-port USB3 Card

See Detailed Parts Information on page 19 for images of these parts.

Connecting the Hardware

Each researcher will need to evaluate the experimental set up to determine the optimal location for the placement of the photometry hardware. The multifiber patch cable will be connected directly to the photometry module and will need to be positioned so the terminating end of this patch cable can be connected to the subject.

Follow these steps to connect the system hardware.

1 Verify that the computer is turned off (powered down).

2 Connect the cameras on the Multi-Wavelength Multi-Fiber Photometry Module (9) to the Computer (16) using the two USB3 cable type A to type B with screwlocks (12).

a It is important to connect the camera(s) to the four-port USB3 card (17) for optimum handling of the video stream; do not connect the camera(s) to any other port(s).

Note: The image of the PC shown below is typical of the units currently being provided with the CineLyzer System. The exact physical configuration of the supplied PC is subject to change over time. The four-port USB3 card may be on the front or back of your PC. If you have any questions regarding cable connections, please contact Plexon Support.

3 Connect the Cameras located on the Multi-Wavelength Multi-Fiber Photometry Module (9) to the Trigger Box (7) using the two Trigger Box to Photometry Camera cables (11).

Note: Make sure to plug the cable from Camera 1 on the Photometry Module into the first Trigger Box connector. The cable from Camera 2 should be plugged into the second Trigger Box connector.

4 Connect the Multi-Wavelength Multi-Fiber Photometry Module (9) to the Trigger Box via the Trigger Box to Photometry cable (8).

Photometry Module

Trigger Box

5 Connect the Trigger Box (7) to a power source with the AC Power cord (1) and Power Supply (2).

6 Connect the Trigger Box (7) to the Computer (16) using the USB cable type A to type B (6).

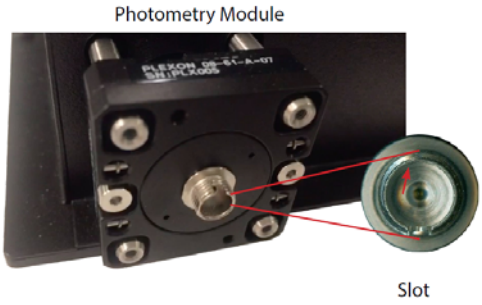
7 Connect the Trigger Box (7) to the Photometry Behavioral Camera (15) using the Trigger Box to Behavioral Camera Cable (5).

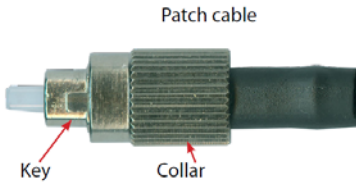
8 Connect the Computer (16) to the Photometry Behavioral Camera (15) using the USB cable type A to type mini-B (14).It is important to connect the camera(s) to the four-port USB3 card (17) for optimum handling of the video stream; do not connect the camera(s) to any other port(s).

9 Connect the provided keyboard and mouse to the computer. It is important to connect the camera(s) to the four-port USB3 card (17) for optimum handling of the video stream; do not connect the camera(s) to any other port(s).

10 Insert the license key into one of the built-in USB ports.

11 Connect the multi-fiber patch cable (3) to the Multi-Wavelength Multi-Fiber Photometry Module (9).





12 Turn on the Trigger Box.

13 The system is now ready for startup and operational testing.

Detailed Parts Information

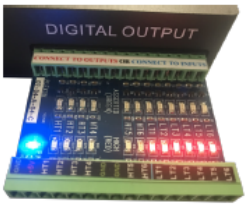
These parts are current as of the publication date and are subject to change.

1. AC Power Cord

2. Power Supply / Plexon 08-06-A-37

3. Multi-fiber patch cable / Plexon 08-60-A-04-C **(optional item)**

4. Digital IO Status Indicator (optional) / Plexon 07-04-A-04-C



5. Trigger Box to Behavioral Camera Cable / Plexon 06-06-A-12-A

6. USB cable type A to type B

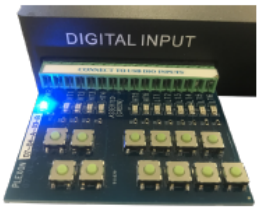
7. Trigger Box / Plexon 07-12-A-00-P3

8. Trigger Box to Photometry Box Cable / Plexon 06-06-A-14

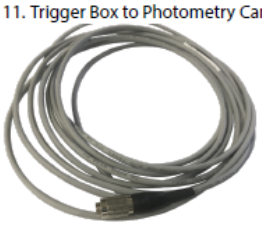
Detailed Parts Information, continued

9. Multi-wavelength Multi-fiber Photometry Module / Plexon 08-61-A-07-A

10. Digital Input Generator (optional) / Plexon 07-04-A-03-B



11. Trigger Box to Photometry Camera Cable / Plexon 06-06-A-13



12. USB3 cable type A to type B with screwlocks (x2)

13. Fiber stub implants / Plexon 08-44-A-10

14. USB cable type A to type mini-B

15. Behavioral Camera / Plexon 07-04-A-24-A

17. Four-port USB3 card

2.3 Startup and Operational Testing

Follow these steps to start the system and perform an operational test. For this operational test, it is not necessary to have the fiber implants or behavioral camera mounted in their final positions. The purpose of this test is to ensure that the system is functioning correctly.

	**CAUTION** **All system components need to be connected, and the Trigger Box power switch needs to be in the "ON" position, before the software starts.** If the software is started first, it will not recognize the photometry system hardware and either will not start or will switch to Files mode.

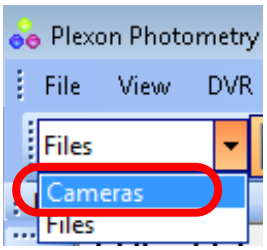
1 Ensure the Trigger Box power switch is in the ON position (up).

2 If not already done, power up the computer and log in.

Note: The behavioral camera as well as Camera 1 and Camera 2 in the Photometry Module should now display green LEDs indicating that power is connected.

3 Start the Plexon Photometry application. The easiest way is to double-click the desktop icon.

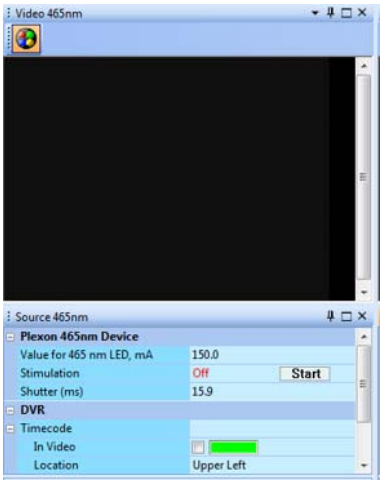
4 When the user interface opens. select **Cameras** mode (from the dropdown menu shown below), if it is not already selected.

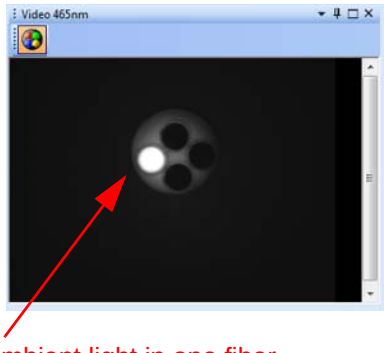


5 If **Cameras** mode is not available in the dropdown menu, or if the system displays any alarms warning you that some of the cables are not properly connected, follow the procedures in Appendix E, Troubleshooting, "Critical Alarm Messages" on page E-9 and "Critical Alarm Messages—Recording Disabled" on page E-12 to clear the problem.

6 View the GUI to see if the photometry video windows appear similar to the example shown below. As seen in this image, the fiber bundles (when not exposed to ambient light) will appear dark.

Point each of the fibers toward any ambient light source and verify the ambient light is observed through the fibers. This process verifies the fibers and patch cable are operational.





7 The branches of the cable are labeled 1, 2, 3, 4 during manufacturing (see below). Use ambient light to identify particular circles in the **Photometry Video** window with the various numbered branches of the cable. For example, you can point each branch toward a bright light in the room, then make a note of the correspondence between the physical branch and the circle that appears in the **Photometry Video** window.

8 Verify there is an appropriate image in the Behavioral Video window. This will verify the behavioral camera is capturing and transmitting properly.

TIP

You can easily restore the default GUI layout at any time

In the **Window** dropdown menu, select **Layout**, then select **Reset to Default Layout**. All the device windows (photometry devices and behavioral camera) will be displayed in the default GUI arrangement.

9 If you are planning to use digital inputs and outputs, two circuit boards—Status Indicator and Input Generator—can be plugged into the Trigger Box for testing purposes as shown in Appendix C, Trigger Box and Digital Input/Output. These boards are not required for normal operation.

10 If only one branch does not pass light then that branch is probably broken. If none of the branches pass light, then they are either all broken, or something else is wrong. For example, the patch cable may not be attached properly. The patch cable should be “hand-tightened” onto the connector on the side of the Photometry Module. If you cannot correct the problem, contact Plexon Support for assistance.

2.4 Verifying LED Function

This procedure verifies the LEDs are capable of emitting light and the light is observed at the fiber ends. Follow this procedure for each excitation wavelength.

WARNING
Never look directly into any optical fibers!

Depending on the wavelength and intensity of light emitted, you risk serious damage to your vision if you look directly into any optical fiber.

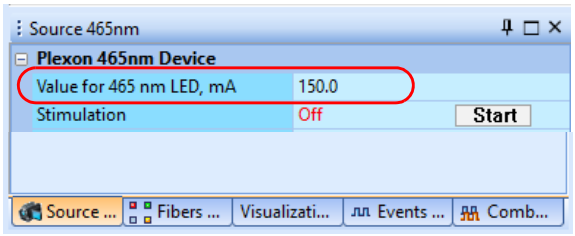
For example, UV light is not visible to the human eye, but it can damage the eye. The Plexon system emits relatively weak UV light, but it is still wise to avoid looking directly into the fibers.

Even if colored light is being emitted, it is possible that other, damaging light, such as UV light could be present.

Do not risk eye damage!

1 In the Source tab, the system will show the current (mA) value for each photometry device. This value will be set to half of its upper limit by default. For now, accept the default values for the LED current. In a later procedure, you can make adjustments to the current values when you measure the actual output power at the ends of the patch cable, as described in Section 4.6, “Test Mode—Measuring and Adjusting LED Output Power” on page 62. You can also make adjustments to the current values after you have created your experiment as described in Section 3.6 Creating a New Experiment on page 34.

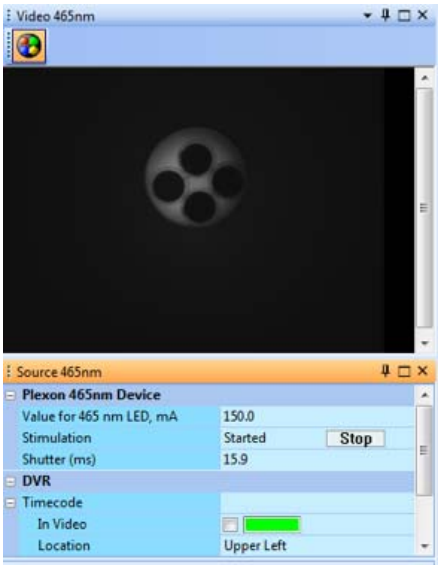
- The upper current limits for the LEDs are as follows:
  - Blue (465nm): 300mA
  - UV (410nm): 1000mA
  - Lime (560nm): 500mA

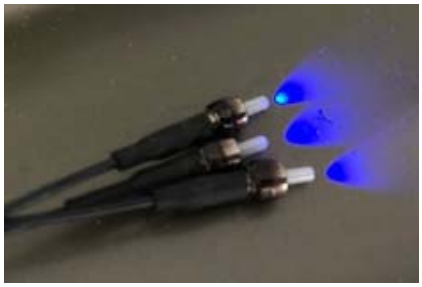


2 In the **Stimulation** item, click the **Start** button (shown in the above image). The fibers should now begin emitting the appropriate color light. (Notice that the **Start** button has changed to a **Stop** button.)

3 Verify the LED excitation light is being emitted at the ends of the fiber branches. The example below shows the test for the blue (465nm) LED.

Note: When you are testing the UV excitation light (centered at 410nm), the emitted light will have a purple tint, but the true UV light will not be visible to the naked eye.



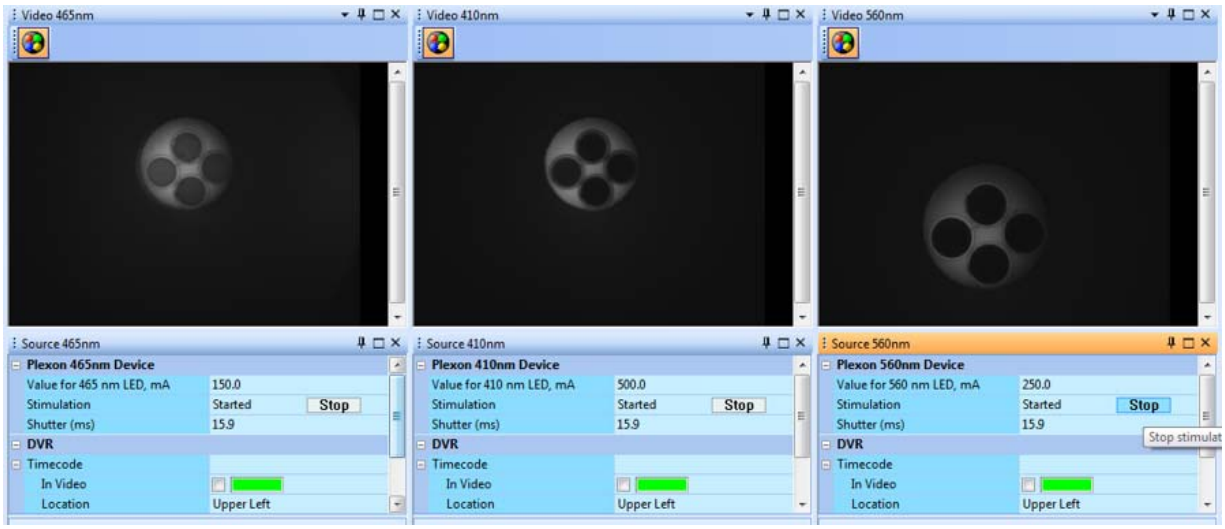


4 Click the **Stop** button to turn off the LED.

5 Repeat Step 2 through Step 4 for the other excitation wavelengths.

6 Start all three of the photometry wavelengths and verify the video windows appear similar to the example below. (This will cause the **Start** buttons to change to **Stop** buttons.)

Notice that the images for the 465nm and 410nm fiber bundles appear identical. This is because those images are recorded by the same camera (Camera 1) in the Photometry Module. The image for the 560nm fiber bundle is typically somewhat different because it is recorded by a separate camera (Camera 2) in the Photometry Module.



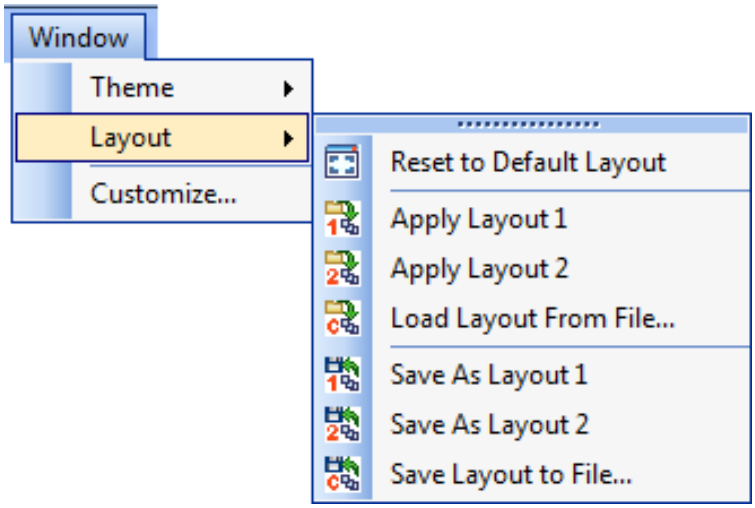
Prior to starting a photometry experiment, it is essential to use an optical power meter (for example, the Plexon light measurement kit) to measure the level of excitation light transmitted out of **each branch** of the multi-branch optical cable. When you are ready to make that measurement, see Section 4.6, “Test Mode—Measuring and Adjusting LED Output Power” on page 64.

2.5 Navigating the User Interface

This section describes the user interface, where you can view and modify video parameter values before starting the recording.

2.5.1 Arranging Windows in the GUI

The default window layout will be suitable for many experiments. To restore the layout to default, use the **Window** dropdown menu at the top of the user interface: **Window** > **Layout** > **Reset to Default Layout**.



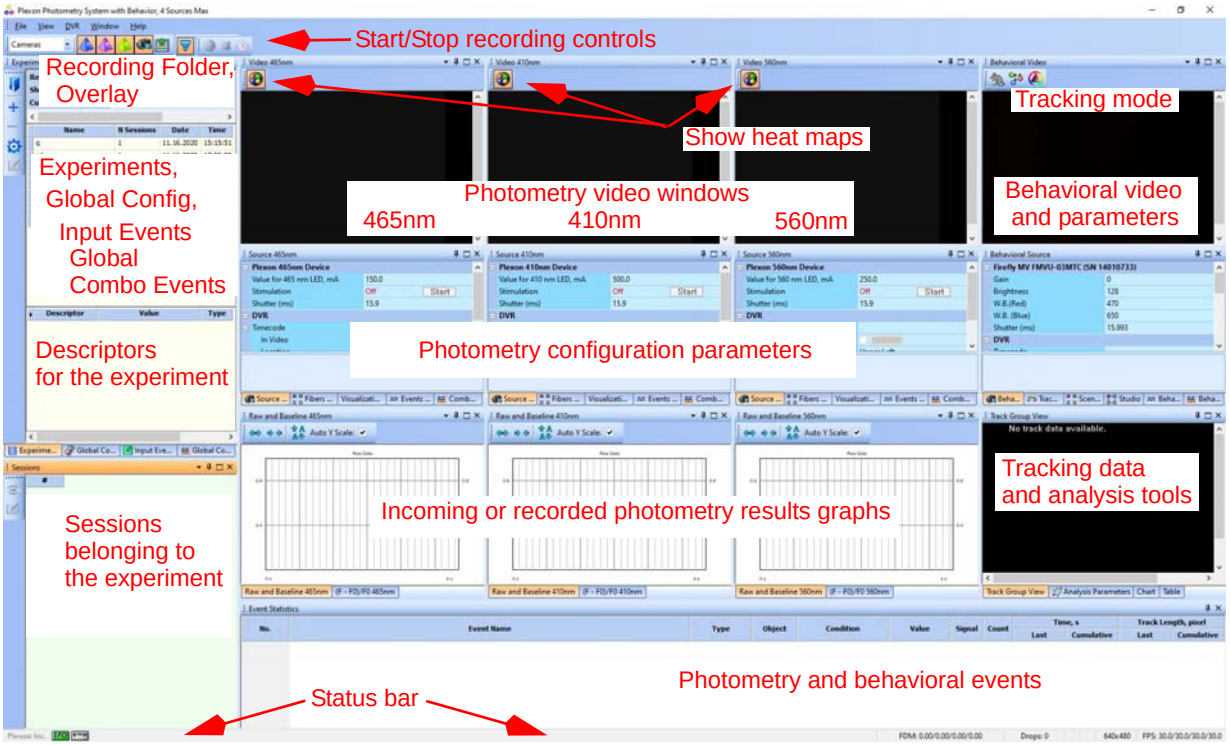
To save an existing layout select **Save Layout to File...** and follow the prompts.

To load a previously saved layout select **Load Layout from File...** and follow the prompts.

For more information on changing the window layout, see "Window Menu" in Appendix B.

2.5.2 Identifying the Areas of the User Interface

This image shows the general location of the windows in the default GUI layout.



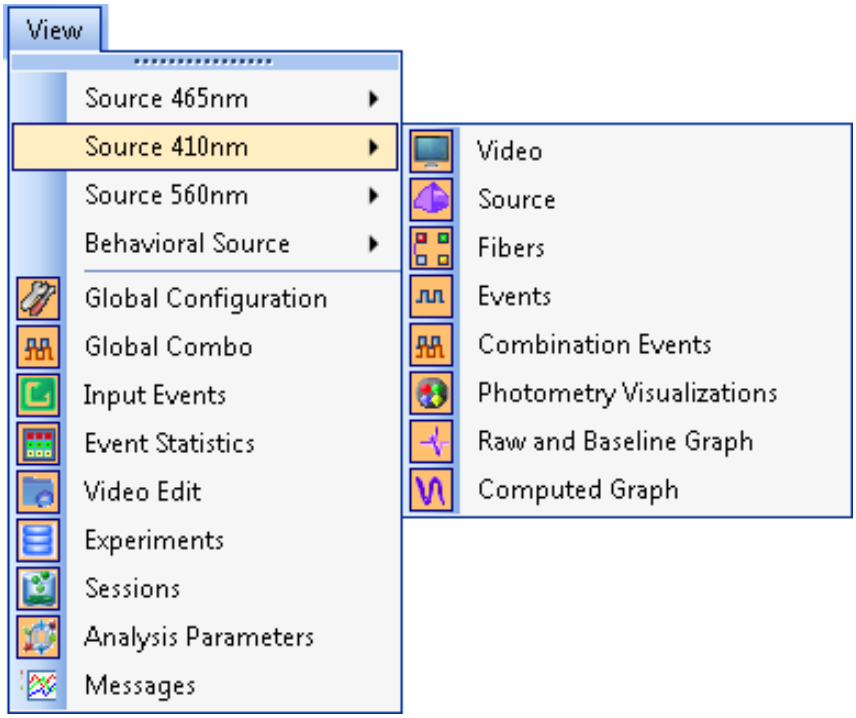
TIP

Use the View menu to see Messages window

There is an additional window that you can display in the interface—the Messages window. This window does not appear by default, but you can select it in the **View** dropdown in the main menu.

2.5.3 Using the View Menu

Use the **View** dropdown menu to quickly access the parameters you want to configure anywhere in the GUI.



2.6 Where to Go Next

Go to Chapter 3, Preparing Your Experiment Database.

Chapter 3
Preparing Your Experiment Database

3.1 Data Storage and Organization

This section provides an overview of data storage and organization in the system.

You can create one or more recording folders (**Recording Folder** in the **Experiments** tab) to contain your experiment settings and data. Each recording folder can contain multiple experiment subfolders (one subfolder for each experiment). Each experiment subfolder contains

- The geometry and other user-specified parameters for the experiment
- Up to 20 user-defined descriptors that uniquely identify the experiment
- Up to 20 session variables that differentiate among the various sessions in the experiment and can be used to group and filter the data from the sessions
- A unique video file for each device (the three photometry wavelengths and the behavioral camera) for each session
- Exported and computed data for the experiment

The disk on which you will be recording should have sufficient free space to accommodate the amount of data you are expecting, as described in Section 3.2, “AVI Video Format, Data Rate, Timestamps and Compression” on page 31.

3.2 AVI Video Format, Data Rate, Timestamps and Compression

This section discusses the format, timestamping, synchronization and compression of video files.

MPEG-4 Recording Processes

During the recording process, the software continually calculates and displays the amount of recording time left until the target hard drive would fill up. As the hard drive capacity is neared, the system stops recording.

The AVI files created by the software are industry-standard AVI-format files that you can play with many standard tools, including Microsoft® Windows® Media Player, which is pre-installed on most Windows computers.

The MPEG-4 AVI format allows the embedding of additional data segments within AVI files. Each embedded data segment has an identification tag. Typically, an AVI file reader that does not recognize a tag for an embedded data segment skips that data segment. The system always produces AVI files with a Plexon® specific additional tagged data segment that contains dynamic data, including the frame timestamp, for each video frame. This Plexon specific nondestructive embedded data is not visible on the video frame. However, the Source tab in the user interface contains an optional setting that can display the timestamp in visible numerals on the video image itself; see Section 4.5, “Configuring Photometry Source Parameters” on page 60 and Section 5.3, “Configuring Behavioral Source Parameters” on page 105.

MPEG-4 Quality, Compression and File Size

The system stores the video recording from the cameras in the database on an internal hard drive. You may start, stop, and timestamp these video files in a way that enables you to subsequently correlate them offline with other data. The system records video into AVI files at a resolution of 640x480 pixels at 30 frames per second.

The system records a very large amount of data during each session. Each photometry video, if not compressed, would generate about 30 GB of data for a one-hour session. Thus, the three photometry videos and the smaller sized behavioral video would generate a total of about 100 GB, and you would be able to store only ten sessions on 1-TB disk space. To reduce disk usage, the system compresses the video using MPEG-4 algorithms before it writes it to the AVI file. In a typical session, the system generates approximately 1.5 GB of compressed data in one hour, including all four AVI files (three photometry videos and one behavioral video). Of course, this is a rough estimate, since, for example, the photometry AVI file size may vary slightly from system to system. The data-recording rate is also related to how much the images change. For example, when there is no stimulation, the photometry images are all black, and are compressed very well, perhaps as low as 120 MB per hour.

The system uses a very high MPEG-4 compression quality (minimum compression) for all four video sources. The purpose for using minimum compression is to preserve the greatest amount of information in the AVI files, in case the researcher needs to remeasure the photometry signals directly from the AVI files. Nonetheless, even this minimum compression process changes the photometry signal significantly. Thus, if you remeasure the photometry signals from video files (in Files mode), you will find them to be significantly less than the original signals that were measured in Cameras mode.

As a practical matter, there is rarely a need to remeasure photometry signals from files. For example, if a researcher disconnects and then reconnects the patch cable during an experiment (not recommended), the fibers might have moved relative to their outlines. If this went unnoticed during recording, they would have been measuring the wrong signal after reconnecting. In that case, reanalyzing the AVI files with adjusted fiber outlines would be a backup method of preserving some data.

3.3 Planning the Database

Follow these steps to plan and organize the database for the experiments you expect to perform.

1. Verify the PC disk has sufficient space for all contemplated recordings, in accordance with the discussion in Section 3.2, “AVI Video Format, Data Rate, Timestamps and Compression” on page 31.
2. Generate a plan for naming your recording folder(s), and subfolders if desired, within the database.
3. Familiarize yourself with the information in Section 3.4, “Setting Parameters for an Experiment with Multiple Sessions” on page 33.
4. Use the **Recording Folder** row in the **Experiments** tab to create and access your recording folder(s) and subfolder(s) according to your plan. See Section 3.5, “Setting the Recording Folder Location” on page 35, for the procedure.

3.4 Setting Parameters for an Experiment with Multiple Sessions

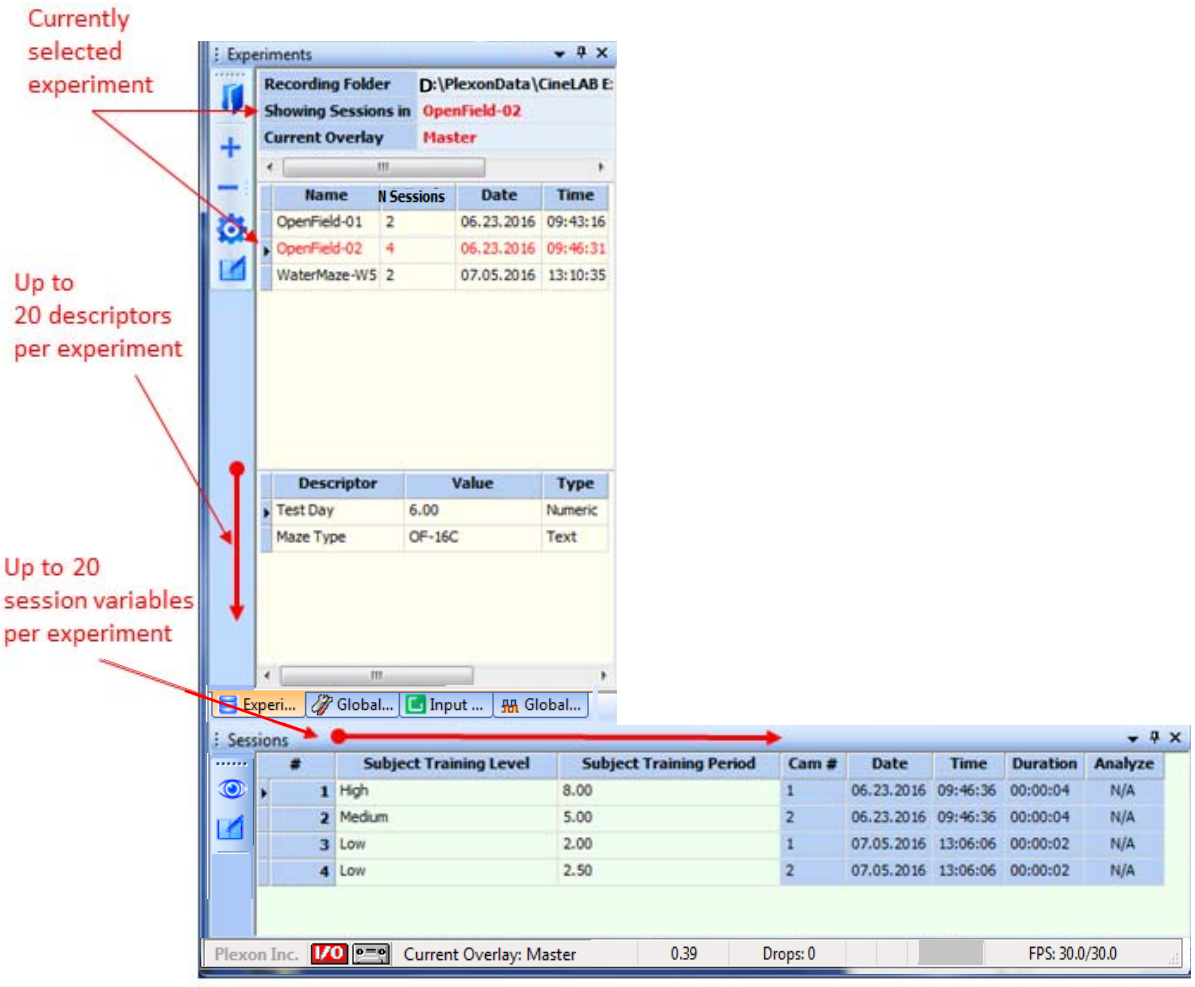
The following diagram shows an example of the setup for an experiment with multiple sessions.

TIP

Reset to Default Layout

It is often helpful to reset the GUI display to the default layout (unless you have created a customized layout that you prefer). The reset ensures that the system is displaying all of the tabs and options you are likely to use in configuring your experiment. In the main window, select **Window > Layout > Reset to Default Layout**.

Note: You must create or select the **Recording Folder** and create or select an experiment (in the **Experiments** tab) to enable all other system functionality. The image below shows you where to configure and view these fields.



The system creates a separate set of files for each experiment that you create. You can define up to 20 different descriptors that characterize and identify each experiment. Within each experiment, you can create up to 20 independent variables that differentiate among the various sessions. Every time you begin a new recording (session), the system creates a new file for that session within the active experiment. For procedures on starting and stopping a recording session, see Chapter 10, Recording and Monitoring.

You can select from among the session variables to group and filter session results when you perform your analysis with the tools built into the system software. See Chapter 11, Analyzing Data and Adding Sessions.

Example with three independent variables

As an example, consider the following experiment. In this example, animals (except the control group) are administered a centrally-active substance that compromises performance on some task, and various compounds are tested for their effectiveness in restoring some behaviors.

In this example, each set of 60 sessions would have three variables—Substance (yes or no), Compound (A or B) and Dosage (number of milligrams or ml).

Sample Experiment – 240 sessions

- 60 sessions: Control group
- 60 sessions: Animals administered the substance, but not treated with any compound
- 60 sessions: Animals administered the substance, then treated with Compound A
- 60 sessions: Animals administered the substance, then treated with Compound B

The animals are put in a maze or other environment where their movements are recorded on video and the animal's positional coordinates are tracked throughout the session. The system records the tracking data along with the photometric fluorescence data.

The system also keeps a record of photometric events, behavioral events, and digital inputs which facilitate analysis of data from the sessions.

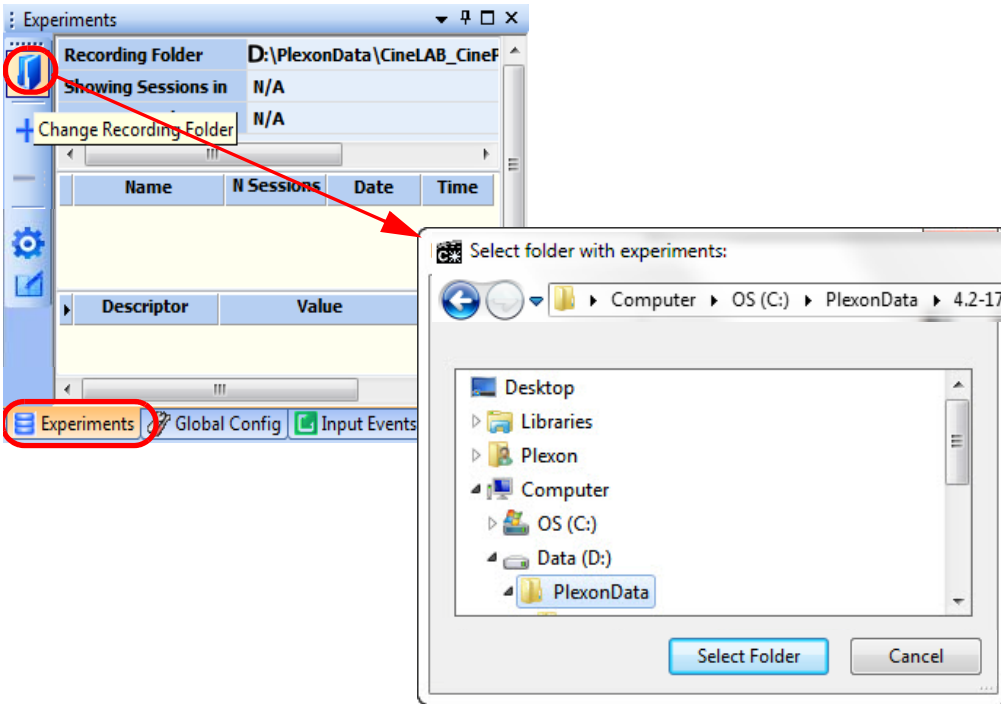
TIP
Use session variables to streamline data analysis

During data analysis, you can use the session variables to group and filter sessions, which makes it easier to visualize results.

3.5 Setting the Recording Folder Location

This section provides the procedure for setting up folders for your video files, data and settings.

1 Click the **Experiments** tab and then the **Change Recording Folder** icon. The browsing window opens.



2 In the browsing window, use standard Windows® methods to select or create the appropriate folder. The factory default location for the Recording folder is D:\PlexonData. You should create a subfolder, for example D:\PlexonData\MyExperiments, to contain all your experiments.

Note: If your PC does not have a D: drive, the **Recording Folder** location defaults to C:\PlexonData.

You can create folders and subfolders according to the needs of your experiments. Every time you create a new Experiment in the user interface, the system creates a new folder specifically for that experiment. All video files, settings and data for that experiment are contained in this folder.

Note: The system automatically looks for existing experiments in the recording folder and displays them in the **Experiments** tab.

3 When you are finished choosing or creating a storage folder, click **OK**.

Note: If the recording drive is not NTFS compatible, a warning message displays.

3.6 Creating a New Experiment

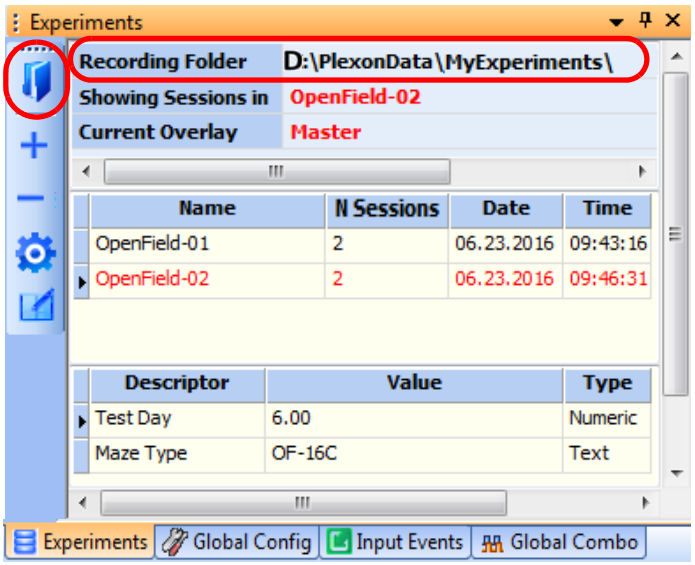
This section explains how to create a new Experiment and add sessions to the experiment, which you can do in either mode (**Cameras** mode or **Files** mode).

All Experiments are saved in the folder that you selected (or created) to be the **Recording Folder** in Section 3.1, “Data Storage and Organization” on page 30.

Note: If you prefer to add sessions to an existing experiment, see Section 3.7, “Selecting a Previously Saved Experiment” on page 44.

The Experiments tab (see the image below) allows you to create one or more recording folders. The system automatically creates a separate subfolder for each new experiment as you add experiments. You can add or delete experiments, attach comments to each experiment, and view the active experiment—the experiment currently being analyzed or to which you are currently adding more sessions. By default, the **Recording Folder** is set to D:\PlexonData. You should create a subfolder, for example D:\PlexonData\MyExperiments, to contain all your experiments. The system automatically looks for existing experiments in the recording folder and displays them in the Experiments tab. The **Change Recording Folder** icon and the current display for the **Recording Folder** are highlighted in the image below.

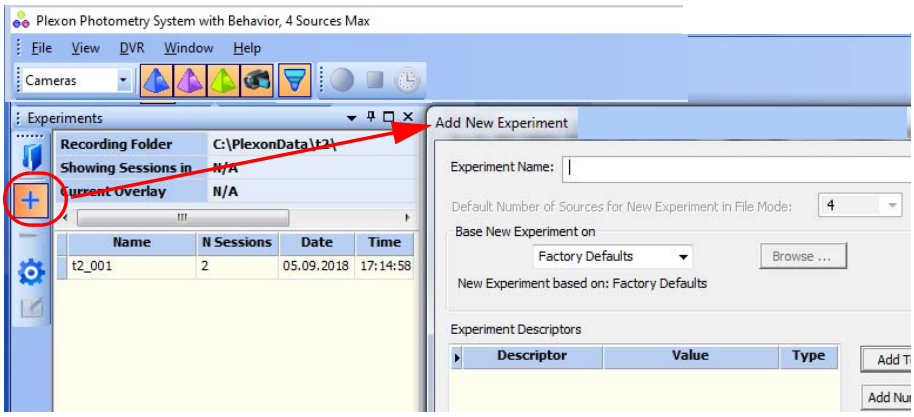
Note: If your PC does not have a D: drive, the **Recording Folder** location defaults to C:\PlexonData.



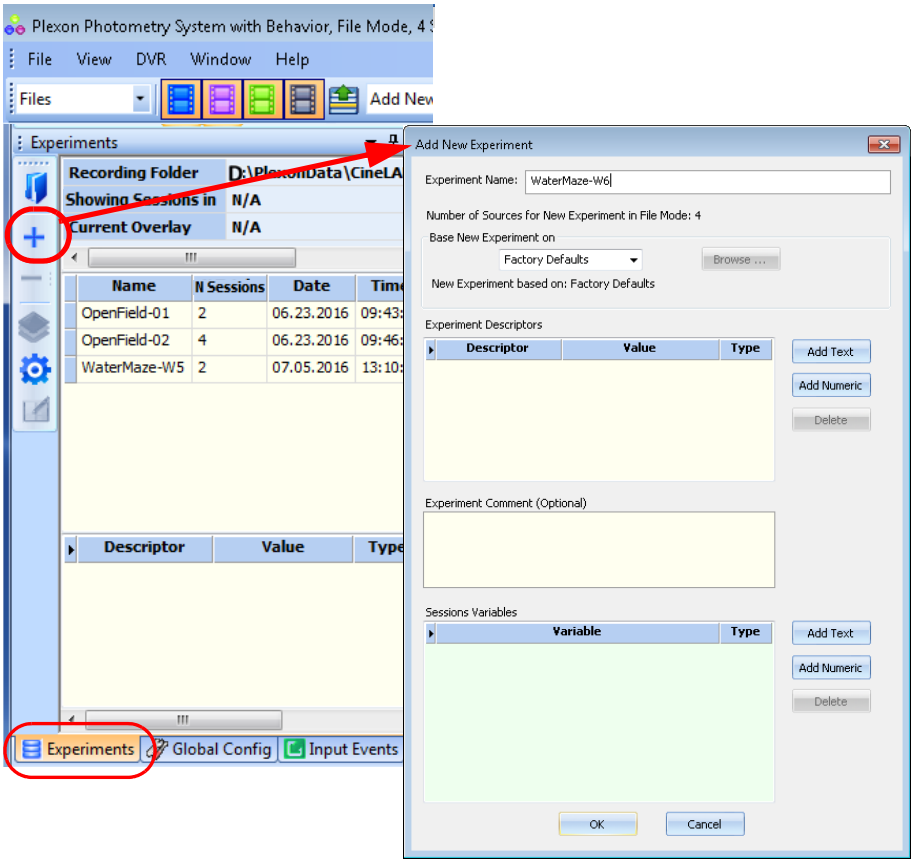
Follow these steps to create a new experiment.

1. Click on the **Experiments** tab. Then click the **Add new experiment** icon to open the **Add New Experiment** dialog box.

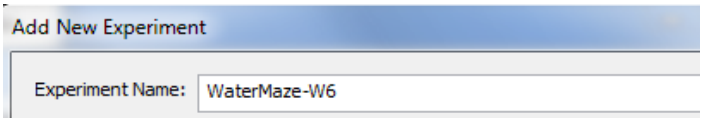
   In **Cameras** mode, the dialog looks like this. The default number of sources is automatically set equal to the number of devices currently active in the system.



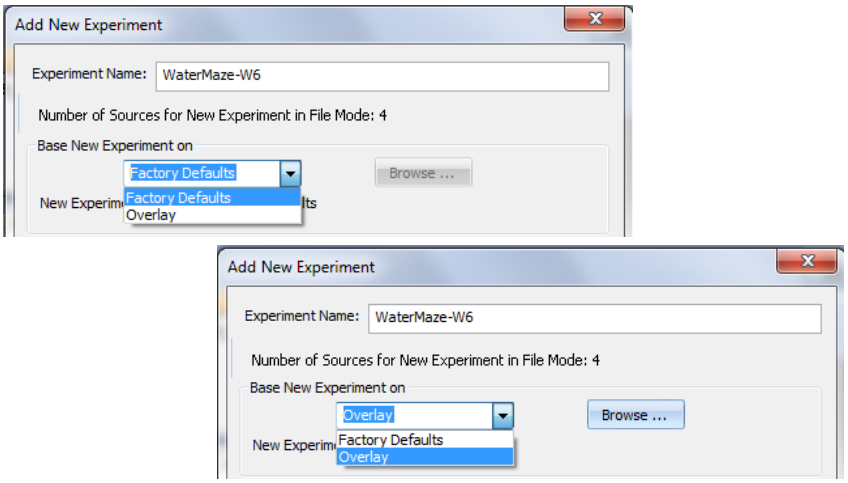
In **Files** mode, the dialog looks like this. The system displays the number of sources that were active when the AVI files were originally recorded.



2 Enter a name for the experiment in the top line of the dialog box.



3 In the **Base New Experiment on** dropdown list, select **Factory Defaults** or **Overlay**. See the image below. (Overlays are explained in the section that follows.)



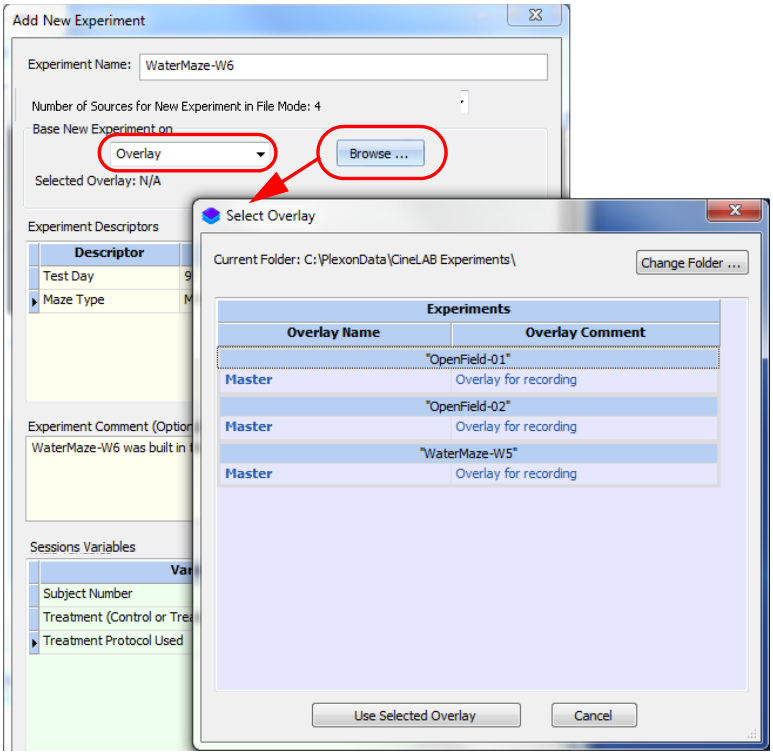
Understanding defaults and overlays

If you select **Factory Defaults**, the system uses the same settings you would obtain from selecting **File > Restore Factory Settings** in the main toolbar. Beginning with those initial settings, you can change any parameters as appropriate for the new experiment.

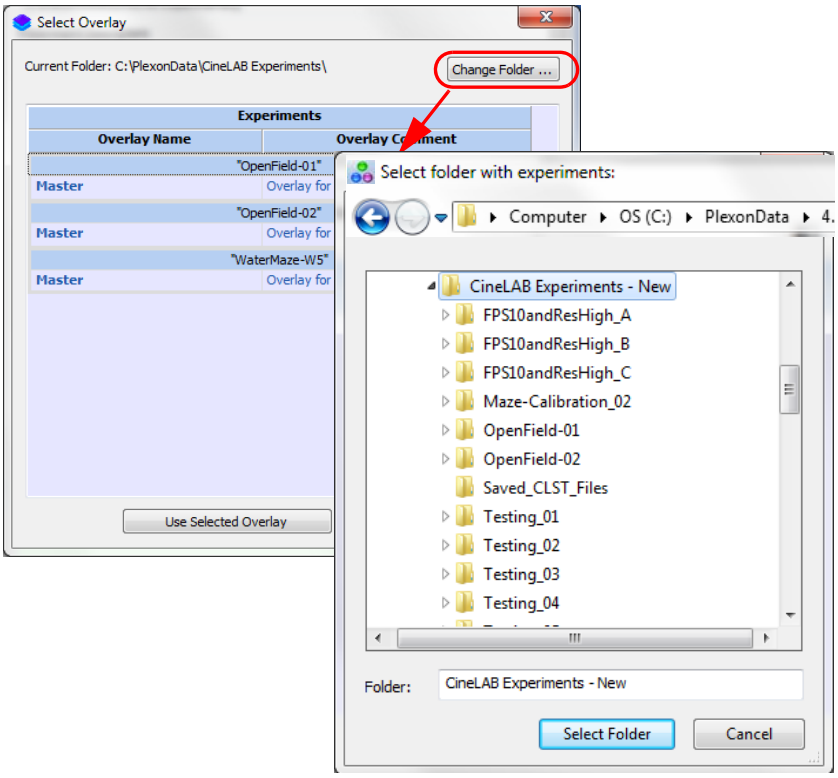
Selecting an Overlay

If you select the **Overlay** option, you will be able to apply settings that were created for a previous experiment, that is, to *overlay* those settings on this new experiment.

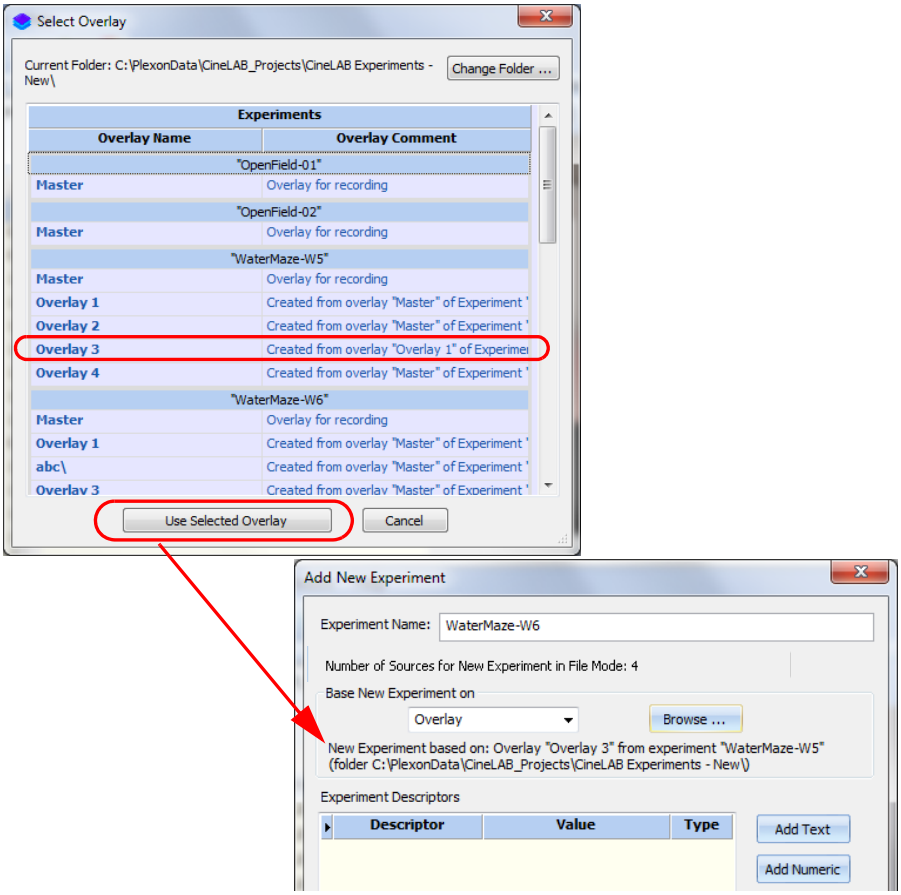
4 If you select **Overlay**, the **Browse** button becomes active; click on this button to open the **Select Overlay** dialog box.



5 Notice the **Change Folder ...** button in the upper right of the **Select Overlay** dialog box. You can click this button to navigate to another folder that contains an experiment with an overlay you want to assign to the new experiment.



6 Click on an overlay (in this example, click on Overlay 3 from the experiment WaterMaze-W5) and then click the **Use Selected Overlay** button. This action will load the geometry and parameter values from the selected overlay (Overlay 3 of WaterMaze-W5) onto the new experiment (WaterMaze-W6). Notice that the **Add New Experiment** dialog box now shows the path to the selected overlay.



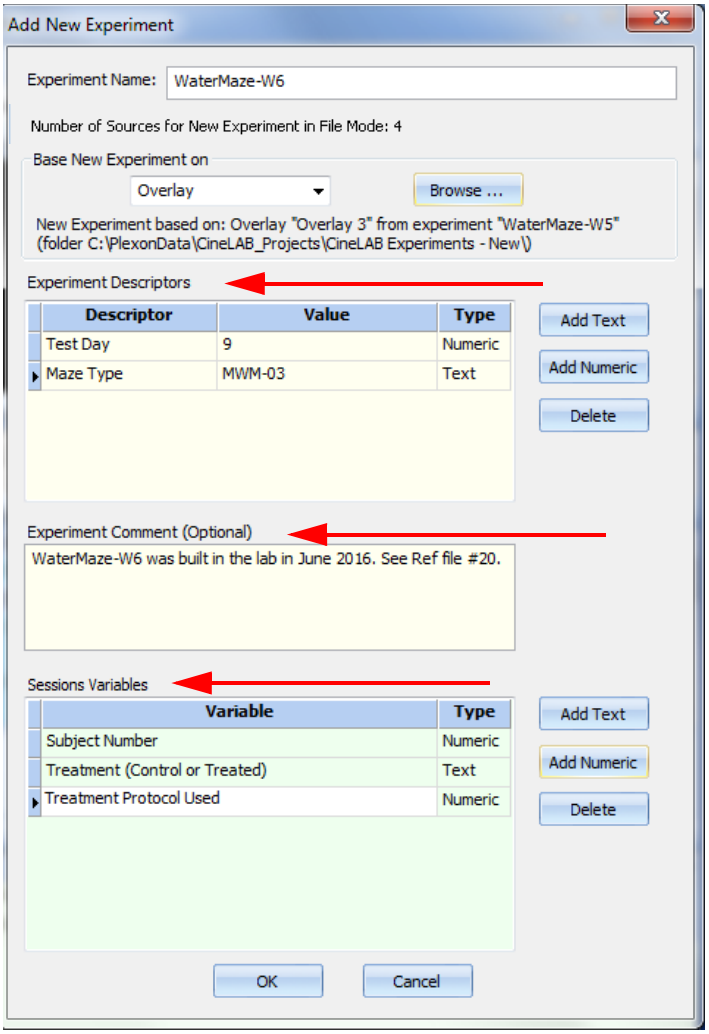
Note: If you select an existing overlay when you create a new experiment, and then start recording, the parameter values in that overlay will be saved as the Master overlay for that experiment. For example, if you load an Experiment 6 Master overlay to Experiment 7, those parameter values are saved as the Master overlay for Experiment 7. In this case, both Experiment 6 and Experiment 7 would have identical Master overlays.

Overlays are described further in Section 11.13, “Using the Overlay Feature during Analysis” on page 290.

Add descriptors and variables

7 Create **Descriptors** for the experiment and **Variables** for the sessions. (See the example below.) You can create up to 20 **Descriptors** for the experiment and 20 **Variables** for the sessions.

You can also add an **Experiment Comment (Optional)**.



- The experiment **Descriptors** identify the features you consider important for this experiment, such as the test day, information about the test subject, number and location of fibers, geometry of the test area, dimensions of a maze, lighting conditions, etc. The experiment **Descriptor** names and values *must* be entered in the **Add New Experiment** dialog box. However, you can modify them later as described elsewhere in this section.

- The session **Variables** apply to every session in the currently selected experiment, and are used later to filter, group and analyze the recorded data. Typically, they are the independent variables with which you wish to compare your test subjects. But you should feel free to add any session variables that are useful for your project. The session variable names must be entered in the **Add New Experiment** dialog box. When you start

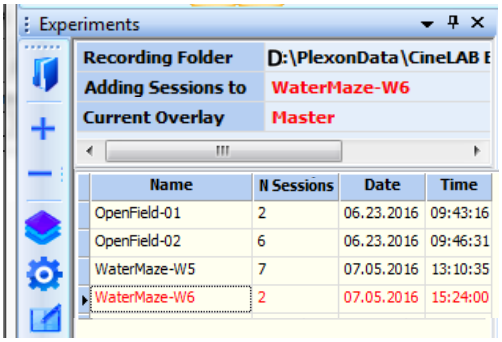
a new recording (i.e., a new session) the system prompts you to enter values for each of the session variables for each behavioral video stream. You can modify session variable values later as described elsewhere in this section.

TIP

Be sure to define session variables when you first create an experiment

If you do *not* define session variables when you create a new experiment, you will *not* be prompted to enter them before each session, nor will you be able to modify them per session later.

8 Click **OK** at the bottom of the **Add New Experiment** dialog. Notice that the new experiment has been added in the Experiments tab.



Note: After an experiment has been created, you can still view the experiment parameters and edit many of them, as described in these sections:

- Section 3.7, “Selecting a Previously Saved Experiment” on page 44,
- Section 3.8, “Editing the Experiment Name, Descriptor and Variable Values” on page 45
- Section 3.9, “Adding and Editing Comments in Experiments and Sessions” on page 48.

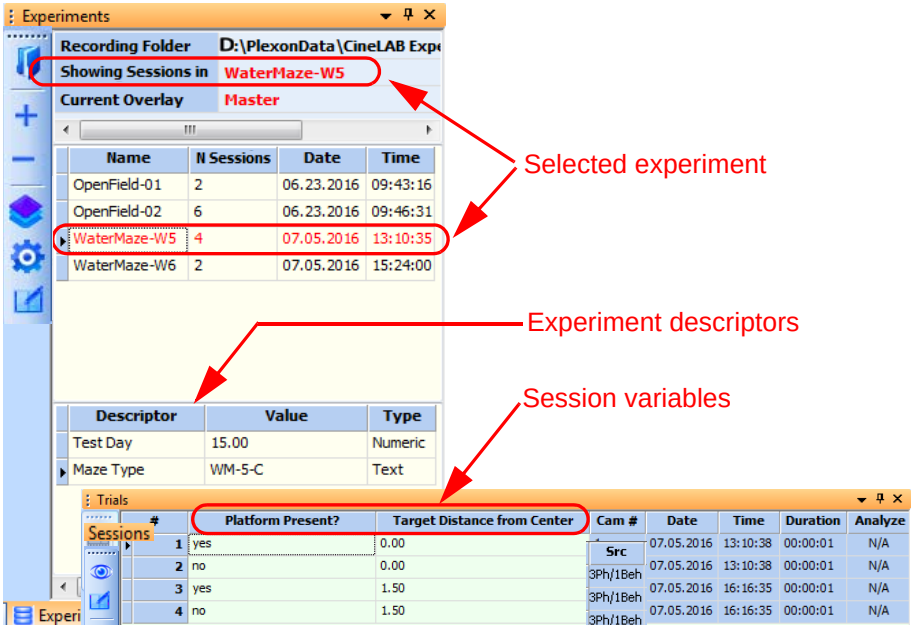
3.7 Selecting a Previously Saved Experiment

1 Click the **Experiments** tab to view a list of existing experiments that are saved in the currently selected **Recording Folder**.

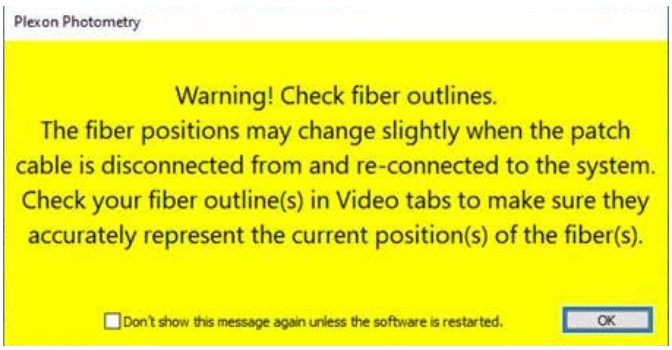
2 In the **Experiments** tab, click on a row to select a specific experiment.

When you click in the row of an existing experiment that you want to run, the system displays the applicable video windows for that experiment and a list of sessions that have already been run for that experiment, if any.

In the example below, the experiment “WaterMaze-W5” has been selected. The experiment descriptors and session variables for this experiment are displayed also.



When you click on a previously recorded experiment, the system displays a pop up warning advising you to check the fiber alignment. Once fibers have been drawn and saved in the GUI for a patch cable, the location of those fibers should not vary. However, if you disconnect the patch cable and then reconnect it, there is a chance that the bundle will be slightly rotated. This can be prevented by always tightening the patch cable "hand tight" using the same force when reconnecting it or by not removing the patch cable at all during an experiment. The warning dialog gives you the option to disable the pop up message after its first appearance.



3.8 Editing the Experiment Name, Descriptor and Variable Values

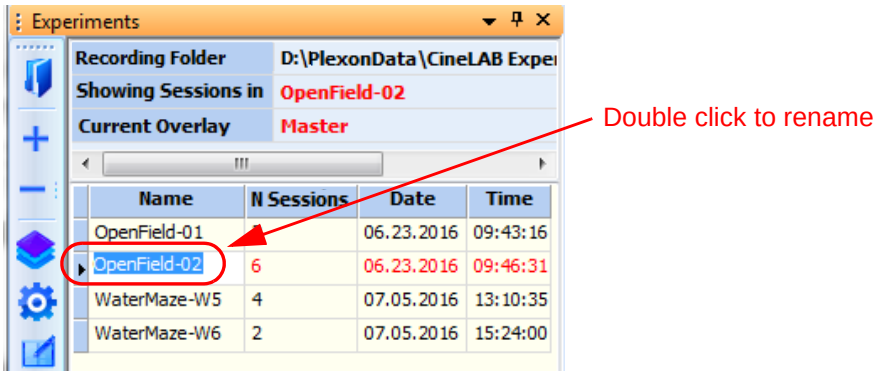
When you create a new experiment (by clicking the + icon in the **Experiments** tab), the system prompts you to enter a name for your experiment, the names of descriptors for your experiment and the name of variables for the sessions. Later, you can modify the name of the experiment, the descriptor names and values, and the values of the session variables. Make these changes in the GUI display, not in the Windows® interface. (Do ***not*** change an experiment name by means of the Windows file-naming function, because the system software will not be able to locate that experiment or file automatically in the future.)

	**CAUTION** **Do not change Experiment names outside the GUI** If you want to change the name of an experiment, do *not* change the name in the Windows environment by changing the folder name for the experiment. The system will not be able to locate the file automatically. Instead, make the change by double clicking the name of the experiment in the **Experiments** tab (as shown below) and typing in the new name.

To change the name of an experiment, the name or value of a descriptor, or the value of a variable, double click on the name or value you want to change, then type in the new value. These methods are described in the following sections.

Changing the name of an Experiment

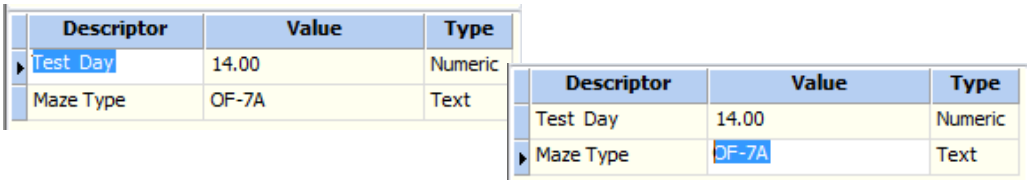
The experiment name ***must*** be entered in the **Add New Experiment** dialog box. However, you can modify the name of an experiment at any time by double clicking the name in the **Experiments** tab and typing in the new name. See the example below, in which the name of an experiment is being modified.



Be sure to change experiment names using the method described above. Do not attempt to change the name in the Windows environment. Also note that the GUI does not allow certain characters in an experiment name. If you enter such a character, it will not be displayed.

Changing an experiment Descriptor or Value

The experiment descriptors identify the features you consider important for this experiment, such as the test day, information about the test subject, LED excitation details, parameters of interest for the photometry fibers, geometry of the test area, dimensions of a maze, lighting conditions, etc. The experiment descriptor names and values *must* be entered in the **Add New Experiment** dialog box. However, you can modify descriptor names and values at any time by double clicking the item and typing in new information. See the examples below, in which the name and value of a Descriptor are being modified.



Descriptor	Value	Type
Test Day	14.00	Numeric
Maze Type	OF-7A	Text

Descriptor	Value	Type
Test Day	14.00	Numeric
Maze Type	OF-7A	Text

Changing the value of a Session variable

The session variables apply to every session in the currently selected experiment, and are used later to filter, group and analyze the recorded data. Typically, they are the independent variables with which you wish to compare your test subjects. But you should feel free to add any session variables that are useful for your project. The session variable names *must* be entered in the **Add New Experiment** dialog box. When you start a new recording (i.e., a new session) the system prompts you to enter values for each of the session variables for each video stream. You can modify variable values at any time by double clicking the item and typing in new information. See the example below, in which the value of a variable is being modified.

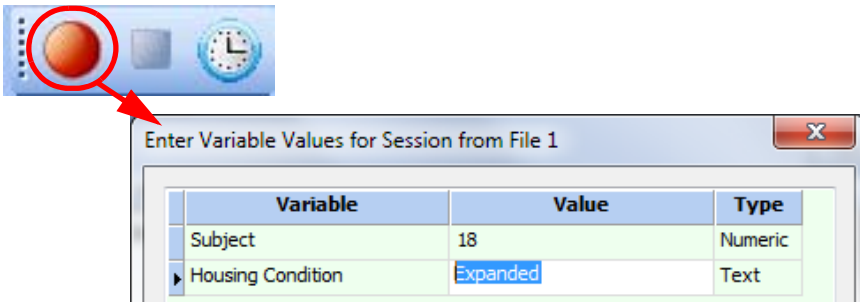
Sessions

#	Subject Number	Treatment (Control or Treated)	Treatment Protocol Used	Cam #	Dat
1	3.00	Control	0.00	1	07.05.2
2	4.00	Treated	7.00	2	07.05.2

Entering the value of Session variables when prompted

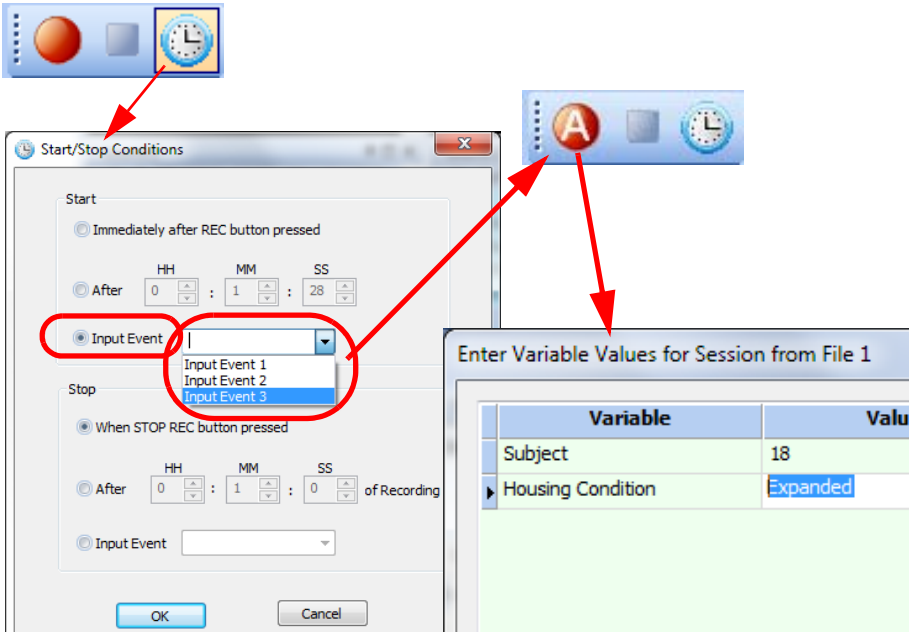
When a new recording (session) is about to start, the system prompts you to enter values for each session variable for the behavioral video stream. The prompt is displayed when the starting condition occurs. See the examples below.

Manual or timed-start recording—when the Recording button is pressed



Input Event starts the recording—when the Arm button is pressed for the first session

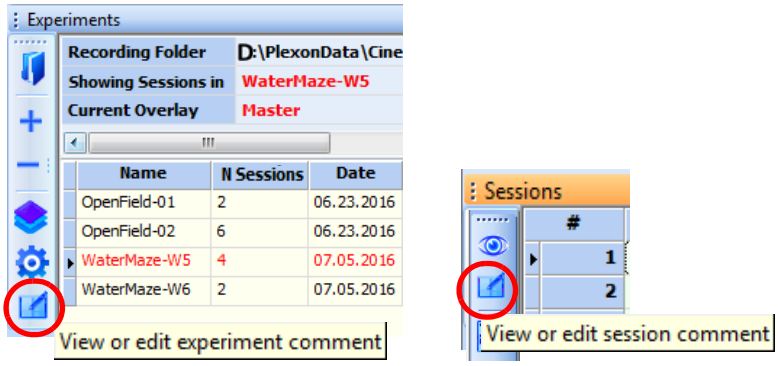
Note: For each subsequent session, the prompt is displayed when the previous session finishes.



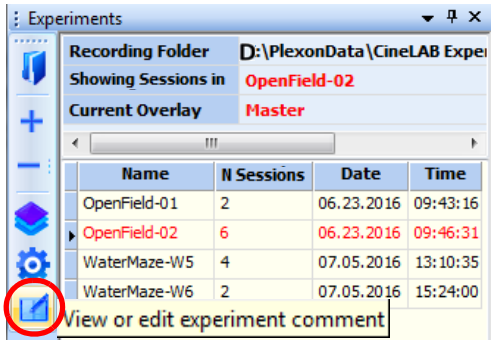
For more information on starting a session based on timing or Input Events, see Chapter 10, Recording and Monitoring.

3.9 Adding and Editing Comments in Experiments and Sessions

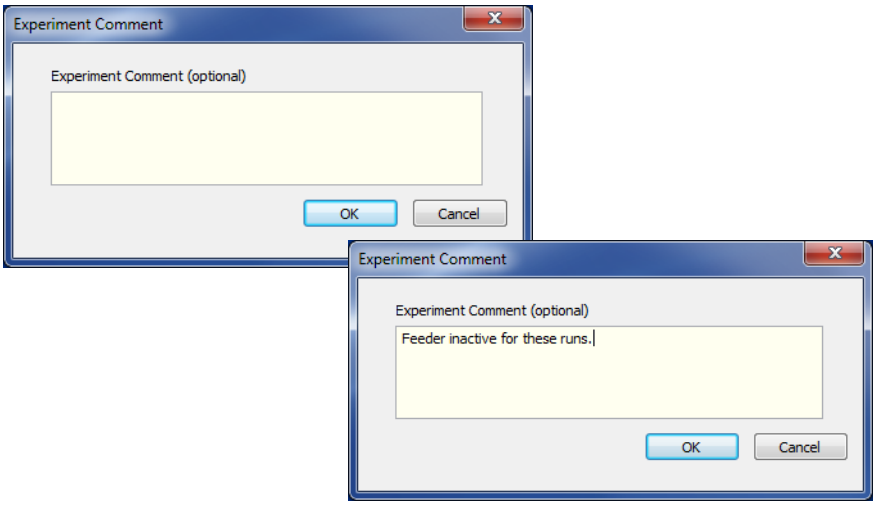
At any time, you can view or edit an Experiment Comment or Session Comment by clicking the applicable **View or edit experiment comment** or **View or edit session comment** icon . See the image below. This function is useful when you are initially describing your experiment or session, and also when you observe an interesting occurrence during a session. For example, you might want to add a comment during a session in which the test subject falls or displays an unexpected behavior.



1 To add a comment to the selected experiment, click the **View or edit experiment comment** icon.

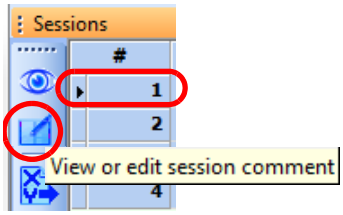


The system displays the **Experiment Comment** dialog box. The comment field might be empty (if no comment has been added yet) or it might contain previous comments.

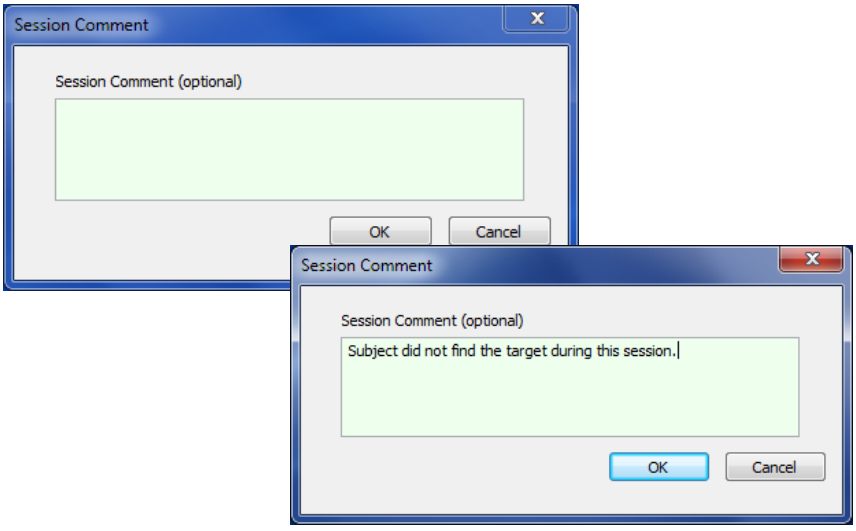


2 Add comments as needed and click **OK**.

3 To add a comment to the selected session, click the **View or edit session comment** icon. (Note that Session #1 is selected in this example.)



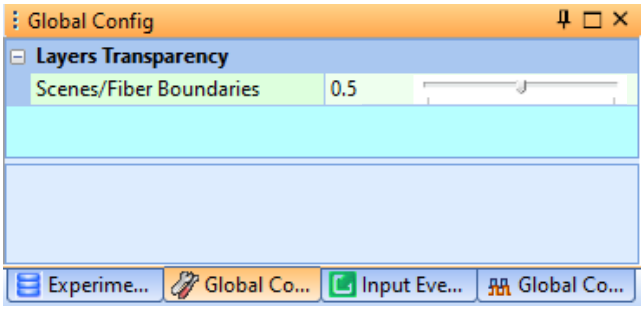
The system displays the **Session Comment** dialog box. The comment field might be empty (if no comment has been added yet) or it might contain previous comments.



4 Add comments as needed and click **OK**.

3.10 Setting Parameters in the Global Config Tab

The image below shows the **Global Config** tab.



You can adjust the transparency of user-defined shapes superimposed on the captured image in the video windows, specifically, Scenes (arena and zones) for the behavioral video, and Fiber Boundaries for the photometry videos. Use the slider to adjust the transparency from 0.0 (opaque) to 0.9 (90% transparent).

If you activate the Tracking function in the behavioral video, the **Global Config** tab displays additional parameters. For those additional details, see Section 7.6, “Setting Parameters In the Global Config Pane (Tracking Enabled)” on page 134.

3.11 Configuring Input Events and Global Combination Output Events

For the functions of the **Input Events** tab, see Section 9.2, “Configuring and Managing Input Events” on page 224.

For the functions of the **Global Combo** tab, see Section 9.3, “Creating Global Combination Output Events” on page 228.

3.12 Ensuring Consistent Parameter Settings in an Experiment

Once there are existing sessions (recordings) within an experiment, certain parameter settings are disabled for that experiment, that is, the settings cannot be changed. This is done so that all the sessions within an experiment are run with consistent parameters. The disabled parameter settings include:

- Number of fiber boundaries (fiber outlines) configured for each wavelength
- Tracking mode in the Tracking toolbar
- Selection of tracked objects in the Tracking tab (for LED or Color Markers tracking)
- **Animals in Area** parameter in the Global Config tab (for LED or Color Markers tracking)

Another way to look at these restrictions is that the above parameter settings can only be selected or changed in an empty experiment (an experiment for which there are no recorded sessions). Furthermore, these parameters can be modified only in **Cameras** mode and **Files/Add New Sessions** submode.

CAUTION

Plan for consistency across sessions

After you start your first recording (session) for a particular experiment, you cannot change the values for certain parameters in that experiment. See the details in the paragraph above.

3.13 Where to Go Next

Go to Chapter 4, Photometry Features and Procedures to configure the excitation wavelengths to be applied by the photometry devices and to configure the photometry fibers.

If you are also using the behavioral camera to record the movements of the subject(s), go to the following chapters, as applicable:

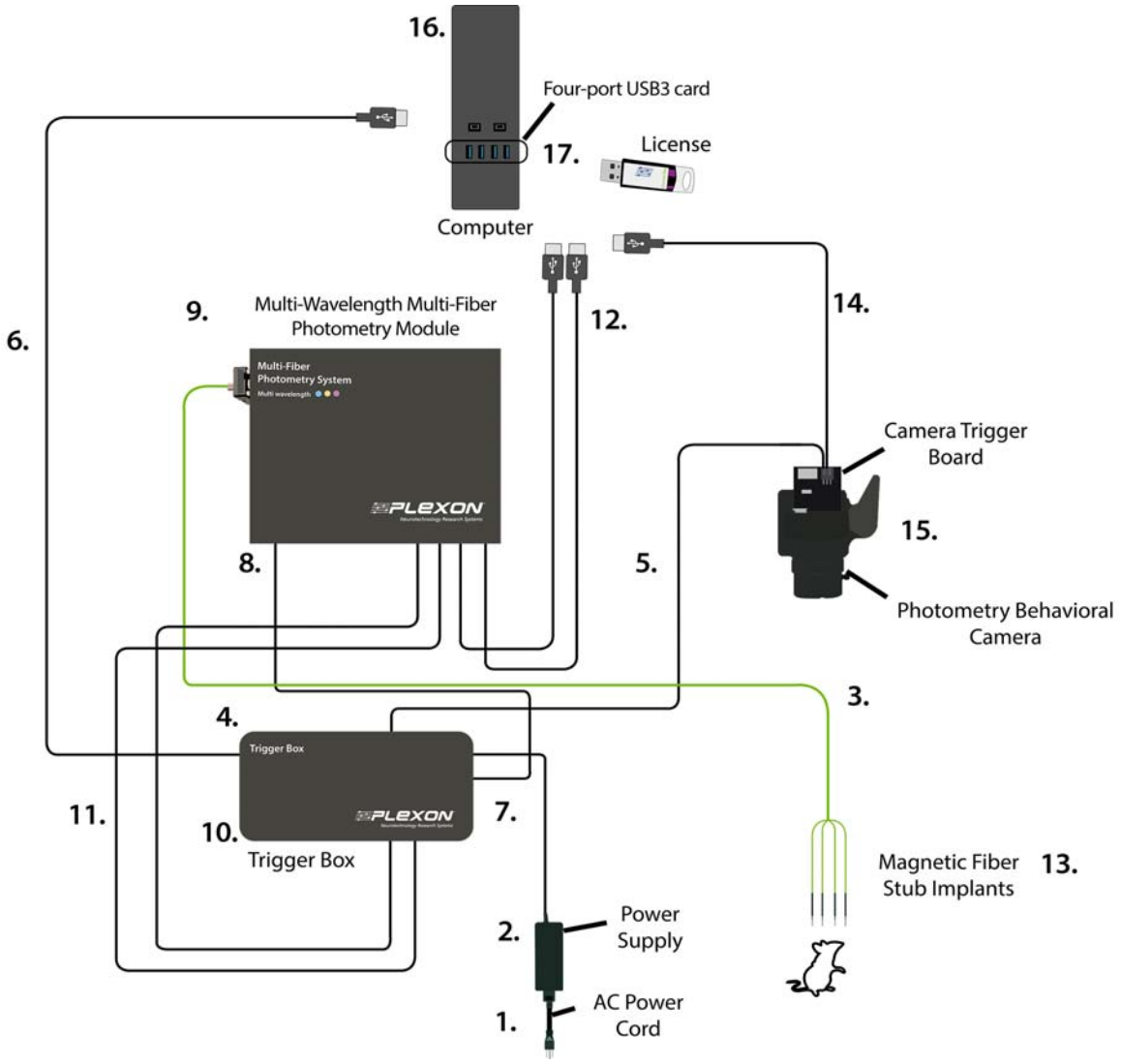
- Chapter 5, Setting Up the Behavioral Camera
- Chapter 6, Calibrating the Arena Dimensions
- Chapter 7, Configuring the Tracking Parameters
- Chapter 8, Behavioral Features and Procedures

Chapter 4
Photometry Features and Procedures

4.1 Photometry Components

This chapter describes the photometry components and functions.

4.1.1 Photometry System Component Diagram



4.1.2 Photometry Module

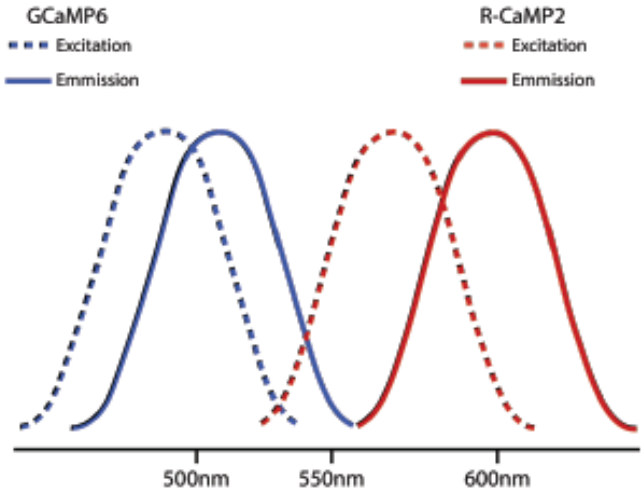
The Photometry Module allows you to measure and record the activity-dependent light emitted by fluorescent reporter cells in the brain in response to an applied excitation wavelength. The system allows you to simultaneously record the activity-dependent reporter fluorescence and (with the behavioral camera) the animal's behavior.

The system includes hardware that emits the excitation wavelength and video cameras that detect and measure fluorescent light emitted by genetically encoded calcium indicators (GECIs) or genetically encoded voltage indicators (GEVIs). It also provides several tools that you can use for photometry analysis. With the photometry functions you can monitor multiple brain regions simultaneously, which is useful in studying the connection between different brain regions, the behaviors that are produced, and the neural correlates of social interaction.

The excitation wavelengths are centered at 560nm, 465nm, and 410nm. The Photometry Module measures and records the emitted fluorescence in two detection ranges, centered at 600nm and 525nm. These excitation and detection ranges were chosen to operate with two of the most popular genetically encoded calcium indicators, GCaMP6 and R-CaMP2. See the graph, below.

Excitation wavelengths:

- 465 nm for selectively activating GCaMP6
- 560 nm for selectively activating RCaMP2
- 410 nm for use as an isosbestic control to detect calcium-independent signals



Detection ranges:
505-545nm
and
580-620nm

4.1.3 Trigger Box

The Trigger Box provides timing and synchronization for all system functions and provides digital input and digital output ports. For details, see Appendix C, Trigger Box and Digital Input/Output.

4.1.4 LEDs, Fibers and Detection Cameras

The Photometry Module consists of optical components (LEDs, filters, dichroic mirrors, cameras and lenses) that filter the excitation light, direct that light down the multi-branch optical cable, filter the emission light coming back up the optical cable and direct that light into the photometry cameras for analysis.

There are three excitation LEDs in the system—465nm (blue) to excite GCaMP6, 560nm (lime) to excite R–CaMP2, and 410nm (UV) to serve as an isobestic control. The light from each LED is transmitted into each of four fibers. Each of the four fibers carries any emitted fluorescence back to the system, where it is detected.

4.1.5 Patch Cable and Fiber Stub Implants (Optional)

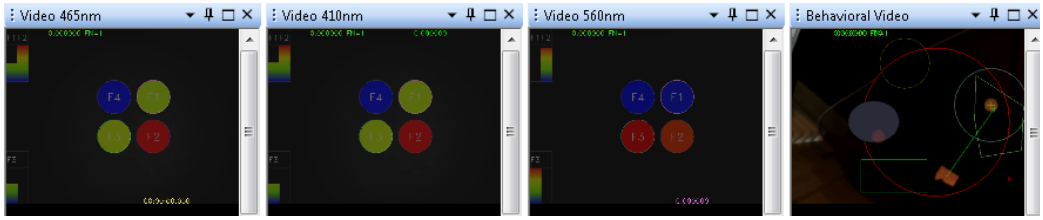
An optical patch cable may be included with the system. It is typically supplied with up to four branches, and with dimensions based on customer requirements. The branching portion of the cable typically will be short if the individual branches are connected to different brain regions in the same subject, or long if the branches are connected to different subjects. Each branch is terminated with a magnetic LC ferrule connector. The magnetic LC ferrule connector is designed to be used with magnetic LC fiber stub implants, but can also be coupled to conventional non-magnetic LC fiber stub implants. Magnetic and non-magnetic branched cable ends are shown in the images below.

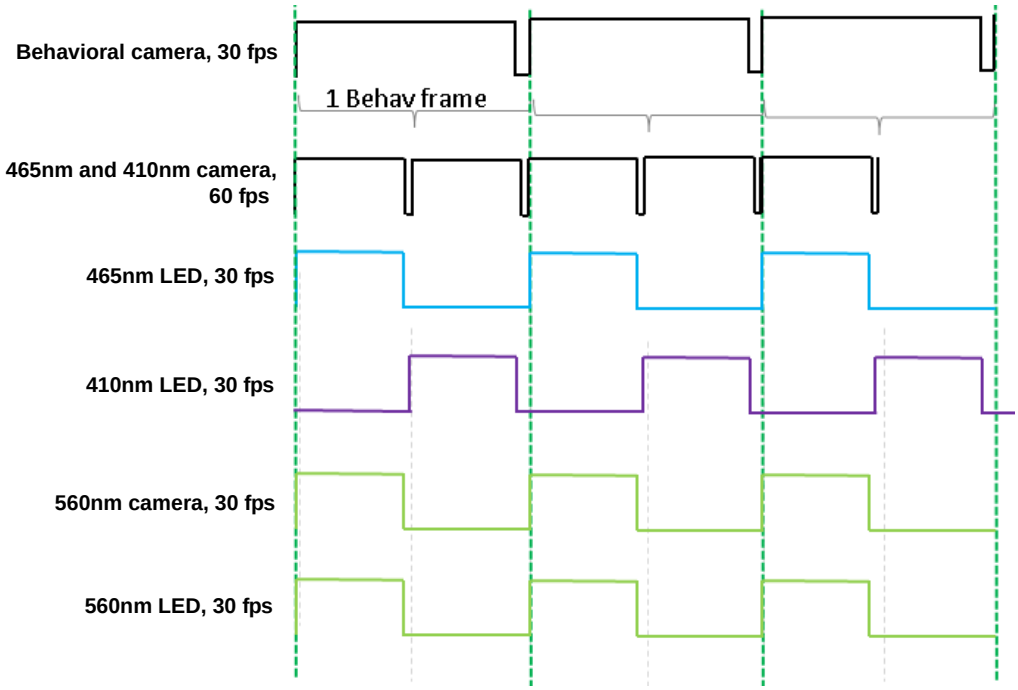
Magnetic and non-magnetic fiber stub implants are available from Plexon.

4.2 Photometry Synchronization and Timing Details

The timing of the LED excitation, fluorescence detection and behavioral video are shown in the following diagram.

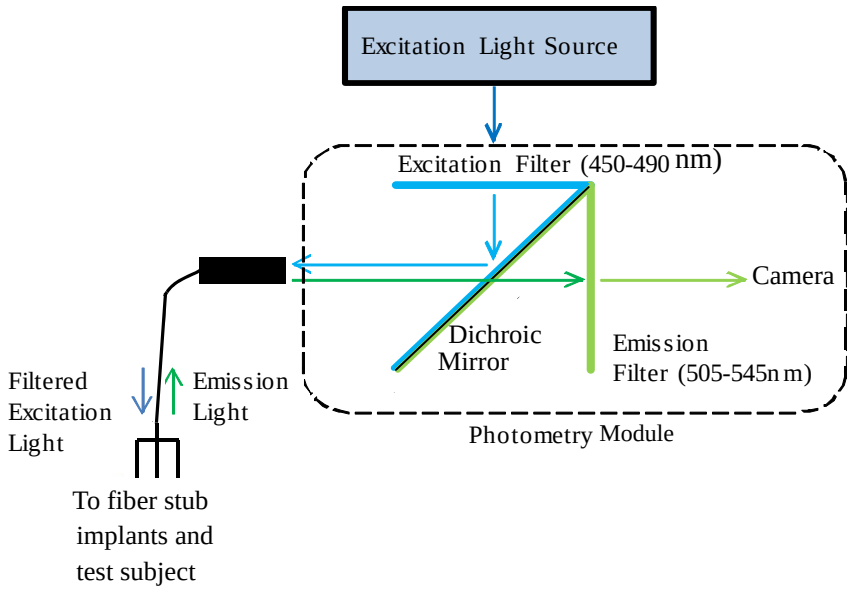
The “465nm and 410nm camera” captures photometry videos that are displayed in the **Video 465nm** and **Video 410nm** windows in the GUI. The “560 nm camera” captures video displayed in the **Video 560nm** window. All cameras are triggered from the same trigger source, and have a common start/stop signal. They all start/stop recording simultaneously and remain synchronized as recording proceeds. This allows you to view and record coordinated behavioral and photometric data.





4.3 Theory of Operation

The system operates as shown in the diagram, with the 465nm (blue) LED used in this example. The patch cable and fiber stub implants are optional items which can be supplied by Plexon.

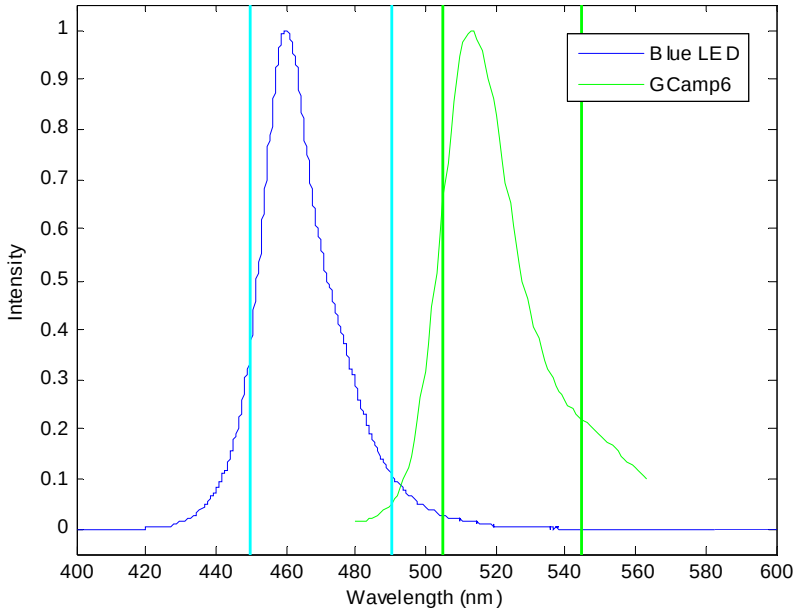


The dichroic mirror in the Photometry Module directs the two light beams. Each fiber branch carries light in two directions:

- Filtered excitation light is carried from the Photometry Module to the subject.
- Green colored fluorescence emitted by GCaMP6 cells is carried to the camera in the Photometry Module.

The excitation light source is a blue LED with a relatively broad spectral output centered around 465nm. This broad light is bandpass filtered from 450 to 490nm and reflected off a dichroic mirror into the optical path. The dichroic mirror reflects wavelengths below 490nm. The filtered blue light travels down the multi-branch optical cable and into the brain where it can excite expressed fluorescent reporters such as GCaMP6. The resulting emitted green light travels back up the optical cable and passes through the dichroic mirror, which passes wavelengths above 505nm. The emission light is filtered from 505 to 545nm to increase specificity of the signal before it is quantified by the camera.

The following graph shows the typical output spectrum of a Blue LED (465nm), the excitation filter bandwidth (vertical cyan lines), a typical GCaMP6 emission spectrum, and the emission filter bandwidth (vertical green lines):



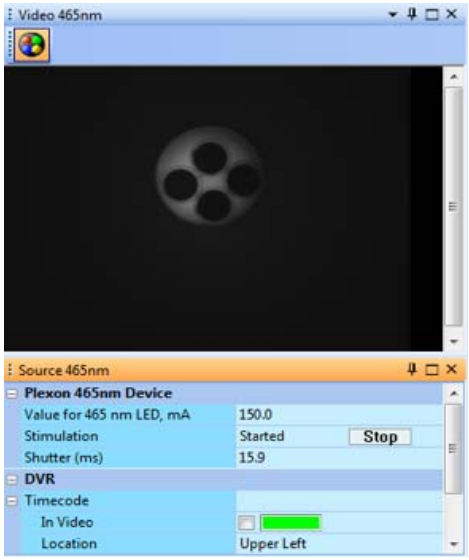
The absolute level of fluorescence observed by the camera in the Photometry Module depends on many factors, for example, the amount of excitation light applied to the input, the efficiency with which the excitation light is coupled into the optical cable, the type and level of expression of the fluorescent reporter molecules present in that tissue, and the efficiency with which the system collects the emitted light.

TIP

Measure the excitation light power at the end of each branch

The actual power level of the light delivered at the end of each branch of the multi-branch optical cable will be much less than the raw output of the LED units. Be sure to measure this power level at each of the fiber ends as described in Section 4.6, “Test Mode—Measuring and Adjusting LED Output Power” on page 64 to be sure it is appropriate for your specific experiment.

The image below shows the blue light exiting the fibers after the blue LED stimulation has been started. In the video displays, the four fibers will appear dark even if there is fluorescent light coming through them, because the amplitude is too small for the human eye to see. However, the Photometry Module is able to detect and record any such fluorescence, and can display the florescence intensity as heat maps in the GUI. The procedures that follow this section explain how to activate each of the LEDs and detect the fluorescence.



WARNING
Never look directly into any optical fibers!

Depending on the wavelength and intensity of light emitted, you risk serious damage to your vision if you look directly into any optical fiber.

For example, UV light is not visible to the human eye, but it can damage the eye. The Plexon system emits relatively weak UV light, but it is still wise to avoid looking directly into the fibers.

Even if colored light is being emitted, it is possible that other, damaging light, such as UV light could be present.

Do not risk eye damage!

4.4 Configuring the Initial Recording Parameters

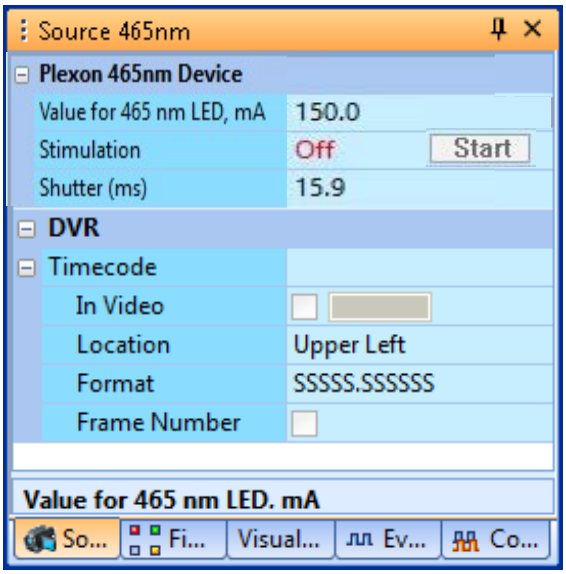
Before you start, verify that you have completed the appropriate hardware assembly and system configuration procedures in the previous chapters, including definition of the recording folder, experiment descriptors and session variables. Be sure to include descriptors and variables that will aid you in organizing and analyzing the photometric data.

4.5 Configuring Photometry Source Parameters

Follow the steps below to configure the parameters in the photometry source tabs. In this procedure, the tabs for the 465nm device are shown in the sample screenshots.

During configuration or operation, if the system displays alarms warning you that some of the cables are not properly connected, follow the procedures in the appendix, “Critical Alarm Messages” on page E-9, to clear the problem.

1. In **Cameras** mode, select or create an experiment.
2. Begin with any one of the photometry devices. Click on the **Source** tab. This tab is used to set parameters for each of the photometry video windows. Each photometry video window has its own **Source** tab with several configurable parameters.

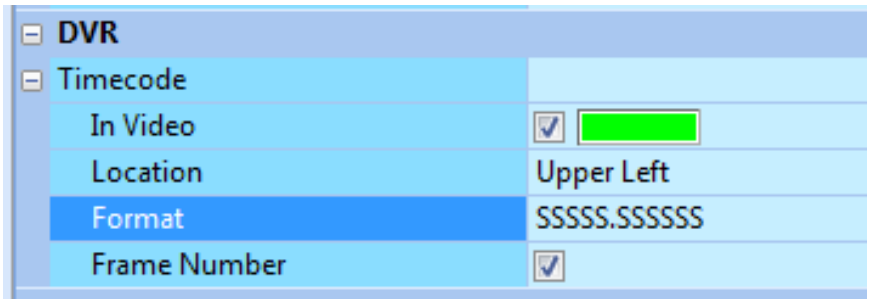


3. For now, accept the default values for the LED current (**Value for 465nm LED, mA**). In a later procedure, you will make adjustments to the current values when you measure the actual output power at the ends of the patch cable, as described in Section 4.6, “Test Mode—Measuring and Adjusting LED Output Power” on page 64.
4. If necessary, adjust **Shutter (ms)**.

   The shutter open durations for the 410nm and 465nm devices are preset to 15.9ms and are not adjustable. The duration for the 560nm device defaults to 15.9ms, but you can increase it up to 33.1ms; typically, red fluorescence is weaker than green fluorescence, so you might wish to increase the 560nm open duration.
5. If desired, you can display the **Timecode** and **Frame Number** in the video stream by selecting the applicable checkboxes.

   In the digital video recording (**DVR)** area of the Source tab, **Timecode** options, select the **In Video** checkbox if you wish to display a time code over the video image. Choose a **Location** and a **Format** setting to configure the

display. Select the **Frame Number** checkbox if you want the frame number displayed along with the time. (The system maintains a time code that tracks the time elapsed since the last time recording began.)



For example, the first image (below) shows the time code in the upper left location in **SSSSS.SSSSSS** format. The second image shows the time code in **HH:MM:SS.SSS** format along with the frame number.

TIP

Timecode checkboxes are automatically synchronized

When you select or deselect the Timecode checkboxes for **In Video** and **Frame Number** in any one of the Source tabs, the system automatically applies the same setting across all four video images. Thus, it is not necessary for you to manually duplicate these settings in the other device Source tabs.

6 Repeat the above steps for the other photometry devices you plan to use for your experiment.

4.6 Test Mode—Measuring and Adjusting LED Output Power

Prior to starting a photometry experiment, it is essential to use an optical power meter (for example, the Plexon light measurement kit) to measure the level of excitation light coming out of **each branch** of the multi-branch optical cable. If the excitation light level is too low, it may not excite enough fluorescence in the tissue of interest to generate robust fluorescence and the resulting signal may be overly noisy. On the other hand, if the light level is too high it may cause excessive bleaching of the fluorophore over the course of the experiment or possibly even lead to saturation of the photometry image.

Note that when an LED excitation source is used, the light output measured at the ends of the optical cable will typically be only a small fraction of the light power measured directly at the output of the source. This is due partly to the excitation filter which excludes both tails of the LED's broad output spectrum (reducing the power, but increasing the proportion of relevant wavelengths in the beam) and partly due to the inherent inefficiency of coupling the single LED light source into the multiple fibers of the optical cable and the small inner diameter of each fiber within the cable.

In normal operation, stimulation LEDs are blinking at 30 fps (see the timing diagram in Section 4.2, "Photometry Synchronization and Timing Details" on page 58). To measure power output, LEDs need to be in constant operation, which is provided in Test mode.

Follow this procedure for each excitation wavelength. How often you repeat this measurement depends on the needs of your experiment.

WARNING
Never look directly into any optical fibers!

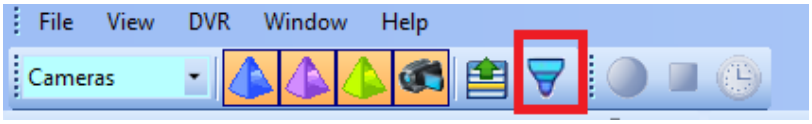
Depending on the wavelength and intensity of light emitted, you risk serious damage to your vision if you look directly into any optical fiber.

For example, UV light is not visible to the human eye, but it can damage the eye. The Plexon system emits relatively weak UV light, but it is still wise to avoid looking directly into the fibers.

Even if colored light is being emitted, it is possible that other, damaging light, such as UV light could be present.

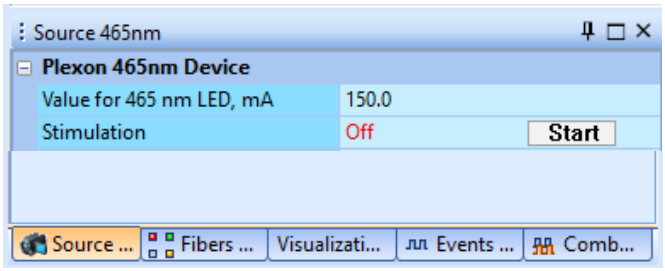
Do not risk eye damage!

1 In Cameras mode, click the **Start LED test mode** button.



Note: Test mode is available only when the system is in Cameras mode. You can start Test mode whether you have selected an experiment or not. When you click the **Start LED test mode** button, all cameras will be stopped along with all other functions not related to the test. You will not be able to select any other experiment or switch experiments. A blinking green bar “LED test mode” will appear at the right side of the Status bar at the bottom of the GUI screen.

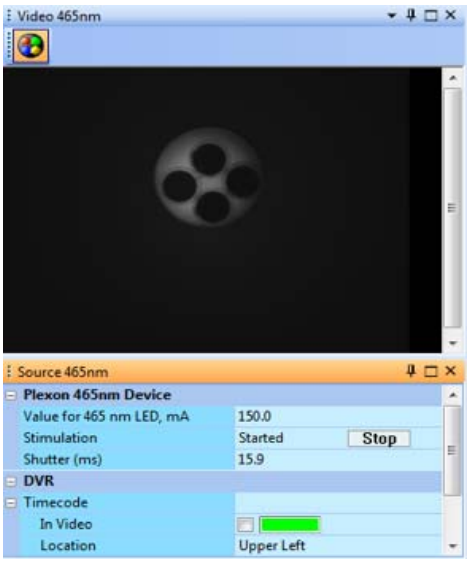
The Source tabs for the photometry devices will become active (or remain active), but the only active items in the Source tabs will be the LED current value (mA) and the stimulation **Start** button.



2 To measure light output of a selected Photometry Source, click the **Start** button in its Stimulation item. The fibers should now begin emitting the appropriate color light in constant mode. (Notice that the **Start** button has changed to a **Stop** button.

3 Verify the LED excitation light is on and is being emitted at the ends of the fiber branches. The image below is for the blue (465nm) wavelength.

Note: When you are testing the UV excitation light (centered at 410nm), the emitted light will have a purple tint, but the true UV light will not be visible to the naked eye.

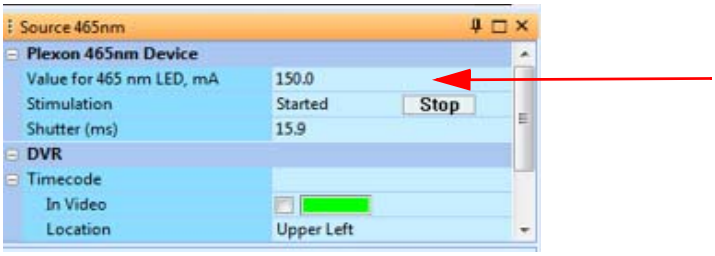


4 One at a time, connect the individual branches of the optical cable to the optical power meter.

5 Measure the power level of excitation light being delivered by each fiber.

6 For each fiber, verify that the power level is sufficient for your experiment. (Keep in mind that the intensity will be further reduced when you connect the branch to the fiber implants.)

If you need to increase or decrease the measured intensity, adjust the value of the current (mA) in the Source tab by double clicking the number and entering a new number.



The current limits of each LED and estimated minimum output power measured at the branching cables are as follows:

Blue (465nm): 300mA — 100µW

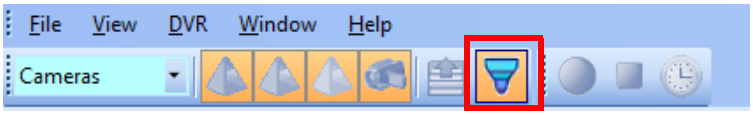
UV (410nm): 1000mA — 100µW

Lime (560nm): 500mA — 30µW

The relation between the input current and the output power is roughly linear, so, for example, 100mA of Blue should yield at least 33µW at the fiber ends. Due to slight variations in patch cables and fiber connections, you can expect some variation in the measured output power levels from one system to the next.

7 Click the **Stop** button to turn off the LED.

8 To exit Test mode, click the Test mode button.

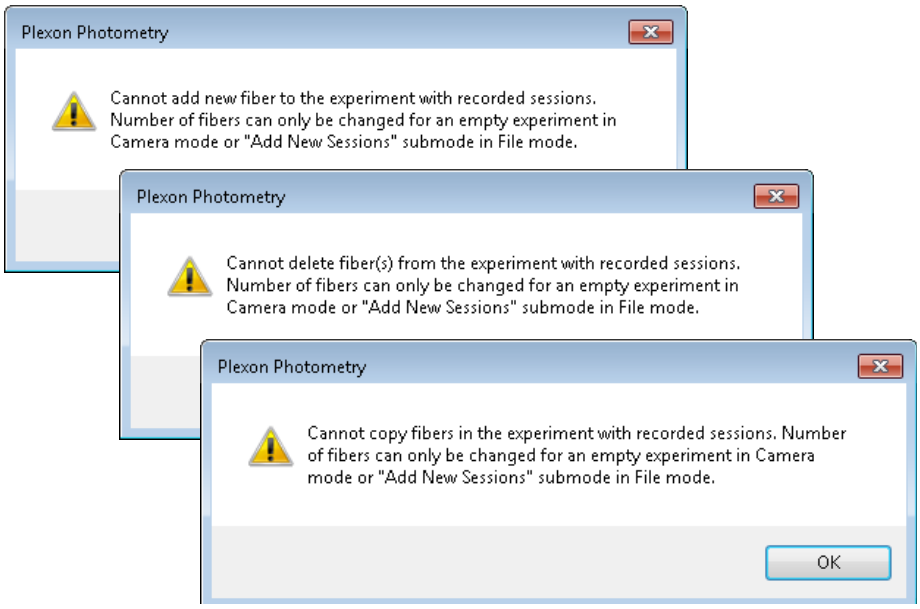


Note: Exiting Test mode turns all LEDs off automatically.

4.7 Configuring (Adding) Fibers

Follow these steps to configure the photometry fibers. In the **Fibers** tab (shown in the images below), each item you configure (Fiber F1, Fiber F2, ...) corresponds to a single fiber branch of the multi-branch optical cable.

Note: You can add, copy or delete fibers only in an empty experiment and only in Cameras mode and Files/Add New Sessions mode. The system does not allow adding, copying or deleting fibers in an experiment after a session has been recorded in that experiment, and it shows messages like the following.

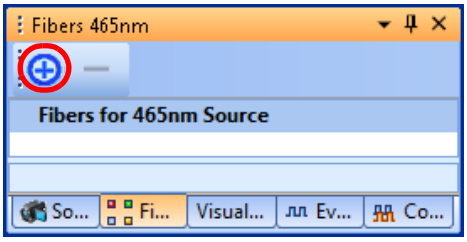


1. Select either the 465nm or the 410nm device to configure first.

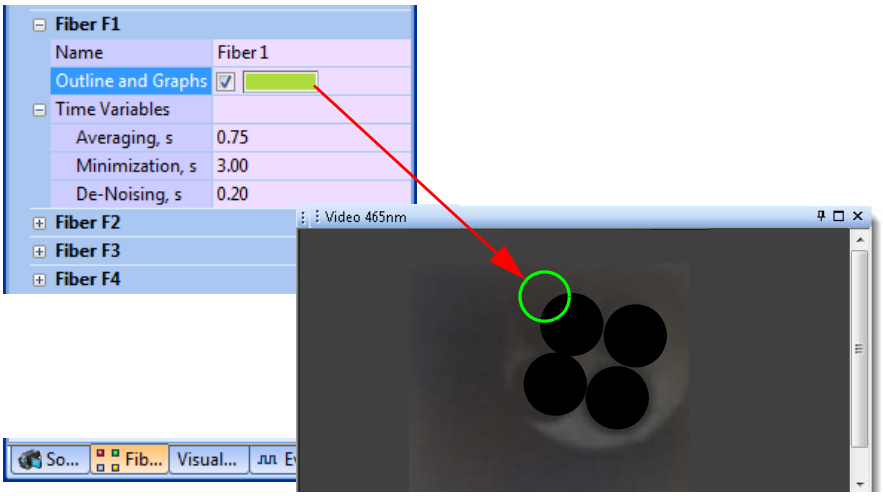
   Each of these devices receives green fluorescence that is recorded by a single detector in the Photometry Module. Therefore, the fiber outlines will be the same for these two devices. (The fiber outlines for the 560nm device are adjusted separately.)

2. In the Stimulation item of the Source tab, click the **Start** button.
   The fibers should now begin emitting the appropriate color light.
   Notice that the **Start** button has changed to a **Stop** button.

3. Click on the **Fibers** tab, then click the "+" in the upper left corner to add a new fiber.



The system will display parameters for a new fiber in the window, and it will place a default fiber outline in all three photometry video windows. (The default outline might not match the size or location of the image the system is receiving from the actual fiber; you will adjust the outline in a later step.)

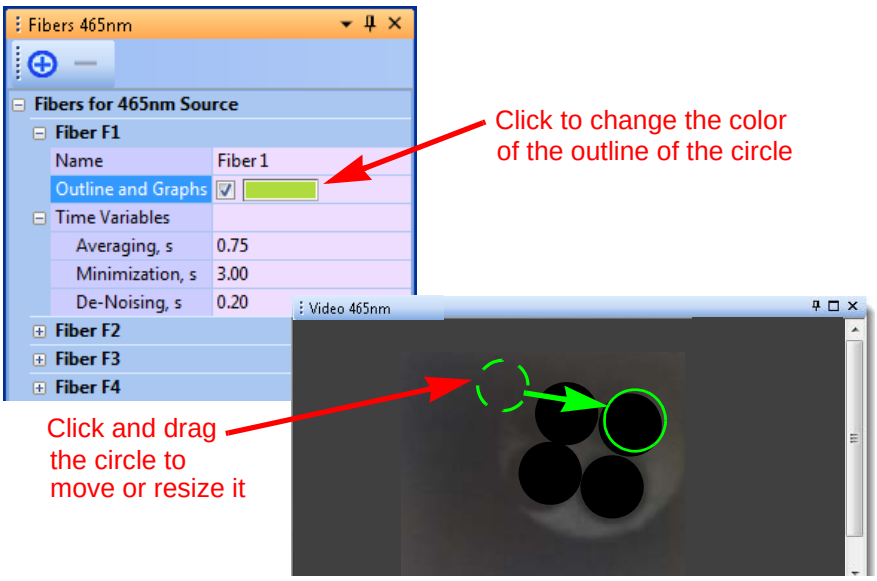


4 To rename Fiber F1 (or any other fiber), double click in the field on the right side of the **Name** row for that fiber and enter the name you want to apply to that fiber.

5 If desired, place a label on each fiber branch. You can identify the individual fibers by pointing them toward ambient light as you view the image in the GUI.

Note: There is no need to adjust the **Time Variables** at this point in the procedure. These parameters will be discussed in Section 4.16, “Photometry Reference—Time Variables” on page 95.

6 Modify the position and diameter of the fiber outline in the **Photometry Video** window so it matches the circular region of the fiber. The system will record the intensity of the fluorescence inside this circular region. You can also change the color of the outline and the associated data graphs that will be displayed later for this fiber.



Notice that the system moves the fiber outline identically in both the 465nm and 410nm windows.

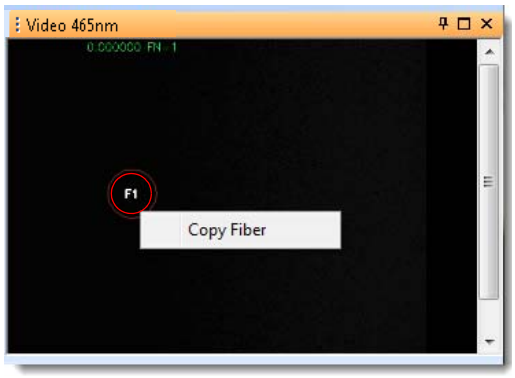
7 If desired, you can change the outline circle color for the fiber. When you do that, the outline color for this fiber will be automatically changed in the fiber settings and video windows for the other two photometry detectors. For example, if you change the outline color for Fiber 1 in the “Fibers 465nm” settings, the same color will be automatically set for Fiber 1 in the “Fibers 410nm” and “Fibers 560nm” settings as well.

8 To create additional fibers for the 410nm and 465nm wavelengths, repeat Step 3 through Step 7 in the applicable Source tabs. (See the tip, below.)

TIP

Copying fibers

In most multi-branch optical cables all of the fibers are essentially the same diameter. Instead of freehand drawing the circles for the second and subsequent fibers, you can copy the circle that you drew for the first fiber. Simply select the circle you want to copy by left clicking it, then right click and select **Copy Fiber** to create a circle of the exact same dimension as the initial fiber.



After this operation is done, the system will automatically add the new fiber to the **Fibers** list in the Fibers tab.

CAUTION

Do not overlap fibers

Never draw a fiber circle that overlaps another (already drawn) fiber circle. This will cause the results to be invalid.

9 In the video window for the 560nm device, modify the sizes and positions of the fiber outlines as needed to match the actual positions of the fibers. the fiber positions in this window do not typically match the positions in the other two photometry windows, because the 560nm images are recorded by a separate camera in the Photometry Module.

10 If desired, you can change the values for the **Name**, **Outline and Graphs** and **Time Variables** for any of the fibers for any of the photometry devices. However, the default values are sufficient for some experiments, in which case this step is not required. (The Time Variables are explained in Section 4.16, “Photometry Reference—Time Variables” on page 95.)

11 **IMPORTANT**—If you are unable to obtain good images of the fibers, for example, if the fiber image appears out of focus or is not fully enclosed in the **Photometry Video** window, contact Plexon Support.

CAUTION

Do not make any adjustments to the optical port mounting plate

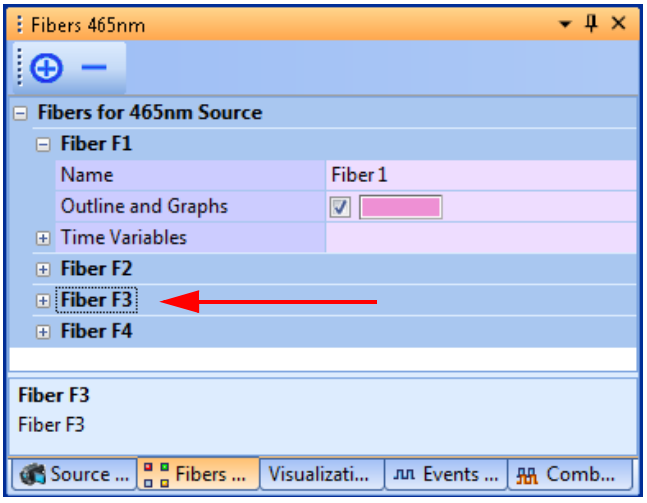
The mounting plate for the optical port in the Photometry Module is positioned and locked down in the factory. You should never attempt to make any adjustments to this plate without first discussing the matter with Plexon Support (+1 214-369-4957 or support@plexon.com). Any errors in adjusting the port can cause a total loss of image and/or damage to the Photometry Module.

4.8 Deleting Fibers

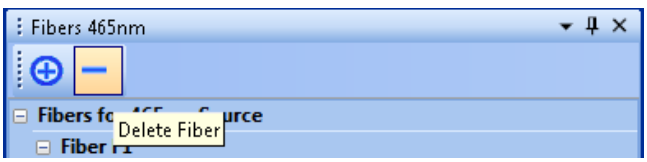
Deleting individual fibers

You can delete individual fibers as described below.

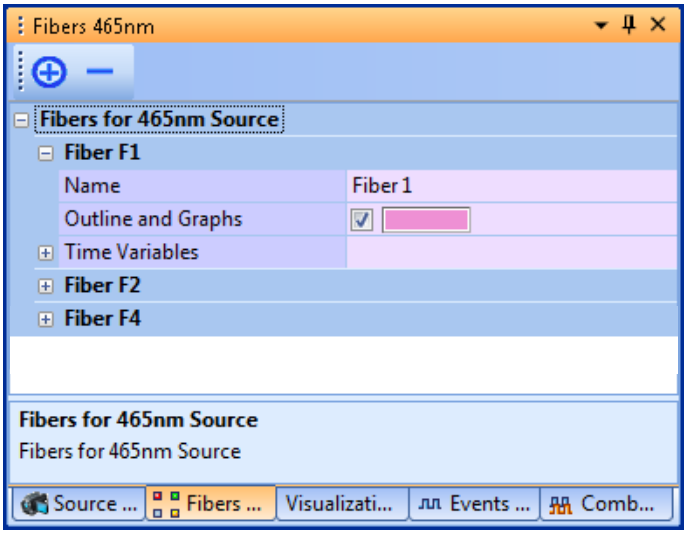
1 Click on the **Fibers** tab, then click on the fiber that you want to delete. In the example below, the user has clicked on Fiber F3 (notice the black dotted line around this fiber name).



2 Click the “—” (**Delete Fiber**) icon.

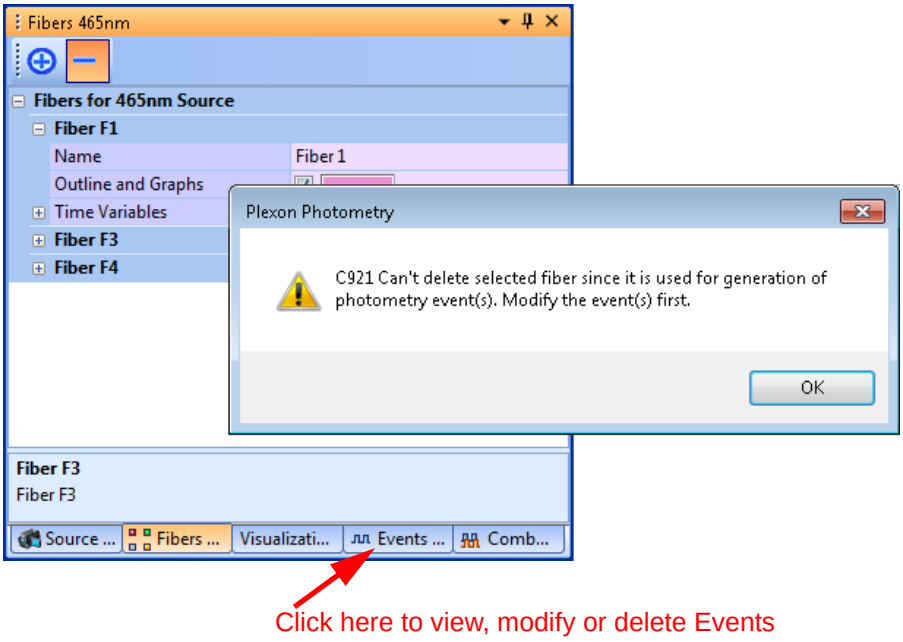


The system deletes the selected fiber (see the example below, after Fiber F3 has been deleted).



Note: Fibers can be deleted only for an experiment without recorded sessions.

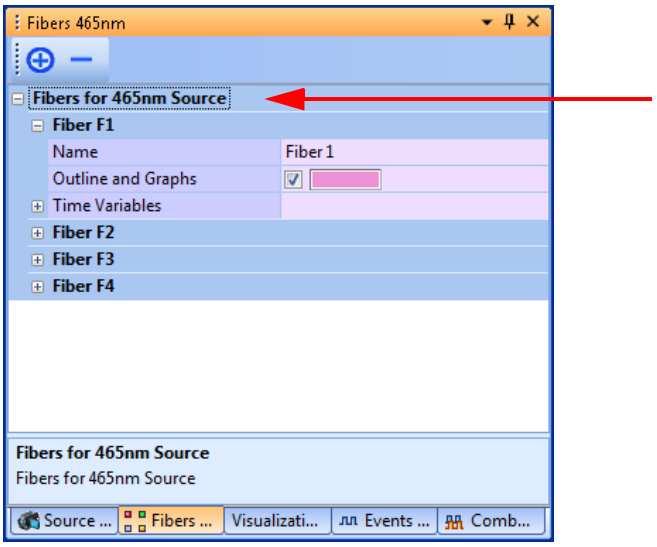
3 If you have an **Event** configured for the fiber you are trying to delete, the system notifies you that the fiber cannot be deleted because the fiber is associated with a photometry event. In that case, modify the photometry event (disassociate the fiber from this event) or delete the photometry event, then the system will allow you to delete the fiber. Note that the fiber might be associated with multiple photometry events, and you will need to modify or delete all of those events.



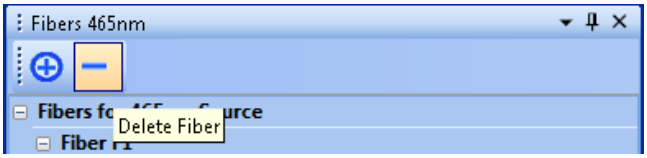
Deleting all fibers

You can delete all fibers as described below.

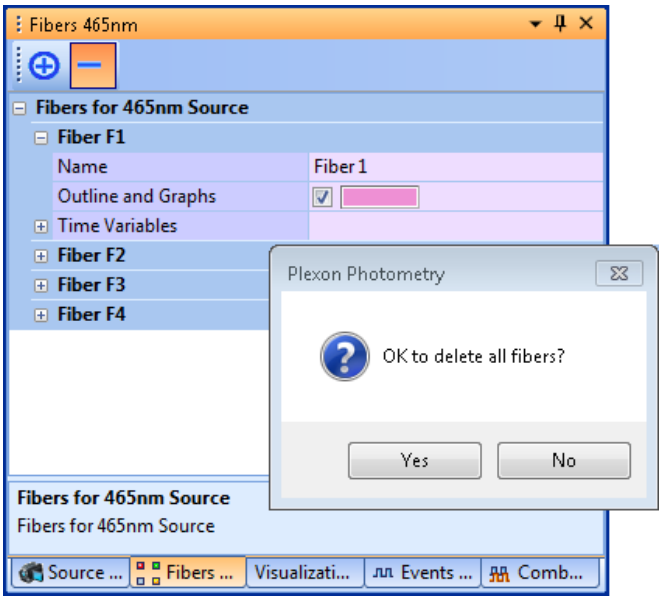
1 Click the **Fibers** row. In the example below, notice the black dotted box displayed in this row.



2 Click the "—" (**Delete Fiber)** icon.

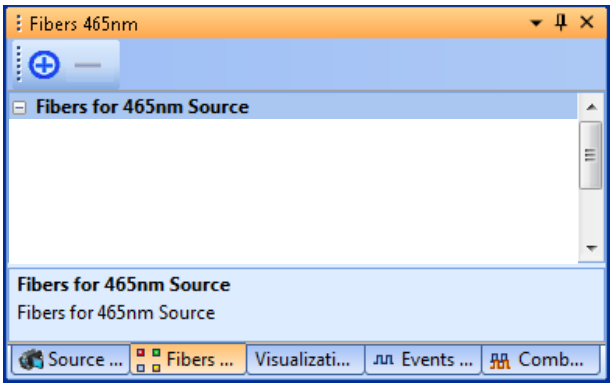


The system displays a caution notice.

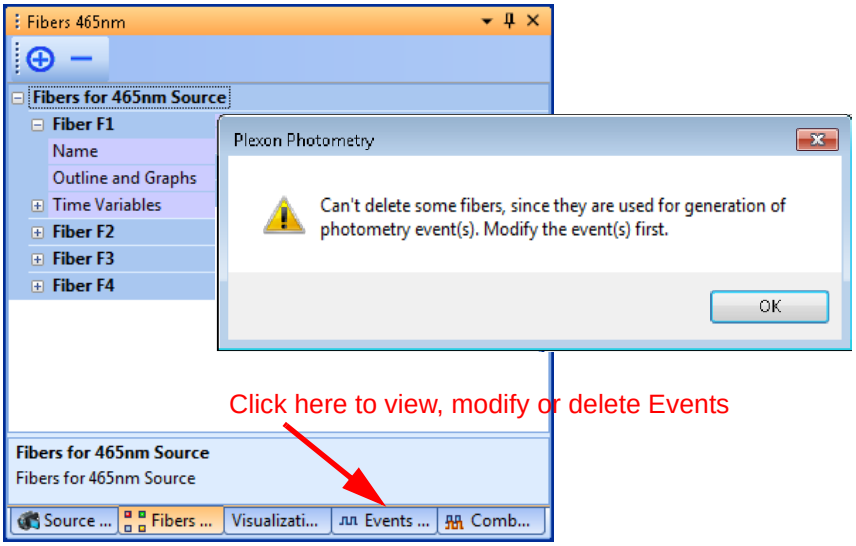


3 If you are sure you want to delete all fibers, click **Yes**. Otherwise click **No**.

If you click **Yes**, the system deletes all fibers.



4 If you have an **Event** configured for any of the fibers, the system notifies you that some fibers cannot be deleted unless the applicable photometry events are deleted (or modified to remove reference to all fibers). In that case, modify or delete all of the applicable photometry events first, then the system will allow you to delete the fibers.



4.9 Working with Photometry Visualization and Graphs

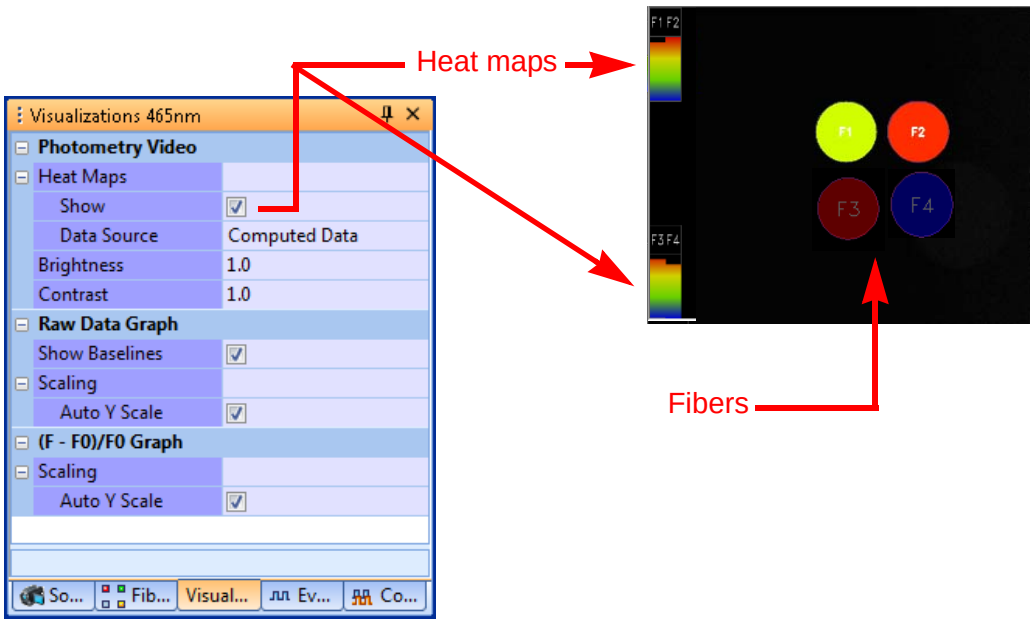
The dialog for the photometry visualization (**Visualization**) tab contains options related to:

- The appearance of the heat maps in the **Photometry Video** window
- The appearance of the data and y-axis scaling in the photometry graphs

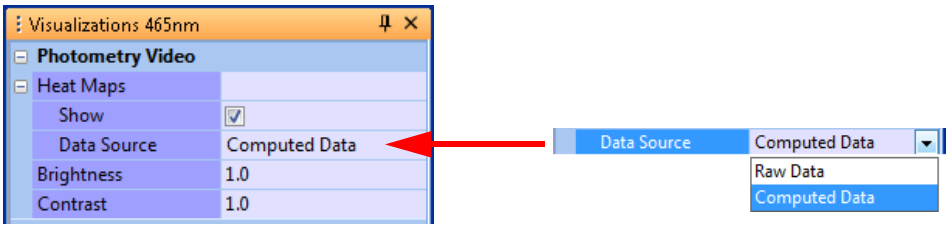
Click on the **Visualization** tab to access these options. By default, all the checkboxes in this dialog are selected.

Select the Heat Maps options:

- Select the **Show** checkbox to display the heat maps for the fibers in the photometry video window.



You can also toggle the heat maps on and off by clicking the heat maps icon .



- **Data Source** (for the heat maps)—During an active session when the GECIs are fluorescing, the system fills each of the fiber circles with a brightly colored heat map corresponding to the fluorescence intensity levels.

— Select **Raw Data** to show intensities of the raw data. In this case, the heat map signals are averaged within the circle visualized on the fiber image.

— Select **Computed Data** to show heat map intensities based on the computed result, (F-F0)/F0, which refers to the computations described in Section 4.16, “Photometry Reference—Time Variables” on page 95.

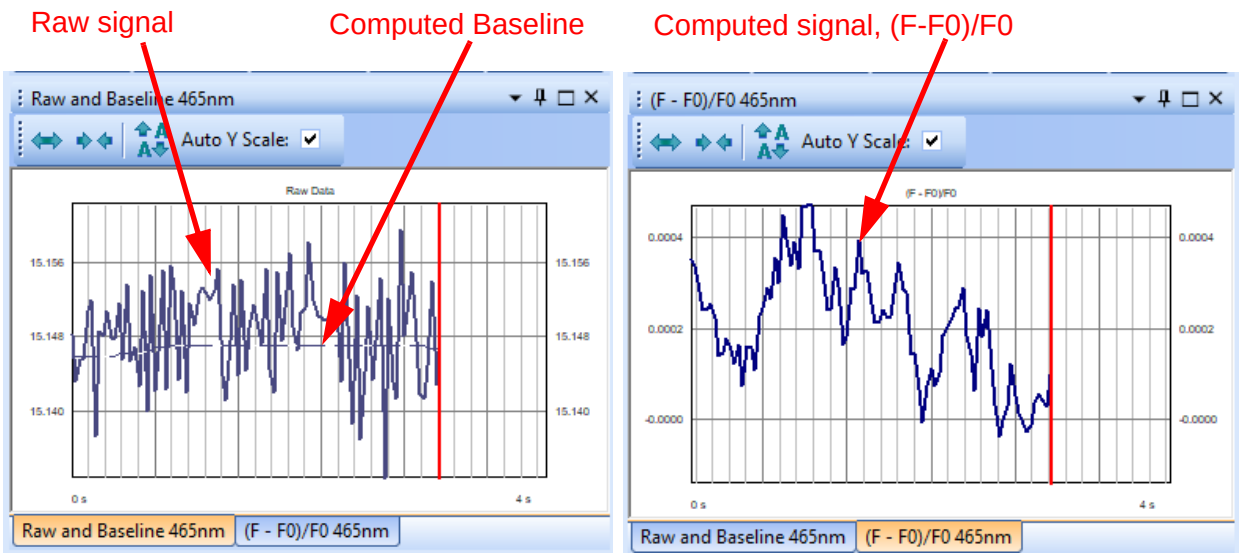
— These heat map intensities (either Raw Data or Computed Data) correspond to the intensity levels displayed in the photometry results graphs (see the screen image below).

- **Brightness** and **Contrast**—Adjust these values as needed to optimize the graphical displays. See the image, above.

Working with the Photometry Graphs

In previous steps, you created fibers in the **Fibers** tabs for each wavelength. During an experiment, when the system is detecting fluorescence, the photometry graphs display information about the intensity of the fluorescence in each of these fibers. For each wavelength, there are two graphs:

- Raw and Baseline (F0)
- (F – F0)/F0

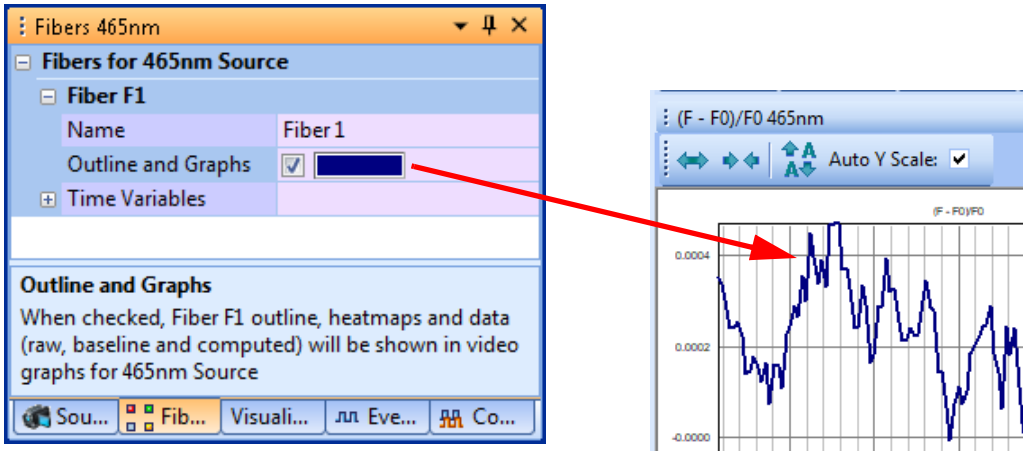


The descriptions and computations for these graphs are explained in Section 4.16, “Photometry Reference—Time Variables” on page 95.

Working with colors

The color of each line in the graph matches the color specified for the corresponding fiber in the **Fibers** tab. When you change the outline circle color for a fiber in the **Fibers** tab, the color for the raw and computed data for this fiber

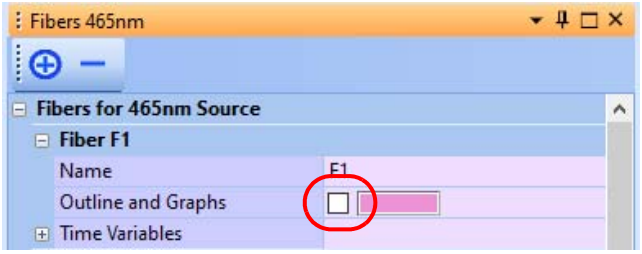
will be changed automatically to match it in both graphs for the corresponding wavelength. For example, the system displays the color (dark blue) for Fiber 1 data in the above graphs according to the color that was set for Fiber 1 in the Fibers tab:



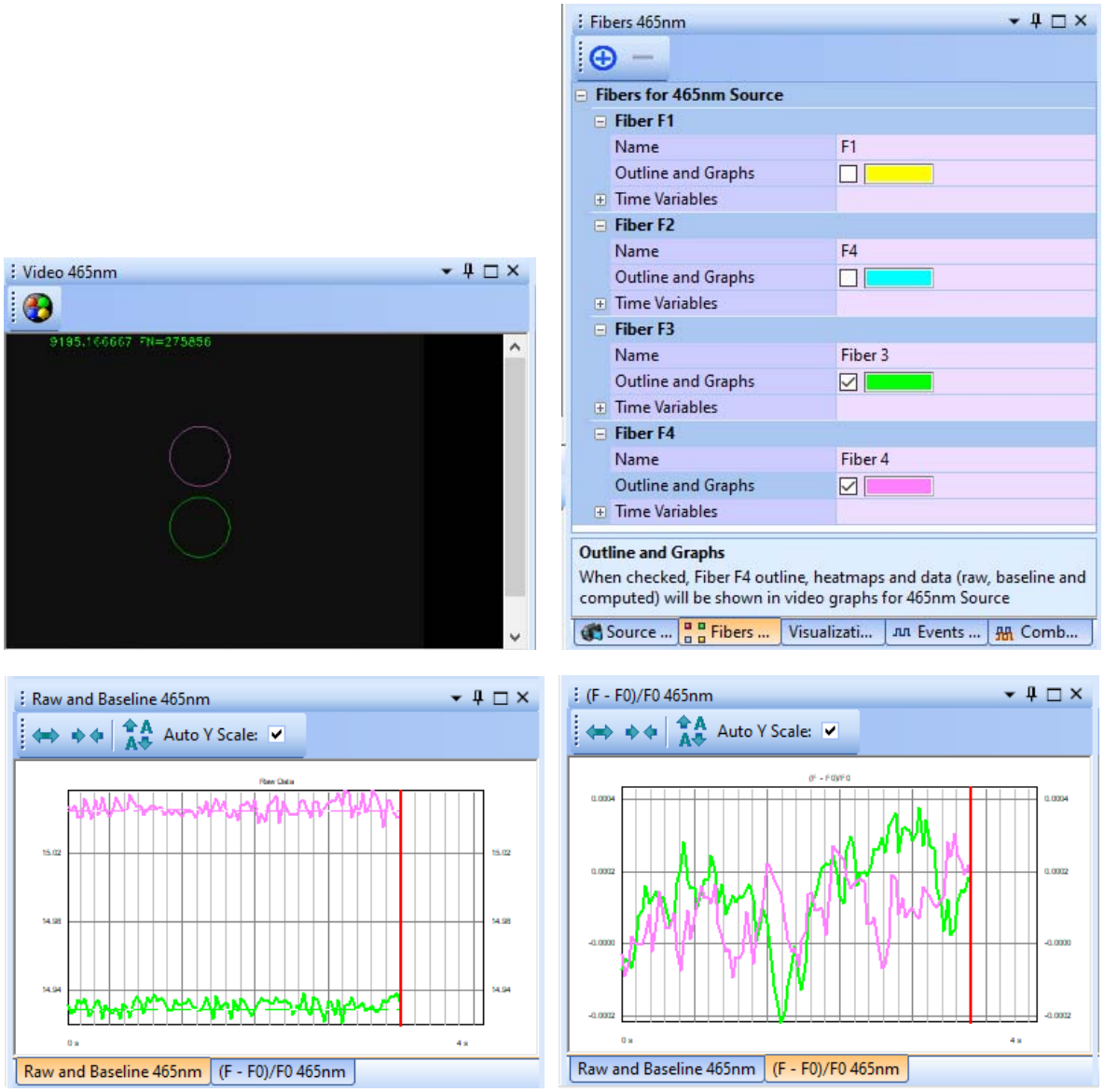
Furthermore, if you change the color for Fiber 1 in any one of the photometry Fibers tabs, the same color will be automatically set for Fiber 1 in the graphs for all three detectors. The same effect would be true for Fibers 2, 3 and 4.

Show/Hide data in the graphs

If you don't want the graphs to show the data for a particular fiber in a particular detector, deselect the checkbox in the **Outline and Graphs** row. (The fiber outline circle in the video will be hidden as well.)



In the example below, four fibers are defined, but the **Outline and Graphs** checkboxes are deselected for Fiber 1 and Fiber 2 in the “Fibers 465nm” settings. Note that this checkbox only affects visualizations in one source—so if you don’t want the data and outlines for Fibers 1 and 2 to show in “Fibers 410nm” and “Fibers 560nm” video windows and graphs, you would need to deselect them there as well.



Show Baselines option for the Raw Data Graph

The baseline for each fiber is a time-averaged value computed as described in Section 4.16, “Photometry Reference—Time Variables” on page 95. You can select the **Show Baselines** box in the Visualization tab to have the baselines displayed in the graphs, or deselected to have the baselines hidden.

Auto Y Scale options for Raw Data and Computed Data

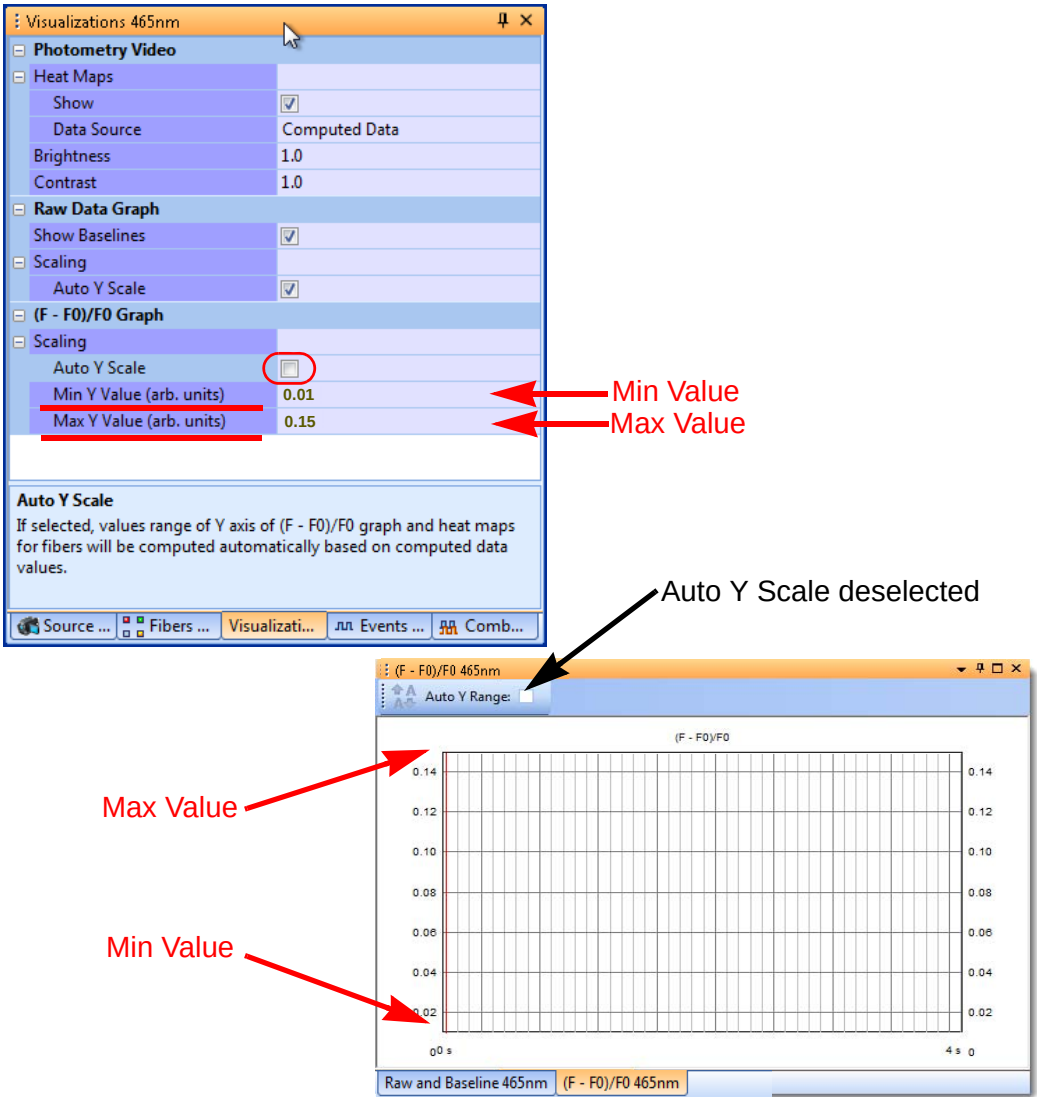
If you select the **Auto Y Scale** checkbox, the system automatically determines the y-axis scaling and labeling for the photometry results graph and the heat maps in the photometry video window based on [1] the incoming data and [2] the photometry event threshold—the **(F-F0)/F0 Threshold** parameter described in Section 4.11, “Creating Photometry Events” on page 84.

TIP

Using the Auto Y Scale checkbox

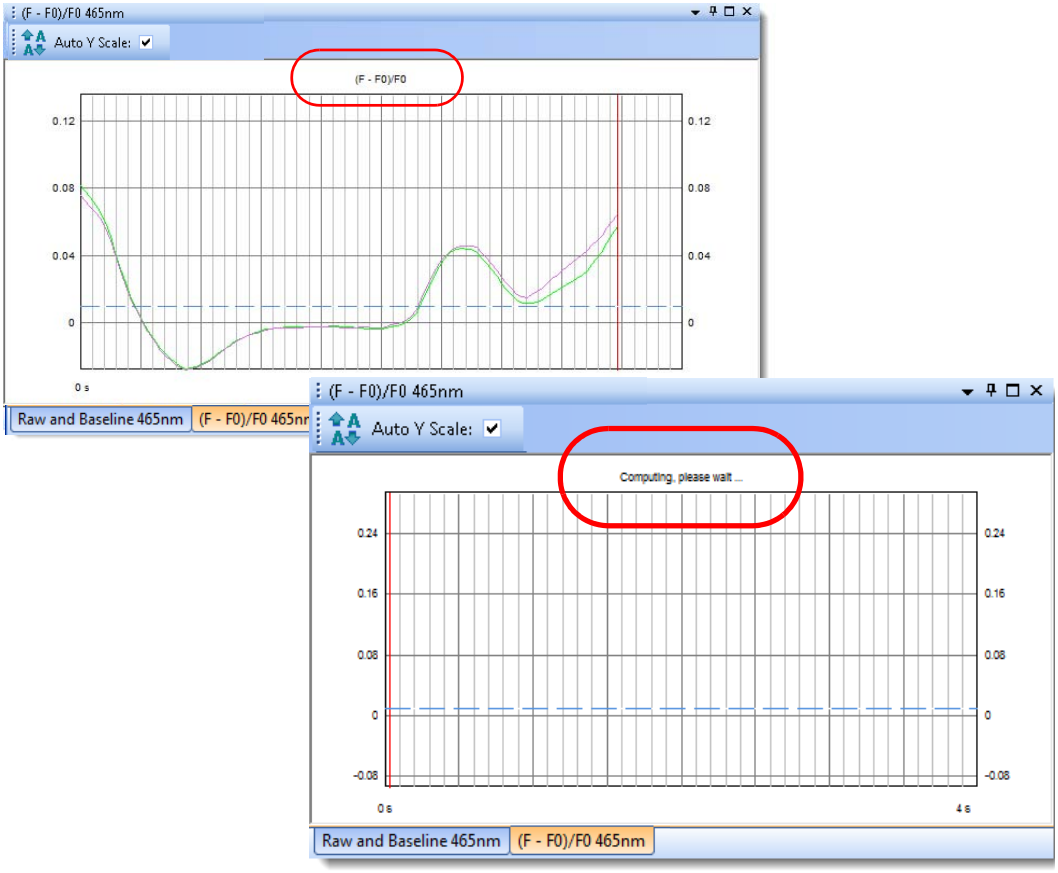
The system automatically scales the Y axis of the graph by considering the minimum and maximum of the signals that have been observed. If there is a large transient change in fluorescence, the maximum or minimum Y values of the graph may take on abnormally large values with the result that the data traces become compressed into a very small region of the graph. If this happens, it may be necessary for you to un-check and then re-check the **Auto Y Scale** checkbox to reset the Y axis limits of the graph.

If you deselect **Auto Y Scale**, the system gives you the option of setting the minimum and maximum values for the y-axis. See the example, below.



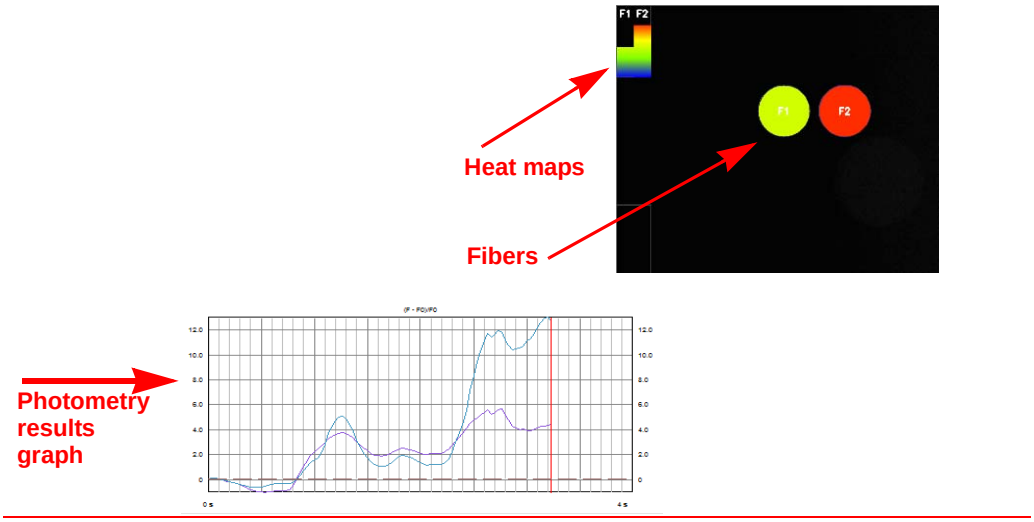
(F-F0)/F0 graph—computing time

The heading in the computed data graph is typically displayed as **(F–F0)/F0**. However, when you add, resize or move a fiber circle in the photometry video window, and the system is gathering data and performing calculations to create the new fluorescence baseline, the heading is displayed as **Computing, please wait...**.



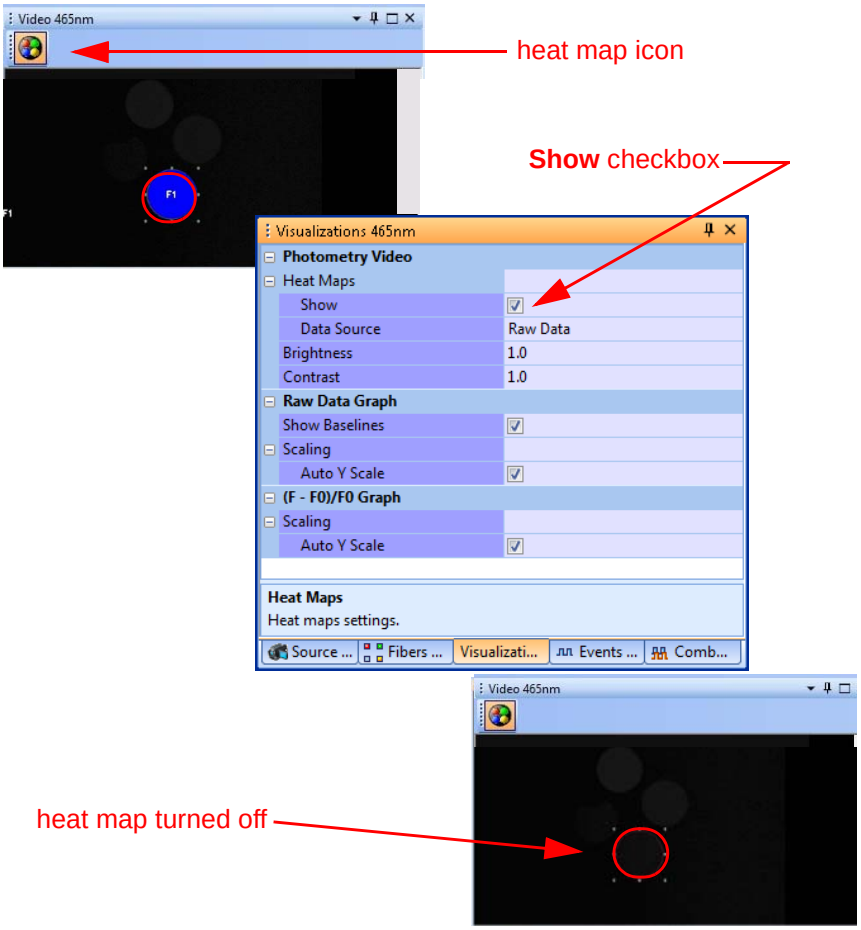
4.10 Adjusting Fiber Locations and Size During Operation

The image below shows a typical heat map and a graph of the photometry results that you might expect to see after the experiment is underway and the GECIs are emitting fluorescence. The photometry video window will display the intensity of the measured fluorescent transients the system receives from the implanted fibers. They are displayed as colored vertical bars, as seen in the image below. (This image shows an example when computed data are selected as a source for the heat maps.)



During an active session when the GECIs are fluorescing, the system fills each of the fiber circles with a brightly colored heat map corresponding to the level of the photometry signal. If you are fine tuning the position of the circle around a fiber, you can, if you prefer, temporarily turn off the heat maps so you can see the fiber boundaries more easily. If you need to adjust the size or position of any circle to fully cover a fiber image, use the procedure below.

1 Temporarily turn off the heat map by clicking the heat maps icon in the photometry video window or by deselecting the **Show** checkbox in the photometry Visualization tab.



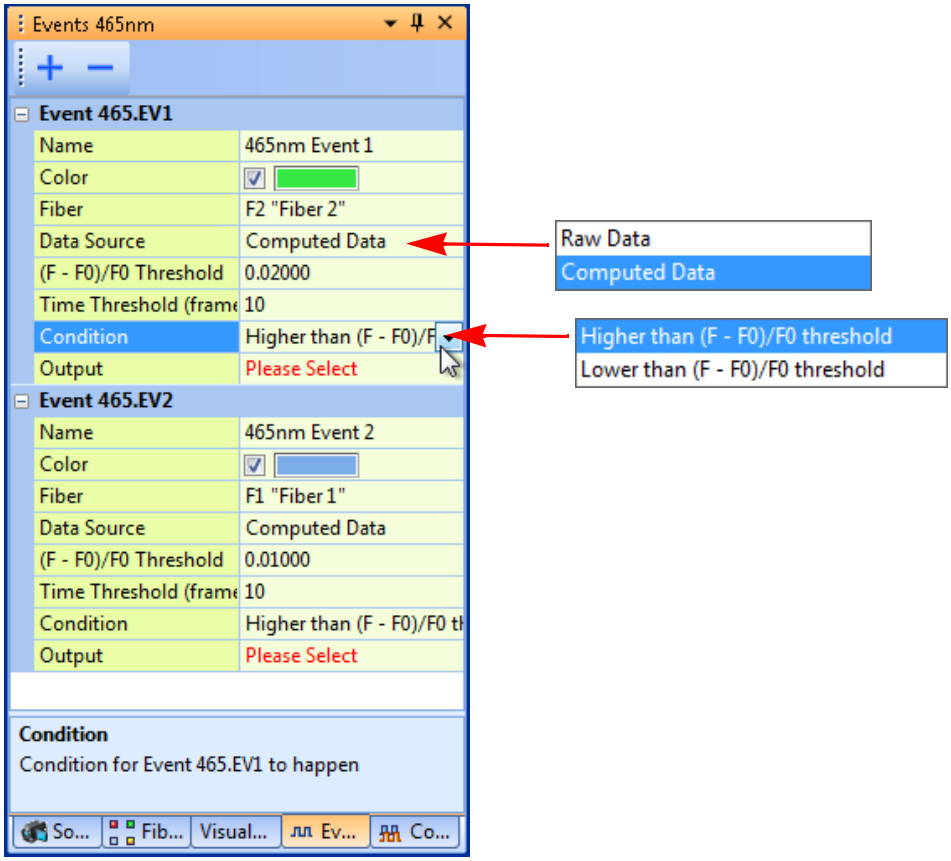
2 Reposition and re-size the circle so it will better enclose the fiber image.

3 Repeat the steps in this procedure until you are satisfied with the size and position of each circle.

4 Select the **Show Heat Maps** box again to display the heat maps.

4.11 Creating Photometry Events

You can create photometry events based on the relative intensity of fluorescence detected from any of the fibers. Click the **Events** tab and create the desired events. The dialog contains dropdown lists and other options as described below.



Enter or select data for each row, as follows.

1. **Name**—The event name that will appear in the Event Statistics tab. It can be modified by double clicking or selecting the current name, then typing in the new name.
2. **Color**—The color that will appear in the Event Statistics tab when the event is occurring. For an example of an Event Statistics tab, see Section 9.4, "Displaying Event Statistics As They Occur" on page 232.
3. **Fiber**—Select the fiber for this event.
4. **Data Source**—Detect the event based on **Raw Data** or **Computed Data**.
5. **(F-F0)/F0 Threshold**—Enter a number from -1.0 to +10.0 (default 0.01) for the relative fluorescent intensity you want to define as the threshold.
6. **Time Threshold (frames)**—Specify the number of frames for which the **Condition** must be met for the event to be true, 0 to 999 (default 10).
7. **Condition**—Specify when the event is true, that is, when the measured relative fluorescent intensity is **Higher than (F -F0)/F0 threshold** or **Lower than (F -F0)/F0 threshold**.
8. **Output**—(Optional) Specify the digital output line you want to use to send a signal from the Trigger Box DIGITAL OUTPUT port to an external device when an event occurs (becomes TRUE). Select the desired **Output** line from the drop down list to be associated with this event. The output line numbers correspond to the line numbers on the connector for the DIGITAL OUTPUT port of the Trigger Box. The software is able to read the DIGITAL OUTPUT settings of the Trigger Box, and will display each output line number with its logic type—high true (HT) or low true (LT).

   For the procedure to specify the **Output**, see Specifying Digital Outputs when Events Occur, below.

4.12 Specifying Digital Outputs when Events Occur

As events occur in the photometry or behavioral video stream, the system can send digital outputs via the Trigger Box DIGITAL OUTPUT port. Digital event logic is set in hardware, and defaults to 6 high-true, and 6 low-true outputs. However, when a system is ordered from Plexon, the Trigger Box can be adjusted to have any combination of high-true or low-true outputs (up to a total of 12). The outputs can be based on a level or a pulse of a user-specified duration.

Configuration of the **Output Line** parameter is optional—You only need to configure it if you want to output a signal.

TIP

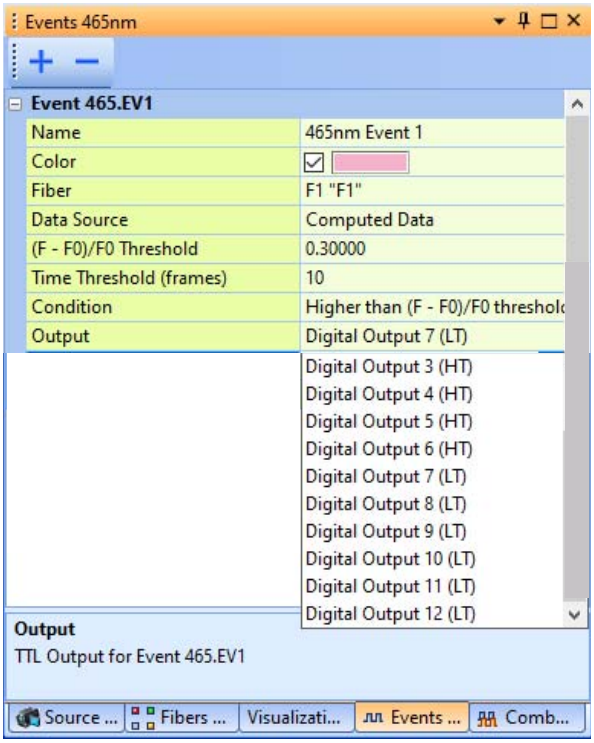
Digital output lines can be specified for all types of events

Digital output lines can be specified for photometry events, photometry combination events, behavioral events, behavioral combination events and global combination events.

Note: The signal logic is described in Appendix C-Trigger Box and Digital Input/ Output.

Note: Outputs can be generated at the camera frame rate (30 frames per second), but not faster than that. For controlling external devices at a very fast rate (for example, LED pulses), you should send a signal to another program to start controlling that device.

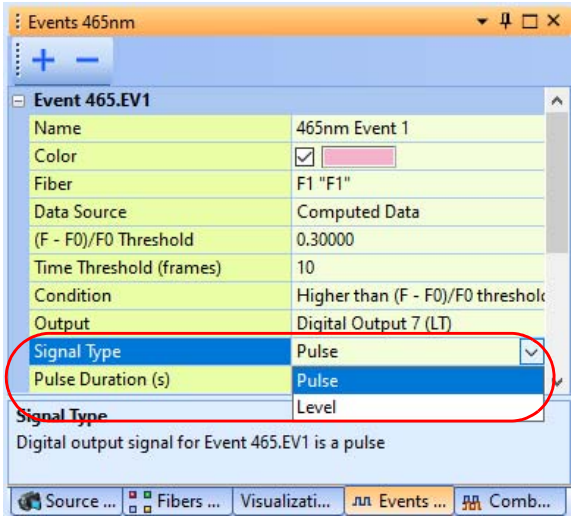
1. Open the Events tab.
2. To specify that a digital output is generated when an event occurs (becomes TRUE), select the desired **Output** line from the drop down list associated with the event. The output line numbers correspond to the line numbers on the connector for the DIGITAL OUTPUT port of the Trigger Box. The software is able to read the DIGITAL OUTPUT settings of the Trigger Box, and will display each output line number with its logic type—high true (HT) or low true (LT).



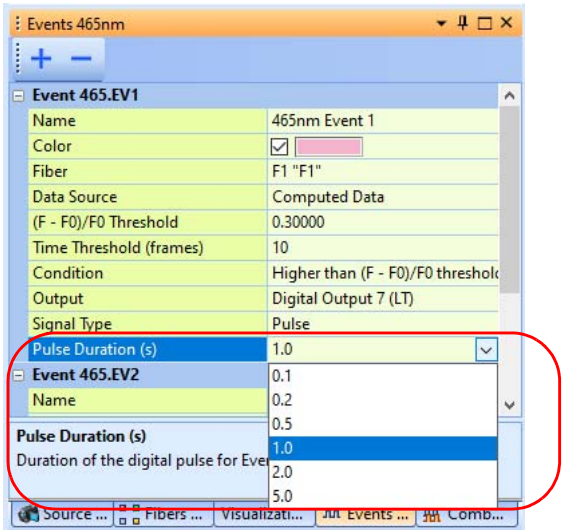
Note: Each digital output line is restricted to one event at a time.

Note that when you select an output line, a new row appears—**Signal Type**.

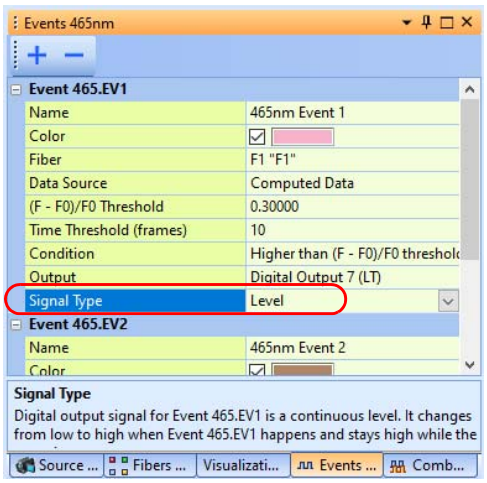
3 Specify the output **Signal Type** as either **Pulse** or **Level**.



4 If the output **Signal Type** is **Pulse**, specify the **Pulse Duration (s)**.

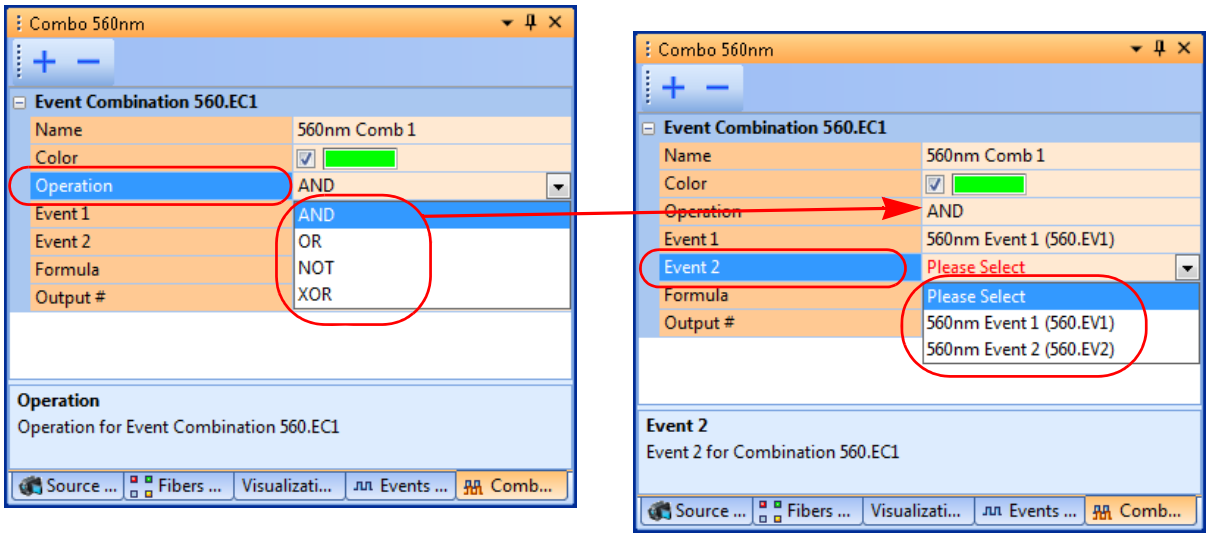


5 If the output **Signal Type** is set to **Level**, the system will assert the output continuously while the condition is true, so there is no additional parameter to set (as there was for **Pulse**).



4.13 Creating Photometry Combination Events

You can create combination photometry events based on a combination of the intensities of the fluorescence detected in multiple fibers. Click the **Combo** tab and create the desired events. The images below illustrate the options.



1 Configure the **Operation** row parameter (see the image above) to specify events that are combinations of one or more existing events on which logical operations are performed. The existing events can be any single or combination event. Select one of the four logical operators: **NOT**, **AND**, **OR, XOR**:

AND—Both of the individual events are true.

OR—Either one of the individual events is true, or both of the individual events are true.

NOT—For single events, an event can be defined which is the opposite of it by using the **NOT** operator. The new event is true when the original event is not true and vice versa (the new event is not true when the original event is true). For example, **NOT**[animal is inside feeding zone] means the animal is not inside the feeding zone.

XOR—Either one of the individual events is true, but not both of them.

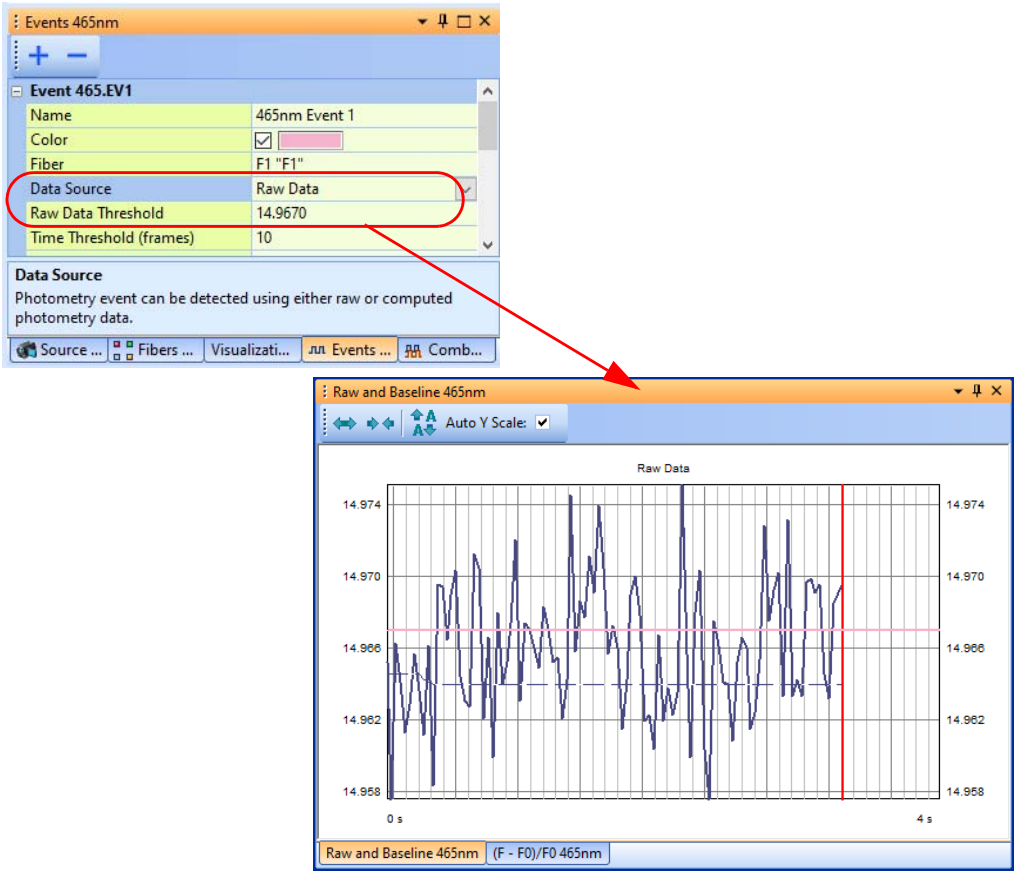
2 For the procedure to specify **Output #**, see Section 4.12, “Specifying Digital Outputs when Events Occur” on page 85.

Note: You can also create *global combination events* across multiple photometry video sources and the behavioral video source. For that procedure, see Section 9.3, “Creating Global Combination Output Events” on page 228.

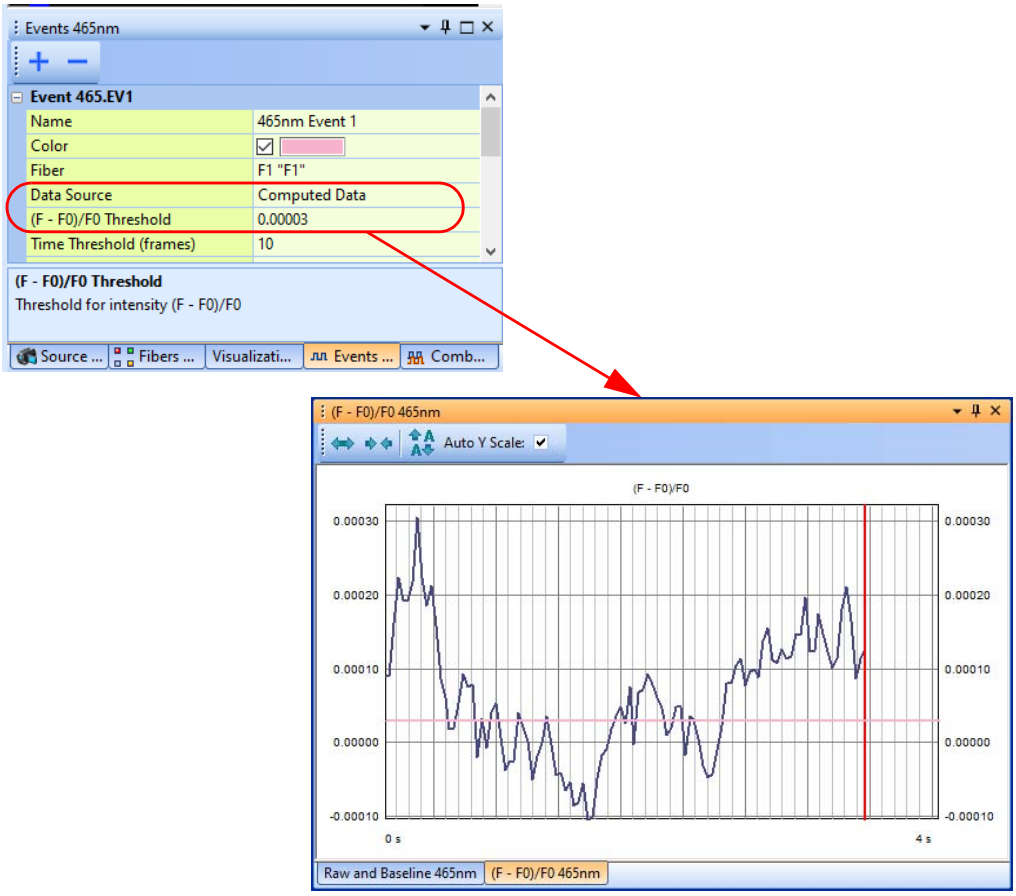
4.14 Showing Event Thresholds On the Photometry Graphs

Photometry events can be based on **Raw Data** or **Computed Data**. Notice in the discussion that follows, the **Raw Data** and **Computed Data** are very different in scale, so the thresholds must be selected separately.

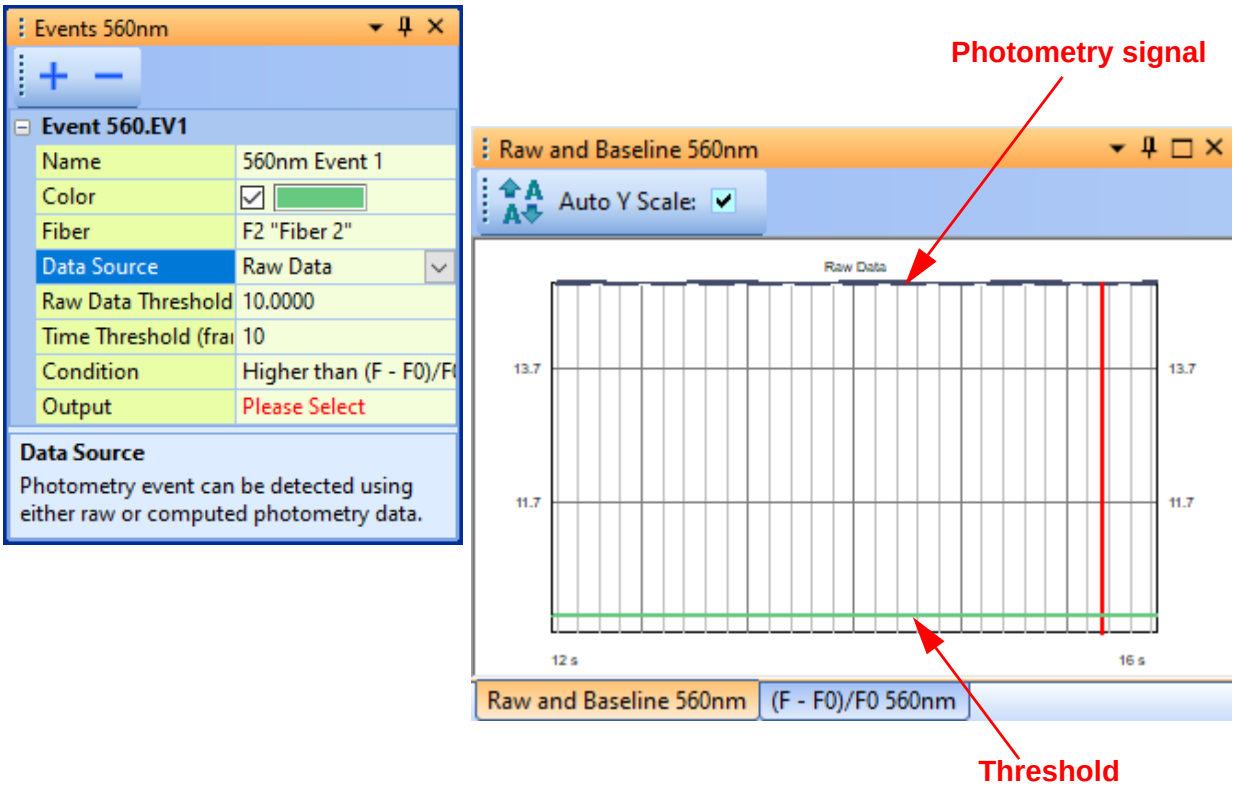
If you select **Raw Data** for a particular 465nm event, for example, the system will display the **Raw Data Threshold** for this event. The threshold for this event will be displayed in the “Raw and Baseline 465nm” graph, but will not be displayed in the “(F – F0)/F0 465nm” graph.



If you select **Computed Data** for this same 465nm event, the system will display the **(F – F0)/F0 Threshold** for this event. The threshold for this event will be displayed in the "(F – F0)/F0 465nm" graph, but will not be displayed in the "Raw and Baseline 465nm" graph.

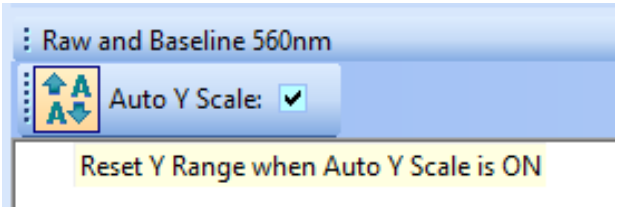


Please note the event thresholds are taken into account to adjust Min and Max Y values on the photometry graphs when "Auto Y Scale" is checked. So, if you see photometry graph all the way at the top or bottom of the graph, and most of the graph space is empty, it means very likely the threshold for one of your events is set much too low or too high for your data. Check the Y values at your graph and adjust event Threshold values. For example:



Set Threshold, for example, to 14.96, and the picture will change:

If you are in Files mode, and playing has not been started yet or is paused/ stopped, and the graph's Y axis did not adjust automatically, click the "Reset Y range" button in the graph toolbar.



4.15 Exporting Photometry Results

This section explains how to export photometry results for individual sessions. You must be in **Files/View Sessions As Recorded** mode to enable export.

For each session (recording) that you create, a row appears in the **Sessions** window at the bottom left of the default GUI layout (red outline below). You can click on an individual row to select a particular session. The selected session is indicated by a small black triangle just to the left of the session number. (In the image below, Session #3 is selected.)

Procedure for exporting all photometry results per frame for a single session into one file

1. Ensure that the system is in **Files/View Sessions As Recorded** mode.
2. To export the photometry data for the selected session, in the toolbar at the left side of the **Sessions** window, click on the **Export all photometry results per frame for a single session into one file** button. A standard file-save dialog box will let you specify the name and location for the exported file. The data will be saved in .csv format.

   Note: If the selected session does not have any photometry data to export, the resulting CSV file can be created, but it will have only frame number and timestamps.

   The exported file will contain data for all of the fibers that you have defined for the Experiment. For example, if you record a session with one fiber defined for a particular wavelength, there will be one data set for that wavelength. If you record a session with four fibers defined for all three wavelengths, there

will be four data sets for each of the three wavelengths (as shown in the example that follows).

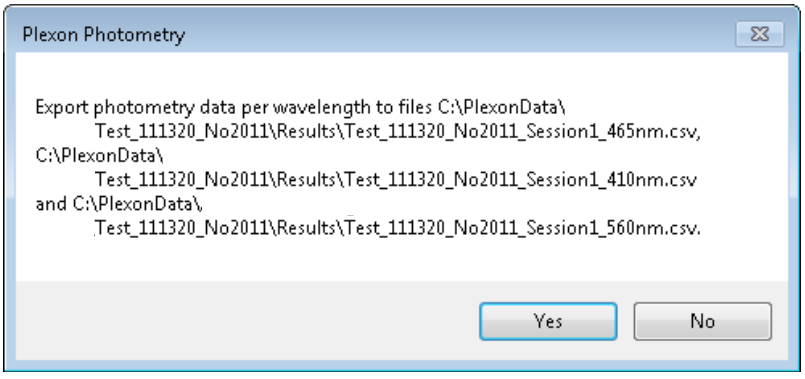
The exported file contains the following data, assuming all four fibers are defined for all three wavelengths in this Experiment:

```
Frame,timestamp,

RawF_465_F1, F0_465_F1, R_465_F1,
RawF_465_F2, F0_465_F2, R_465_F2,
RawF_465_F3, F0_465_F3, R_465_F3,
RawF_465_F4, F0_465_F4, R_465_F4,

RawF_410_F1, F0_410_F1, R_410_F1,
RawF_410_F2, F0_410_F2, R_410_F2,
RawF_410_F3, F0_410_F3, R_410_F3,
RawF_410_F4, F0_410_F4, R_410_F4,

RawF_560_F1, F0_560_F1, R_560_F1,
RawF_560_F2, F0_560_F2, R_560_F2,
RawF_560_F3, F0_560_F3, R_560_F3,
RawF_560_F4, F0_560_F4, R_560_F4
```

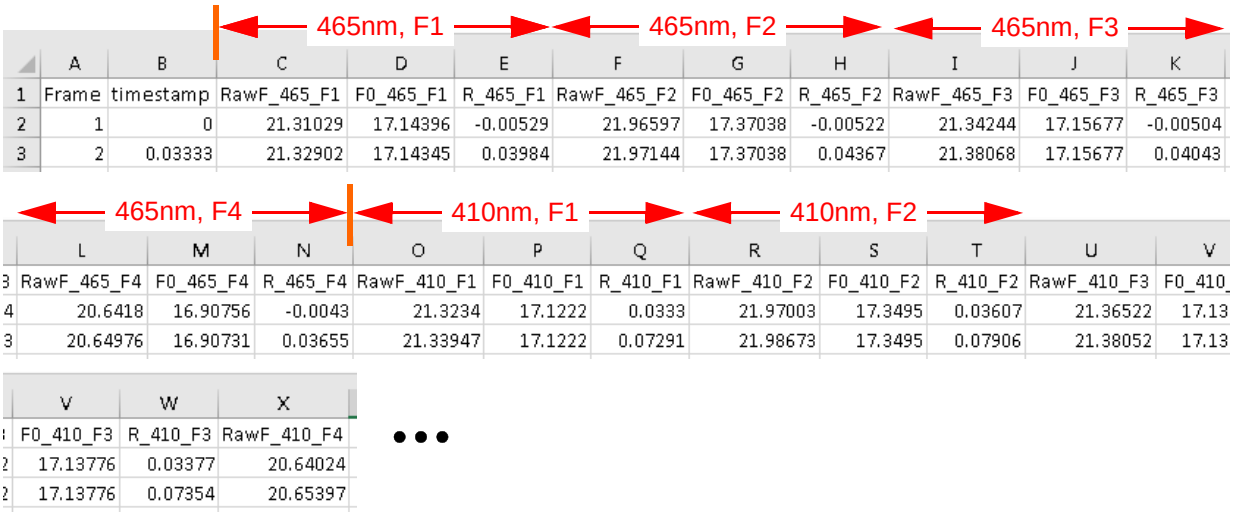
RawF is the raw signal, F0 is the baseline, and R is the filtered relative increase in fluorescence. For a detailed description of these data types (RawF, F0 and R), see Section 4.16, “Photometry Reference—Time Variables” on page 95.

Procedure for exporting all photometry results per frame for a single session into a separate file for each wavelength

1. Ensure that the system is in **Files/View Sessions As Recorded** mode.
2. To export the photometry data for the selected session, in the toolbar at the left side of the **Sessions** window, click on the **Export all photometry results per frame for a single session into a separate file for each wavelength** button. A dialog box, similar to the example below, will appear. After you click Yes, the data will be saved in .csv format.



The exported photometry results include RawF, F0 and R for each fiber for each wavelength. The CSV file has the following headings when opened in a spreadsheet. (In this example, only a portion of the large spreadsheet is shown.)



	A	B	C	D	E	F	G	H	I	J	K
1	Frame	timestamp	RawF_465_F1	F0_465_F1	R_465_F1	RawF_465_F2	F0_465_F2	R_465_F2	RawF_465_F3	F0_465_F3	R_465_F3
2	1	0	21.31029	17.14396	-0.00529	21.96597	17.37038	-0.00522	21.34244	17.15677	-0.00504
3	2	0.03333	21.32902	17.14345	0.03984	21.97144	17.37038	0.04367	21.38068	17.15677	0.04043

	L	M	N	O	P	Q	R	S	T	U	V
3	RawF_465_F4	F0_465_F4	R_465_F4	RawF_410_F1	F0_410_F1	R_410_F1	RawF_410_F2	F0_410_F2	R_410_F2	RawF_410_F3	F0_410_
4	20.6418	16.90756	-0.0043	21.3234	17.1222	0.0333	21.97003	17.3495	0.03607	21.36522	17.13
3	20.64976	16.90731	0.03655	21.33947	17.1222	0.07291	21.98673	17.3495	0.07906	21.38052	17.13

	V	W	X
1	F0_410_F3	R_410_F3	RawF_410_F4
2	17.13776	0.03377	20.64024
2	17.13776	0.07354	20.65397

• • •

4.16 Photometry Reference—Time Variables

This section explains the calculations involving the time variables in the Fibers tab—τ1 (Averaging), τ2 (Minimization), and τ0 (De-Noising). It covers these topics:

Using Raw Data for heat maps

When you select **Raw Data** (in the Visualizations tab) for the heat maps, the system displays colors based on instantaneous fluorescence values for each frame, and these values are encoded in color inside the heat maps. Because this signal is noisy, you will see very fast changes in the heat maps.

Using Computed Data data for heat maps

When you select **Computed Data** for the heat maps, the system displays colors based on values that have been computed according to the algorithms described in this section. In this case the heat map signal you are seeing in the current frame is the result of computation over T = t_averaging + t_minimization + t_de-noising (for each fiber). In other words, to get the filtered fluorescence value in the current frame, the system must gather the data over time T. So, there is a delay. The value “F – F0” is the level of fluorescence after removal of the background noise. Thus, even after stimulation is stopped, there will be a nonzero value displayed in the heat maps for a short time, T.

If you change any value involved in the computations, such as a timing parameter, or if you move a fiber, you will see the heat maps do not show immediately, and the display indicates “computing, please wait …”. The reason for that is that the system is gathering data for a time T before it can display the very first computed point.

Note: The calculations in this section are based on the algorithm in the reference listed at the end of this section (see Additional reading).

Spatial average / raw signal

For each photometry fiber, the user defines a circular region in the photometry video corresponding to the imaged end of a single optical fiber (see Section 4.7, "Configuring (Adding) Fibers" on page 68). The spatial average of the pixel intensities in this circular region is computed and this average intensity is considered to be the raw fluorescence signal (F_{RAW}) for that fiber as a function of time.

Temporal average and baseline

The change in the calcium fluorescence signal is computed relative to the background level (the baseline). Since the fluorescence signal and the baseline, $F_{BASELINE}(t)$, can be changing in time (for example, in a phenomenon known as bleaching), the system uses an algorithm to adjust the baseline value dynamically. The value is computed in a two-step process.

First, the temporal average of the raw fluorescence (F_{AVG}) is computed over a sliding window of width τ_1, specified in seconds:

$$F_{AVG} = \frac{1}{\tau_1}\int_{t-\tau_1}^{t} F_{RAW}(t)dt$$

The default value of τ_1 is 0.75s.

Next, $F_{BASELINE}(t)$, is computed as the minimum of the temporal average in a window defined by a parameter called τ_2:

$$F_{BASELINE}(t) = \{min\ F_{AVG}(x) \,|\, (t - \tau_2) < x < t\}$$

The default value of τ_2 = 3s.

Note: $F_{BASELINE}$ is displayed as F0 in the GUI.

Relative change in fluorescence

The (unfiltered) relative fluorescence for time *t*, ΔF/F(*t*), is computed as:

$$R(t) = \Delta F/F(t)_{unfiltered} = \frac{F_{RAW}(t) - F_{BASELINE}(t)}{F_{BASELINE}(t)}$$

De-noising the results

Finally, the relative change in fluorescence is smoothed with an exponentially weighted moving window. The exponential weighting is described by a time constant, τ_0, and the width of the averaging window is set to five time constants ($5*\tau_0$). The final result is:

$$\int_0^{5\tau_0} R(t-\tau_0)\cdot e^{-\frac{|t|}{\tau_0}}$$

Note: This final result is available after $\tau_1 + \tau_2 + \tau_0$ delay, i.e., when all preliminary computations are finished.

Additional reading

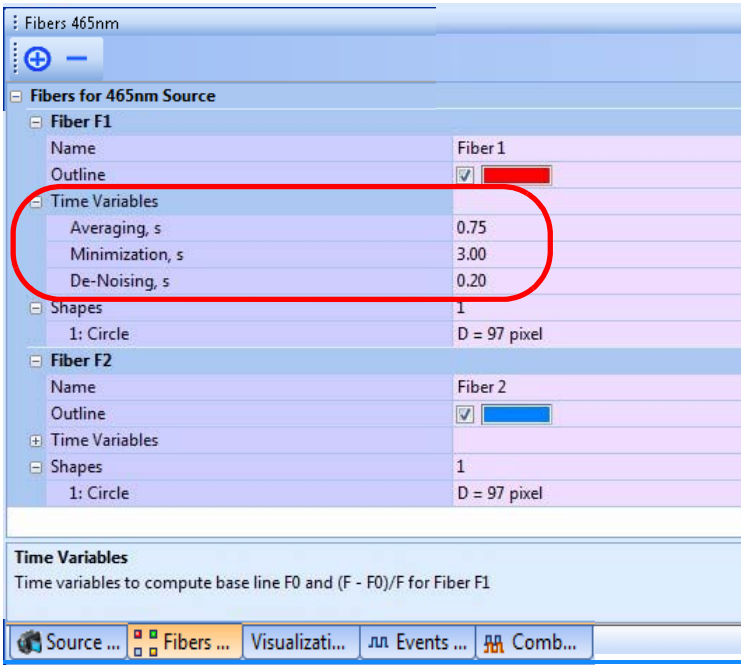
H. Jia, N.L. Rochefort, X. Chen, A. Konnerth, In vivo two-photon imaging of sensory-evoked dendritic calcium signals in cortical neurons, *Nat. Protoc.*, 6 (2011), pp. 28-35.

Adjusting the photometry analysis parameters (Time Variables)

The default photometry analysis parameters are taken from the reference article listed above. The reference suggests that they provide “effective filtering for 30 Hz imaging.” However, you can adjust any of these analysis parameters on a per fiber basis, as described below.

1. Click on the “**+**” signs if necessary to expand the controls for the particular fiber you want to adjust, and then expand the **Time Variables** for that fiber to expose the controls as shown below.

2. You can type in new values for τ_1 (Averaging), τ_2 (Minimization), and τ_0 (De-Noising). The values are in seconds. Double click on a value, then enter the new value.



4.17 Recommendations on Adjusting Time Variables

Although values of the timing variables will always be a somewhat subjective choice, there are several recommendations.

4.17.1 Baseline Computation— “minimization” (τ_2) and “averaging” (τ_1) variables

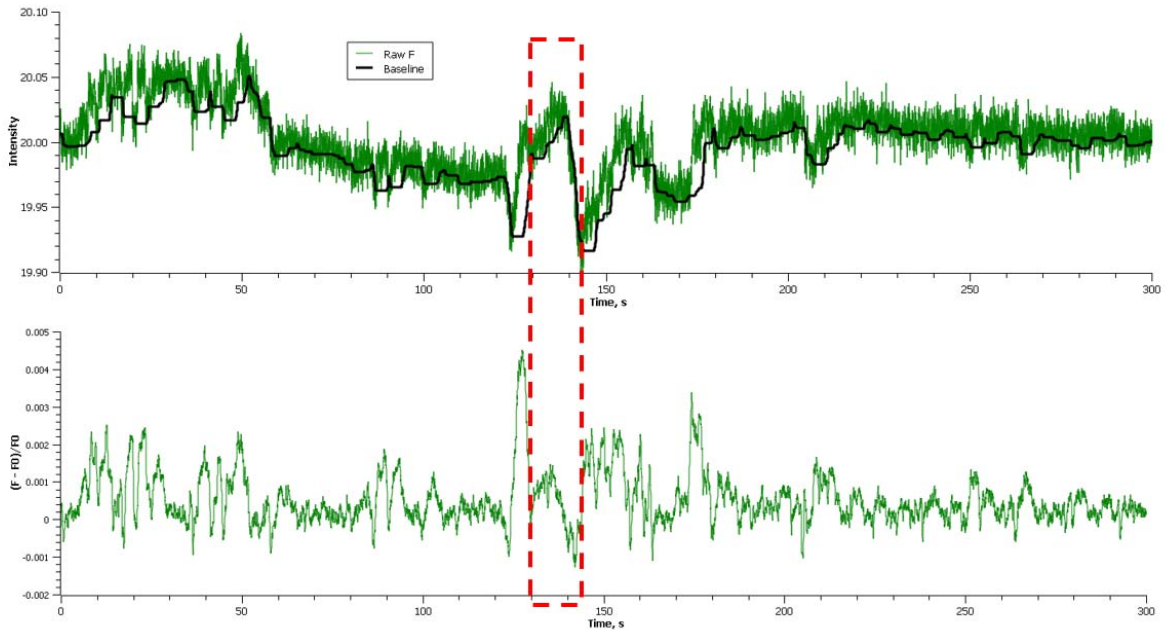
The general rule for the baseline is that it must smoothly follow the tendency of the signal, but not “repeat” the shapes of the peaks.

“Minimization” is the most important parameter to achieve this goal, and it has to be adjusted first. It is very important to have an idea about the shape of your fluorescent signal—specifically about the maximum width of the peaks—to choose the correct value for τ_2, since it has to exceed or at least be equal to the

maximum duration of the peaks. Otherwise, the resulting signal will be distorted—actual peaks can be lost completely or partially.

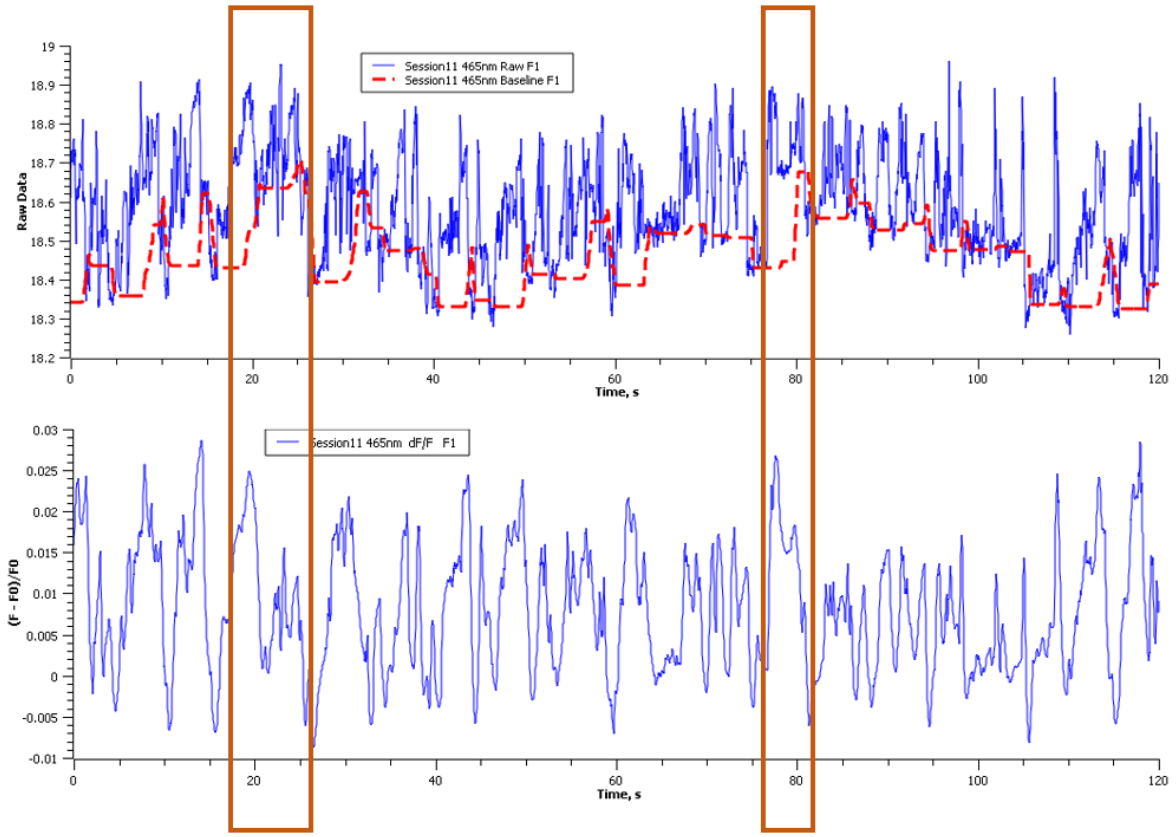
Example 1 below shows how τ_2= 3s (default value) causes the large peak in the resulting dF/F signal to become much smaller than the little parasite peak next to it. The reason for that is the baseline being flat where the small parasite peak is, and completely repeating the shape of the “good” large peak in the raw signal.

Example 1

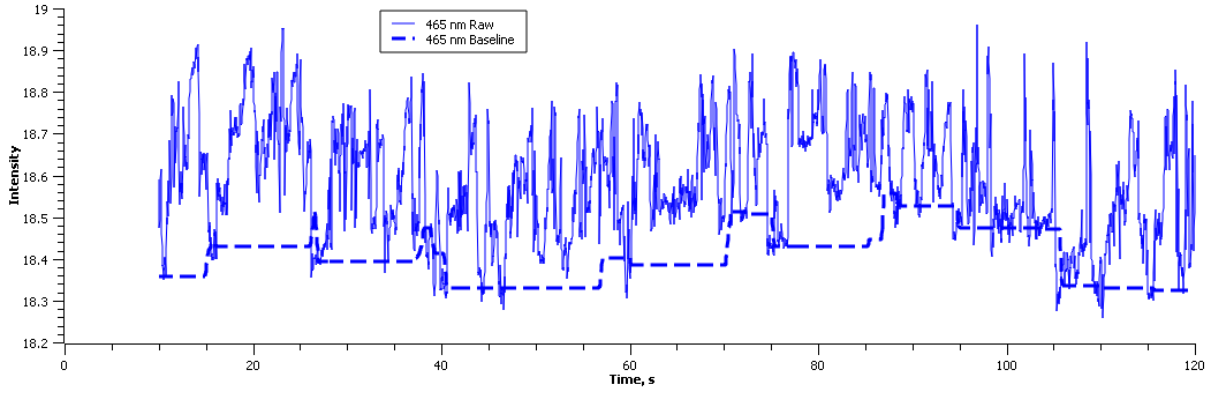


Here is another example where too small a value of τ_2 (3 ms) compared to the width of the peaks distorts intensity between parts of the same peaks.

Example 2

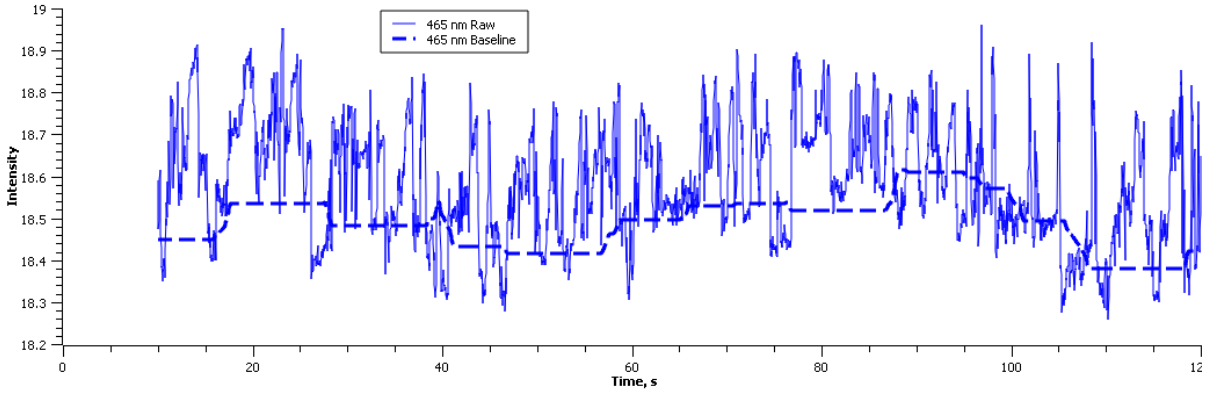


For Example 2, τ_2= 10.0 s seems more reasonable, and with default values of τ_1= 0.75s and τ_0= 0.2s ("de-noising") we would get this result:



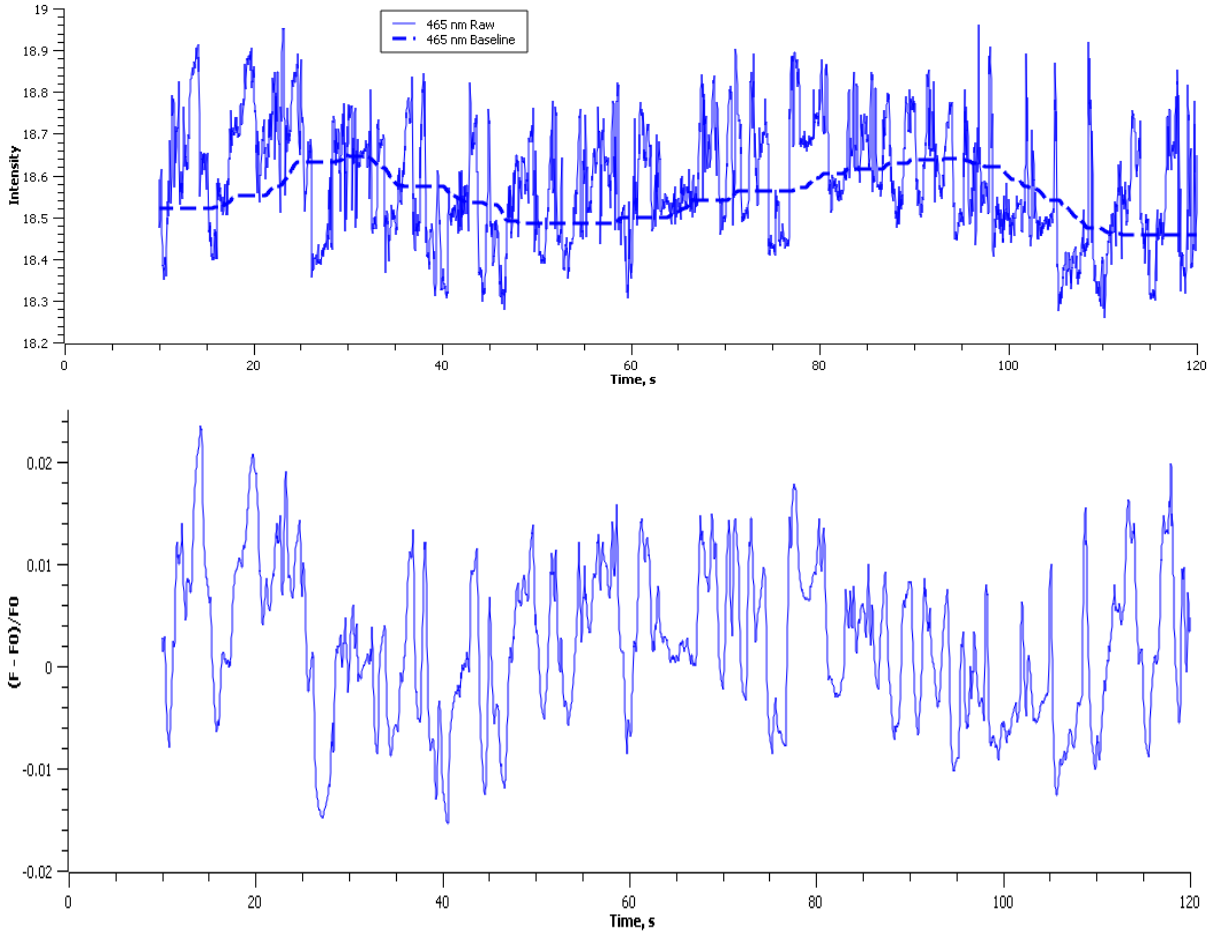
However, we still keep seeing some "peaks" in the baseline, and now we can increase τ_2 to smooth it some.

This is what we would get with τ_1= 3.0s, τ_2= 10.0s, and τ_0= 0.2s



And if we increase τ_1 to 10 s, we would get the baseline we can go with for future computations of dF/F. We don't want the baseline to be completely flat, so there is no need for further increase of τ_1 and τ_2.

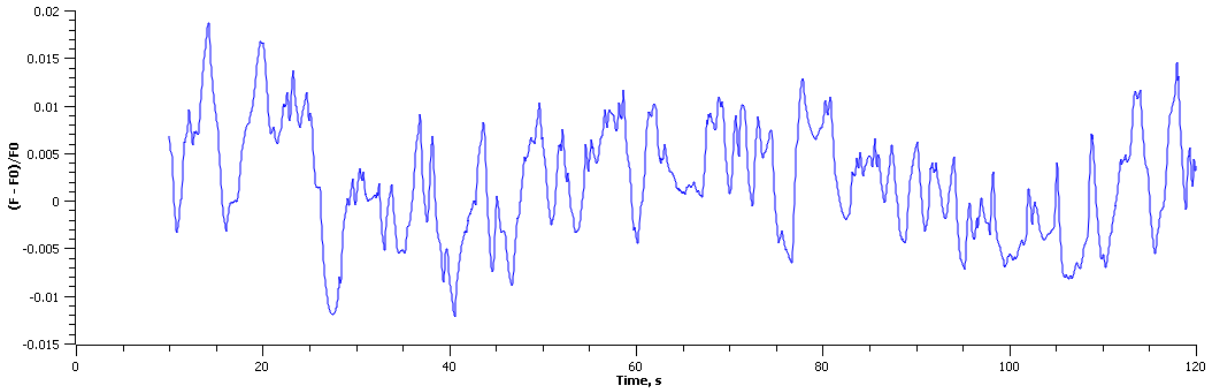
With τ_1= 10.0s, τ_2= 10.0s, and τ_0= 0.2s, we get the following:



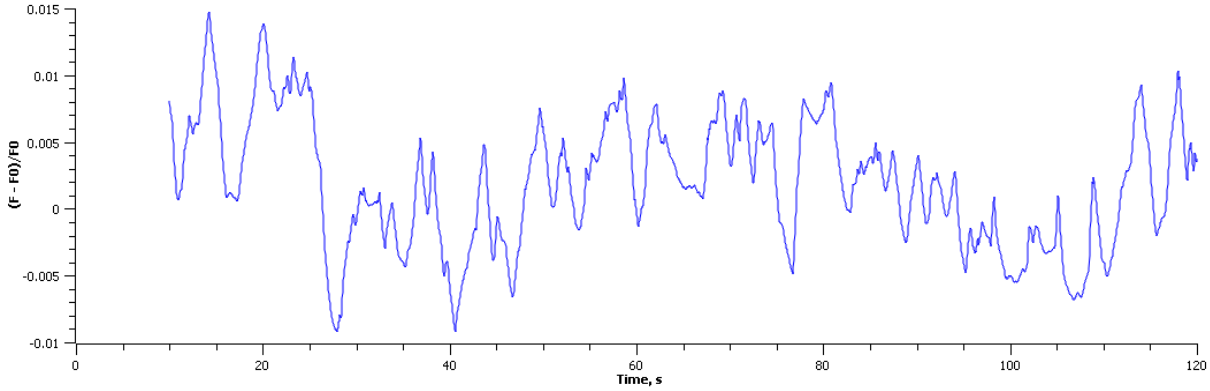
4.17.2 De-noising the dF/F result

Below you can see how an increase of τ_0 changes the computed dF/F result.

With τ_1= 10.0s, τ_2= 10.0s, and τ_0= 0.5s:



With τ_1= 10.0s, τ_2= 10.0s, and τ_0= 1.0s:



Further increase of τ_0 in this example would cause smoothing out the features of the signal itself, and thus is not recommended for this particular case.

4.18 Where to Go Next

If you are using the behavioral camera to record the movements of the subject(s), go to the following chapters, as applicable:

- Chapter 5, Setting Up the Behavioral Camera
- Chapter 6, Calibrating the Arena Dimensions
- Chapter 7, Configuring the Tracking Parameters
- Chapter 8, Behavioral Features and Procedures

Otherwise, go to Chapter 9, Configuring and Viewing Global Events.

Chapter 5
Setting Up the Behavioral Camera

5.1 Before You Start

Be aware of these important cautions on camera care and arena lighting.

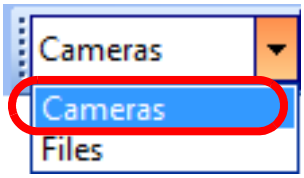
	**CAUTION** **Avoid possible damage to camera** Do not connect or disconnect the behavioral camera while the computer power is on, as doing this might damage the camera. Always shut down the PC completely before you connect or disconnect the camera.

	**CAUTION** **Lighting Conditions** If lighting conditions change enough during recording that ‘objects too large’ or some other condition occurs, the position data may be meaningless.

5.2 Mounting the Behavioral Camera and Adjusting the Image

In the basic startup procedure (Section 2.3, “Startup and Operational Testing” on page 20), you turned on the behavioral camera and verified that video was being displayed in the GUI. The steps shown below will assist you in positioning the camera and obtaining the video quality you want for your experiment.

1 Ensure that **Cameras** is selected in the video source dropdown list on the main toolbar. Camera setting options do not display in the user interface unless **Cameras** is set in the main toolbar.



Note: If the **Cameras** option is not present in the menu, it means that the system has not detected the behavioral camera. If this occurs, turn off (power down) the computer, then verify that the camera is properly connected to the computer via a USB cable. For most camera

models, when the computer is powered up, the camera should display a green LED indicating that power is connected**.**

TIP

Consider calibration requirements

Plan the mounting of the behavioral camera with calibration in mind. Calibration of linear dimensions (in inches or centimeters) works most accurately when the camera is orthogonal to the arena. The calibration procedure is explained in Chapter 6, Calibrating the Arena Dimensions.

2 Read Appendix A, Optimizing Behavioral Camera Positioning, to understand the detailed requirements for camera mounting, cabling and removal. This information is essential for positioning the camera and obtaining the best results from the system.

3 Set up the camera as described in Appendix A.

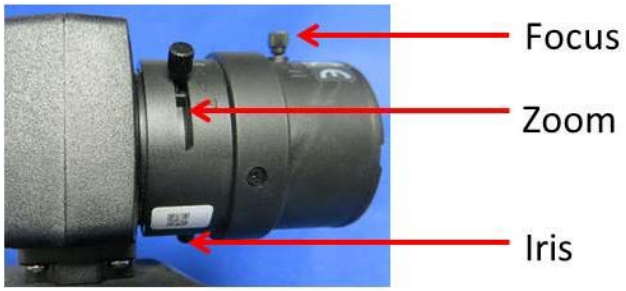
TIP

Ensure the camera is set up for optimum imaging

In general, it is best to position the camera as far from the experiment as possible, then zoom as much as possible in order to fill the field of view with the area of interest without distortion. Appendix A-Optimizing Behavioral Camera Positioning explains how to compute the distance from the camera to the arena so that the whole arena is visible.

4 Obtain an initial video image from the camera using the default parameter values and changing only the physical adjustments (iris, focus and zoom) on the camera lens.

Open and close the iris on the camera to make the image brighter or darker for the best possible contrast between the background and your test object. Refer to the image below to identify the components of your camera (typical camera shown).



5 If the camera experiences either of the following problems, take the action recommended here:

a If there is no Behavioral Source window corresponding to the camera, go to the **Window > Layout** dropdown menu and select **Reset to Default Layout**.

b If there is still no video, go back to Section 2.3, “Startup and Operational Testing” on page 20 to resolve the problem.

TIP

Make the Video Image Larger

If the default video image is not large enough to make your desired adjustments, drag the video window out of its frame to another area on the monitor. Then adjust the window size in the normal way.

6 If the previous steps do not result in a good image on the camera, change values of the camera parameters in the Behavioral Source window. The available parameters and values depend on the camera model. Typically, they include **Gain**, **Auto Brightness**, **White Balance** (**W.B. Red and W.B. Blue**), and **Shutter (ms)**.

If additional troubleshooting is necessary, see Appendix E, Troubleshooting.

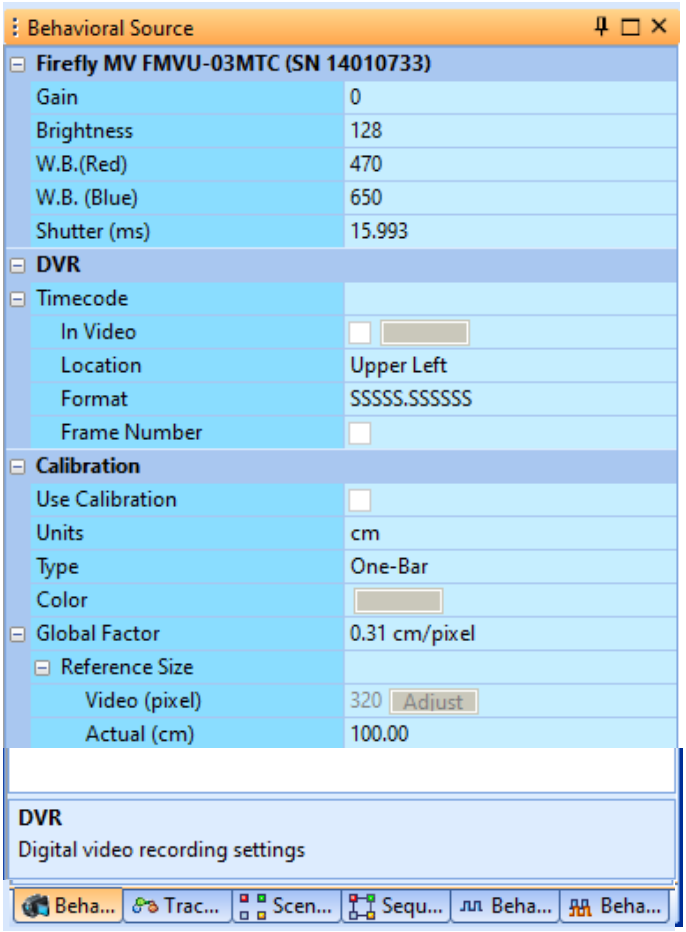
7 Optional—Calibrate the physical dimensions in the Behavioral Video field. (Calibration works most accurately when the camera is positioned orthogonally to the arena.)

Note: In many experiments, it is useful to calibrate the video image dimensions so that the ratio of centimeters to pixels (or inches to pixels) is known. For the procedure, see Chapter 6, Calibrating the Arena Dimensions.

5.3 Configuring Behavioral Source Parameters

The **Behavioral Source** tab is used to set parameters for the video stream on the behavioral camera or video file. This tab contains parameters that relate to optical settings for the camera (such as gain, brightness, white balance and shutter speed), labeling/timestamping of frames and calibration.

1 With the video image properly set up and focused in the Behavioral Video window, click on the **Behavioral Source** tab under the Behavioral Video window to view the parameters. The following steps configure these settings.



2 In the camera area of the Source tab (which displays the camera name, model and serial number) adjust the controls to obtain an acceptable image on the screen. The specific controls vary depending on the camera model.

Typical adjustments may include **Gain**, **Auto Brightness**, **White Balance**, and shutter speed (**Shutter (ms)**).)

TIP

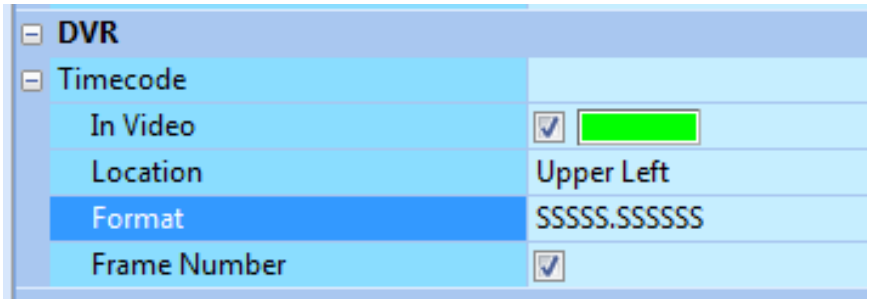
Reducing blurs and color streaks in the video

If the experimental subject moves quickly, blurs and color streaks can appear in the video. You can reduce or eliminate these problems by increasing the shutter speed—**Shutter (ms).**

Although it may seem confusing, for historical reasons the terms “increasing shutter speed” and “reducing shutter open time” are synonymous.

The maximum shutter speed for the behavioral camera is 15.993ms, and it can be closed more if you prefer.

3 In the digital video recording (**DVR)** area of the Source tab, **Timecode** options, select the **In Video** checkbox if you wish to display a time code over the video image. Choose a **Location** and a **Format** setting to configure the display. Select the **Frame Number** checkbox if you want the frame number displayed along with the time. (The system maintains a time code that tracks the time elapsed since the last time recording began.)



For example, the first image (below) shows the time code in the upper left location in **SSSSS.SSSSSS** format. The second image shows the time code in **HH:MM:SS.SSS** format along with the frame number.

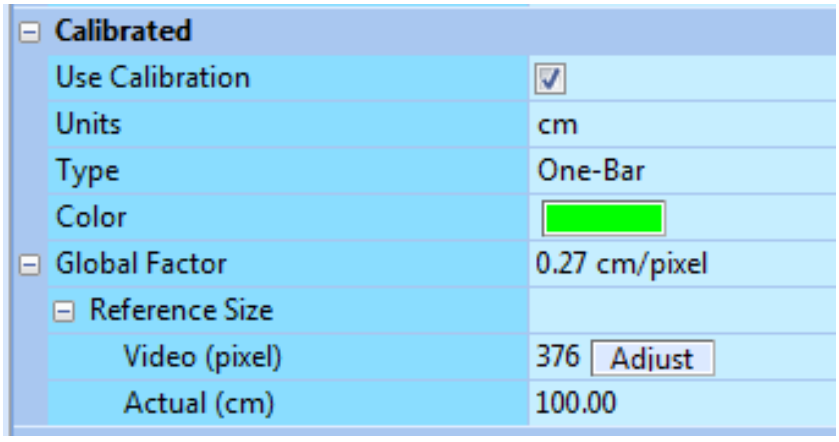
TIP

Timecode checkboxes are automatically synchronized

When you select or deselect the Timecode checkboxes for **In Video** and **Frame Number** in any one of the Source tabs (any of the three photometry Source tabs or the Behavioral Source tab), the system automatically applies the same setting across all four video images. Thus, it is not necessary for you to manually duplicate these settings in the other device Source tabs.

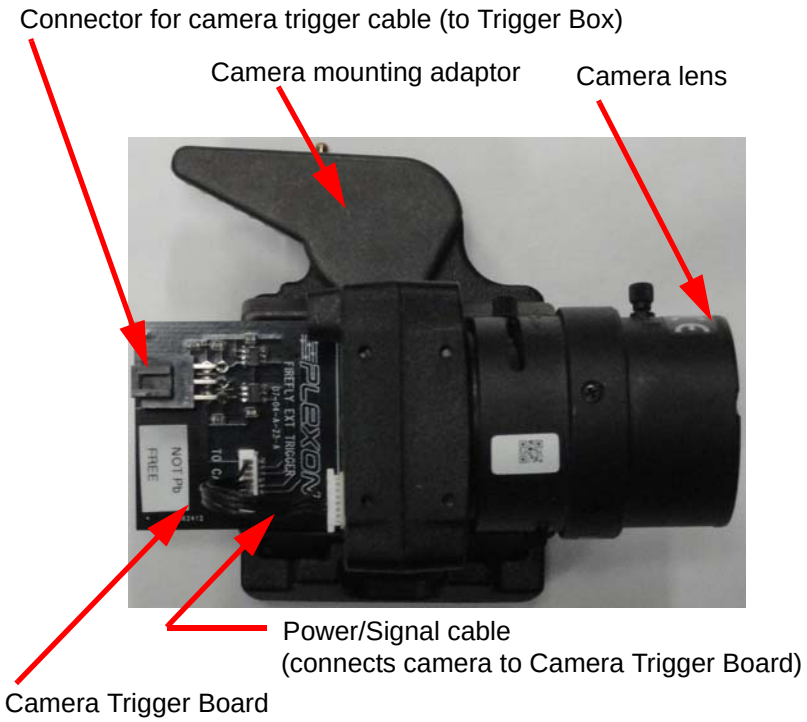
4 (Optional) If not already done, calibrate the image for the behavioral camera as explained in Chapter 6, Calibrating the Arena Dimensions. You must check the **Use Calibration** checkbox to enable the calibration function. Note that the heading in this section of the Source tab changes from “Calibration” to “Calibrated” when you select this checkbox.

Note: Once you set a calibration, it will automatically be applied to all future sessions. You can make individual session adjustments after recording.

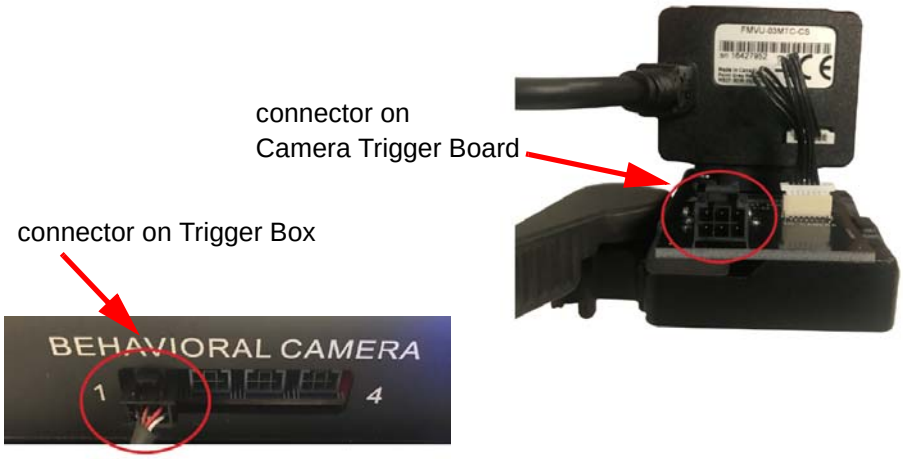


5.4 Camera Trigger Board and Trigger Cable

This section shows the connections for the Camera Trigger Board.

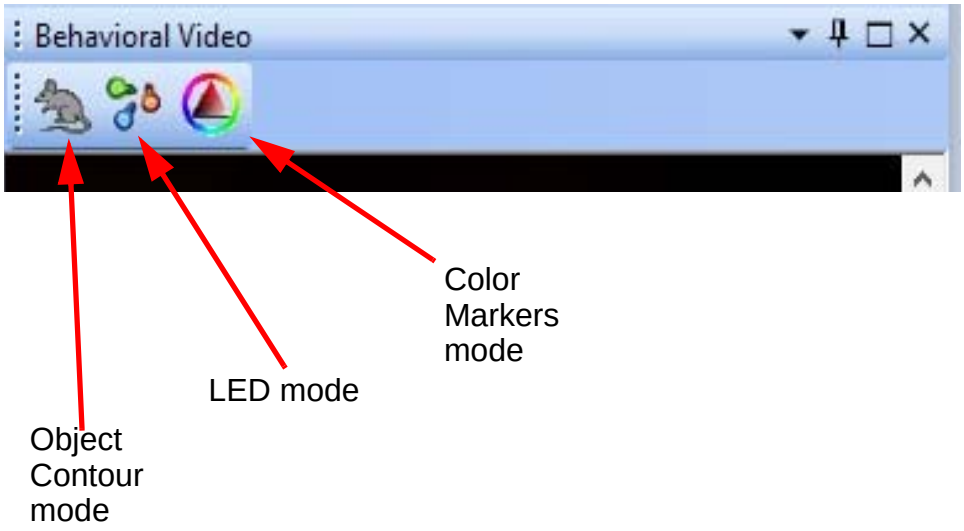


The camera trigger cable connects the Trigger Box to the Camera Trigger Board on the behavioral camera. This signal provides timing to the camera.



5.5 Toolbar Icons in the Behavioral Video Window

The image below shows a close-up view of the tracking toolbar. You can select the type of tracking to use in your experiment. For details on these different modes, see Chapter 7, Configuring the Tracking Parameters.



5.6 Where to Go Next

Go to the following chapters, as applicable, for further configuration of the behavioral camera:

- Chapter 6, Calibrating the Arena Dimensions

Note: You can calibrate the physical dimensions of the arena in centimeters or inches. If you do not calibrate the arena, the system reports physical dimensions in pixels.

- Chapter 7, Configuring the Tracking Parameters
- Chapter 8, Behavioral Features and Procedures

After you have completed the configuration of the behavioral camera, go to Chapter 9, Configuring and Viewing Global Events.

Chapter 6
Calibrating the Arena Dimensions

6.1 Before You Start

Verify that you have completed the procedures in Chapter 5, Setting Up the Behavioral Camera.

6.2 Preparing for Calibration

In many experiments, it is useful to calibrate the video image from the behavioral camera so that the ratio of centimeters to pixels (or inches to pixels) is known. This section describes how to perform the calibration procedure on an active camera.

Note: Calibration works most accurately when the camera is positioned orthogonally to the arena.

When to Perform the Calibration Procedure

For any experiment, you can perform the calibration procedure at any time, even between sessions. The system will apply the new calibration settings to all future sessions. This feature is useful if the camera is accidentally moved and you need to reposition it, or if you want to use a different method of calibration (2-bar vs. 1-bar, as described later in this chapter).

6.3 Calibration Procedure

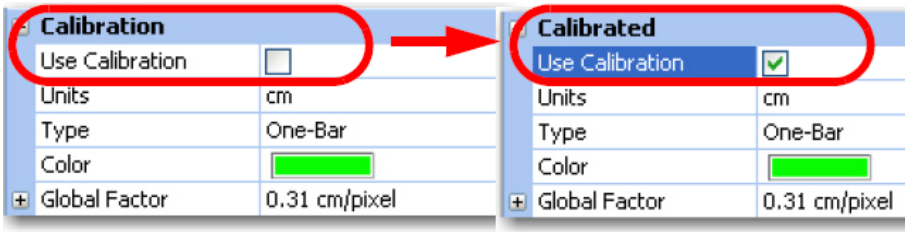
Follow these steps to calibrate the dimensions in the arena.

Selecting Calibration Units and Type

Calibration parameters are located in the lower section of the **Source** tab.

1. Open up the **Calibration** section (if it is not already open) by clicking on the "**+**" sign.

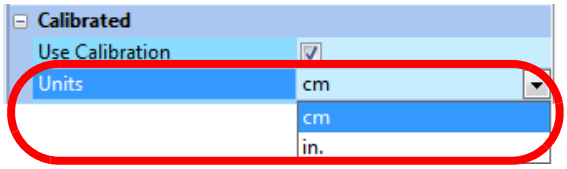
2. Check the box labeled **Use Calibration**. Note that the title of the section changes from **Calibration** to **Calibrated**. This is because the current calibration **Global Factor** is now being applied.



Note: The calibration parameters are applied only if the **Use Calibration** checkbox is checked. If the box is unchecked, the system ignores the calibration parameters.

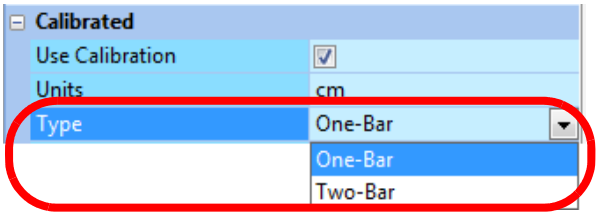
	**CAUTION** **To extract tracking and behavioral data in metric units, check "Use Calibration" before recording** If you want the tracking coordinates and behavioral event data to be extracted in metric units (not in pixels) in **Files/View Sessions As Recorded** mode, make sure **Use Calibration** is checked before you start recording.

3 Select the unit of measure (**Units**) - either cm for centimeters or in. for inches.



4 Select the method of calibration (**Type**) - either **One-Bar** or **Two-Bar**.

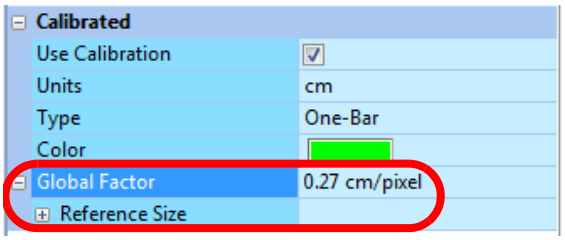
Note: There are two methods of calibration—Single axis (also called One-Bar) and dual axis (also called Two-Bar). Select the method that is consistent with the geometry of your arena.

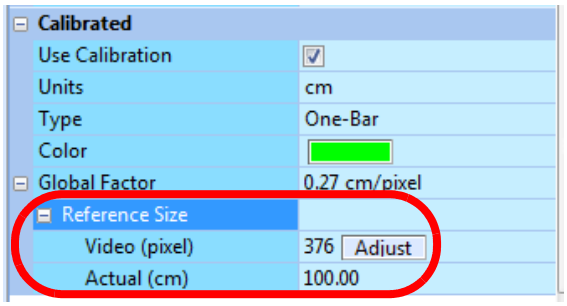


Setting Calibration Parameters with One-Bar Method

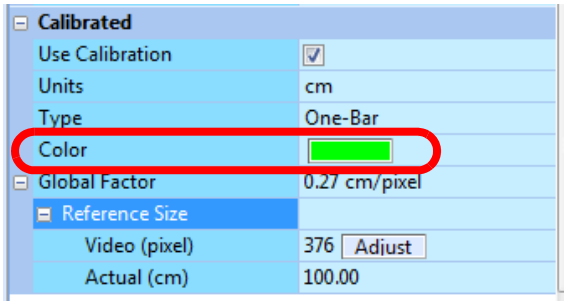
5 When **One-Bar** has been selected:

a Click the "**+**" sign next to **Global Factor** and then the "**+**" next to **Reference Size** to expand their subsections, if needed. If they are already shown as "**-**" signs, this is not needed.





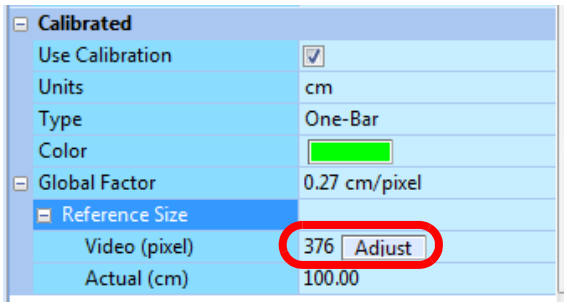
b If the current color in the box next to the **Color** item will not provide good contrast with the video image, click in the box and select a suitable color. The color selected is the color of the measurement bar used for calibration.



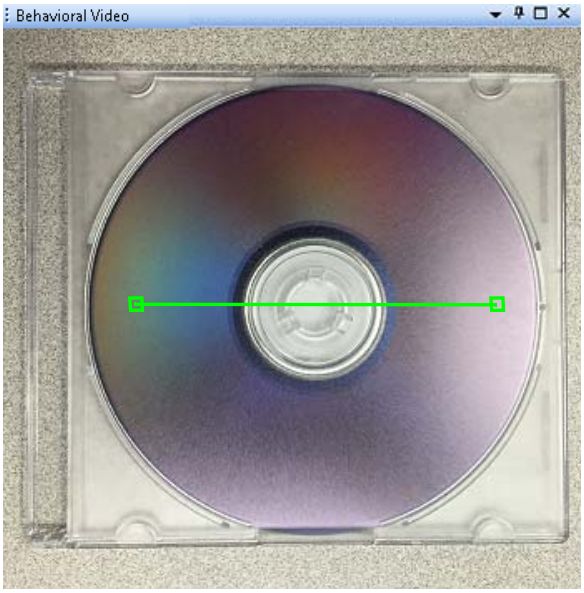
c Choose a feature in the experimental area whose video image extends over much of the field of view. As an illustration, the outside diameter of the disk in the image below will be used as the desired dimension.

d Measure its longest dimension in the units desired, and record it for reference. In this example, assume that the diameter of the disk measures 30.5 cm.

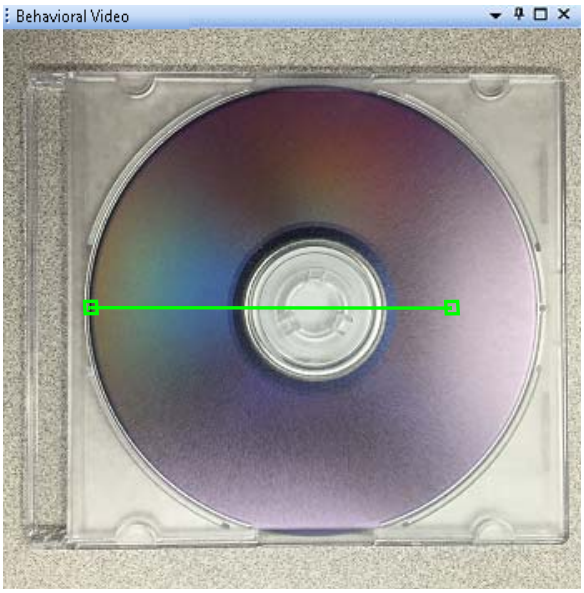
e Click the **Adjust** button. The cursor will go to the video image and a line will appear in the color selected. Select one end of the colored line and move it to one end of the feature just measured. Select the other end of the line and move it to the other end of the feature.



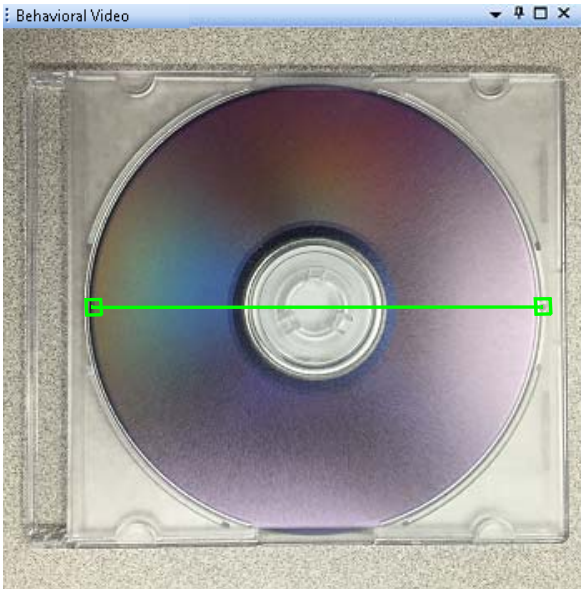
In the image above note the green line with the box on each end. This is the sizing bar. Move the sizing bar vertically to the desired location, in this case, the center of the disk.



In the next image note, that the left end of the sizing bar has been moved to the left edge of the disk.



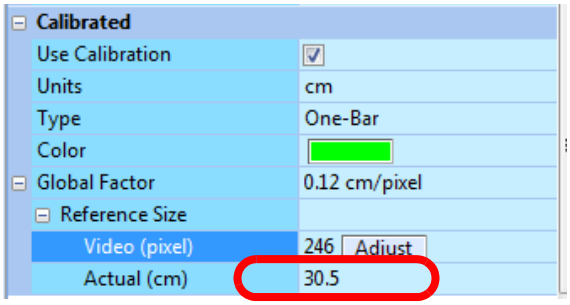
Select the right end of the sizing bar and move it to the opposite edge of the disk and ensure the sizing bar crosses the center of the disk.



In the above image note that the sizing bar now measures the diameter of the disk.

f **Right-click** to record the length of the bar in pixels.

g In the row labeled **Actual**, double click on the number and enter the actual dimension of the object under the sizing bar (30.5 cm in this example).



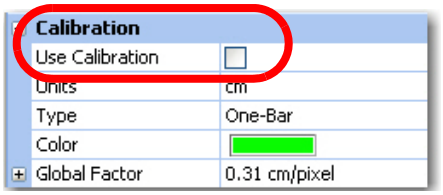
In the image above note that the **Actual** setting has been changed to the measured diameter of the disk (30.5 cm) and the system displays the new Global Factor; in this example, it is 0.12 cm/pixel, which is 30.5 / 246.

The system is now calibrated to the measured distance.

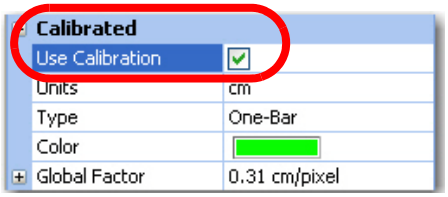
h Click all three "**-**" signs once the adjustment is satisfactory if you want to hide the adjustments.

i If you have not already done so, be sure to click the **Use Calibration** checkbox. Be aware that the positional coordinates will be extracted from the AVI file in pixels if the **Use Calibration** checkbox was not checked during recording. See the examples below.

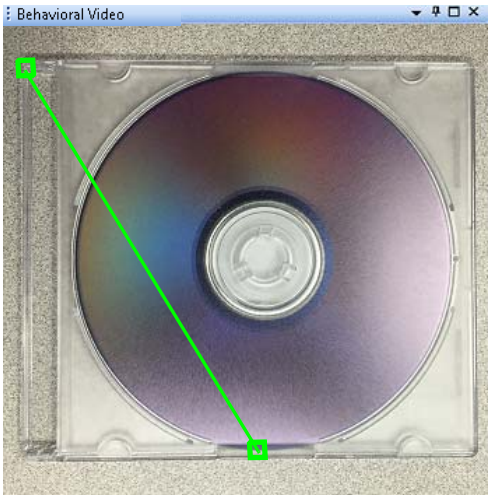
Use Calibration unchecked
when recording started -
Extracted coordinates will be in pixels



Use Calibration checked
when recording started -
Extracted coordinates will be in cm



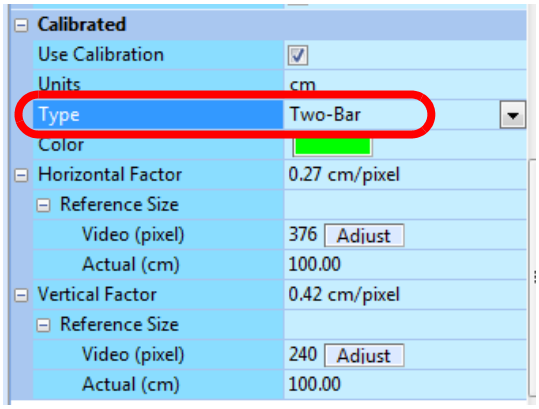
The example above assumes that the known (or most convenient) dimension on the object was oriented in the horizontal direction. In some experiments, the most convenient dimension might be in some other direction. The system allows you to orient your calibration line in any direction. For example, you could orient your line as shown below.

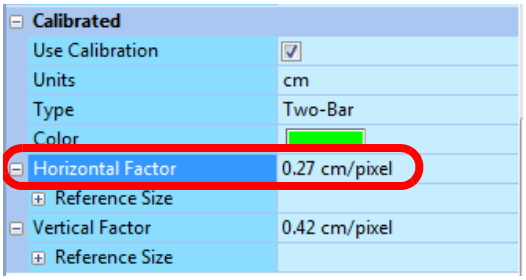


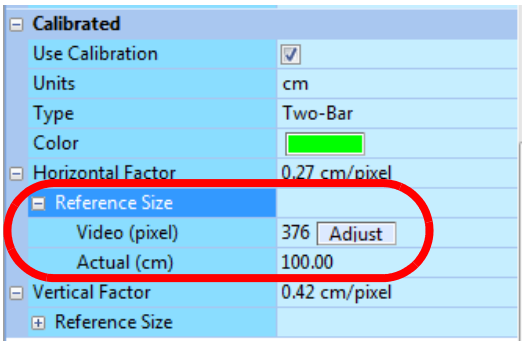
Setting Calibration Parameters with Two-Bar Method

6 When **Two-Bar** has been selected:

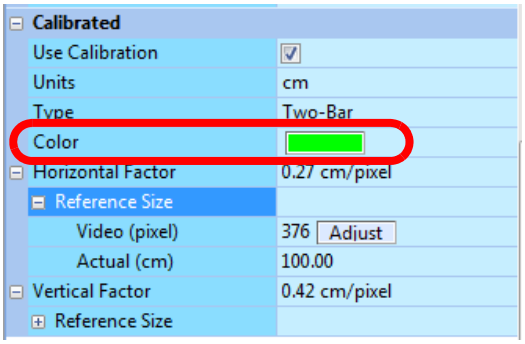
a Click the "**+**" sign next to **Horizontal Factor** and then the "**+**" next to **Reference Size** to expand their subsections, if needed. If they are already shown as "**-**" signs, this is not needed.







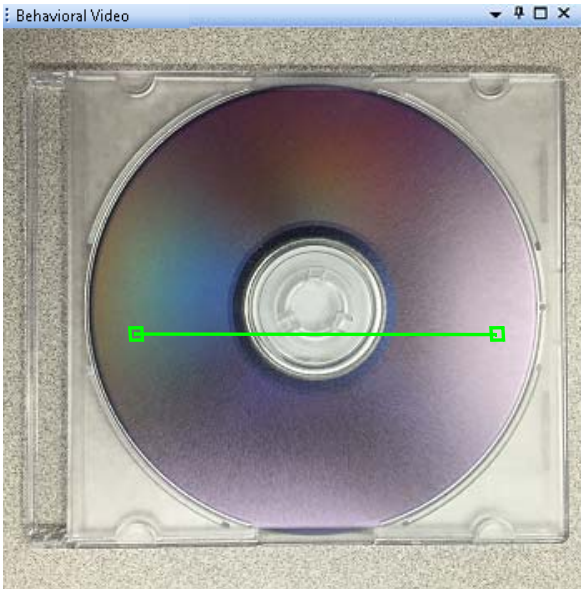
b If the current color in the box next to the **Color** item will not provide good contrast with the video image, click in the box and select a suitable color.



c Choose a horizontal feature (horizontal relative to the video image) in the experimental area whose video image extends over much of the field of view. In this example, the width of the plastic case will be used.

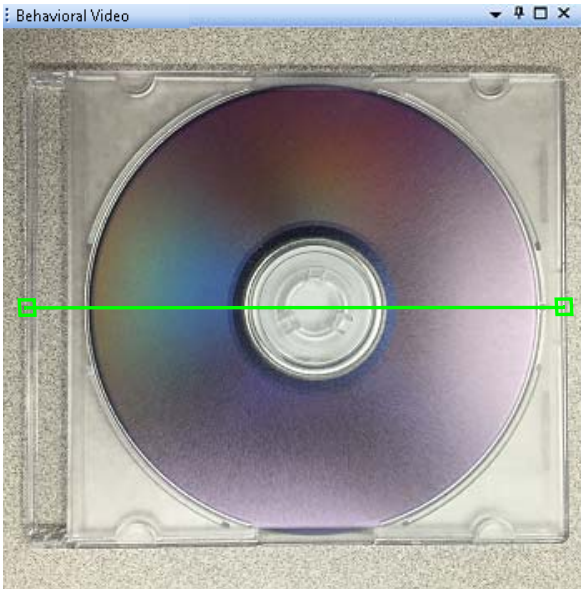
d Measure the feature's horizontal dimension in the units desired, and record it for reference. In this example, the width is 41.0 cm.

e Click the **Adjust** button in the **Horizontal** section. The cursor will go to the video image and a horizontal line will appear in the color selected.



In the image above note the green line with the box on each end. This is the sizing bar.

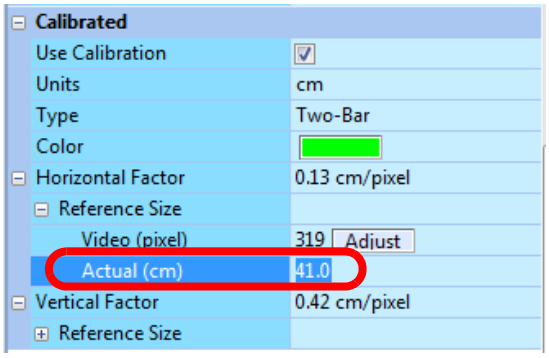
f Select the horizontal sizing bar and move it vertically so that it rests on the desired feature in the frame (in this example, it will be moved to the center of the disk). Select one end of the sizing bar and move it over the desired feature in the image. Repeat this process for the other end of the sizing bar. In this example, the ends of the sizing bar will be placed at the edges of the plastic case.



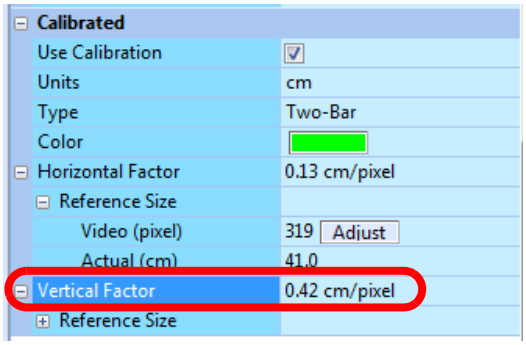
In the above image note that the sizing bar now measures the horizontal width of the plastic case.

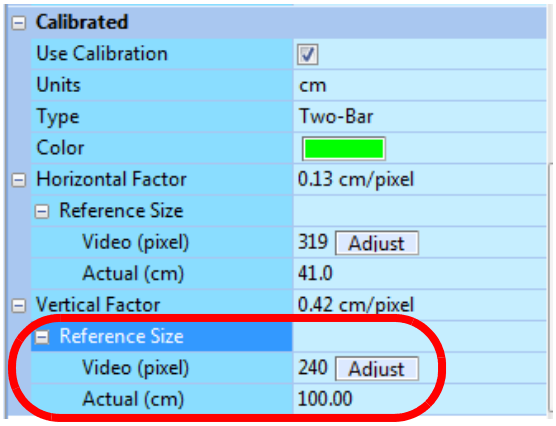
g **Right-click** to record the length of the bar in pixels.

h In the row labeled **Actual**, double click on the number and enter the actual dimension of the object under the sizing bar (41.0 cm in this example).

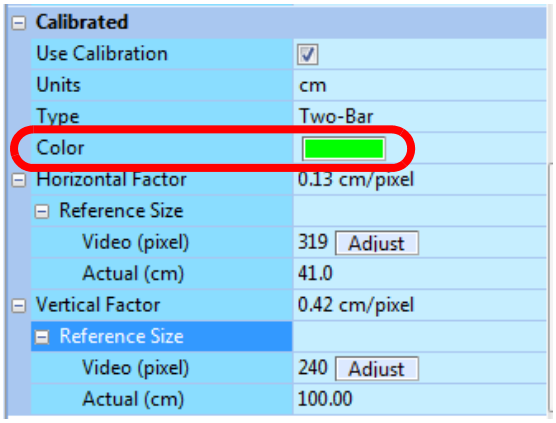


i Click the "**+**" sign next to the **Vertical Factor** and then the "**+**" next to **Reference Size** to expand their subsections, if needed. If they are already shown as "**-**" signs, this is not needed.





j If the current color in the box next to the **Color** item will not provide good contrast with the video image, click in the box and select a suitable color.



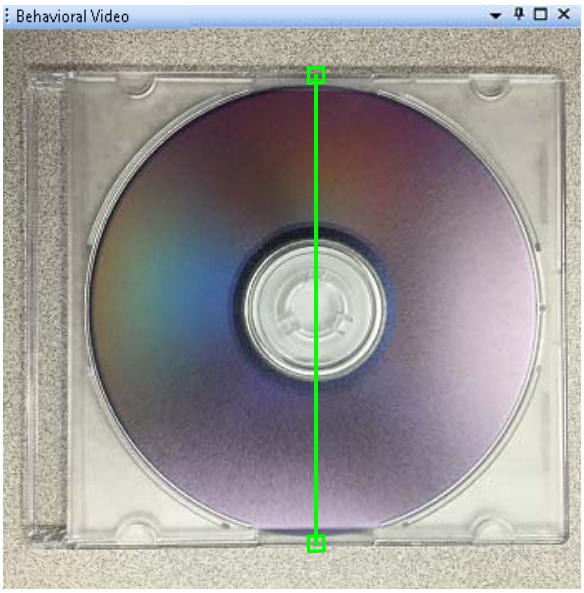
k Choose a vertical feature (vertical relative to the video image) in the experimental area whose video image extends over much of the field of view. In this example, the height of the plastic case will be measured.

l Measure the feature's vertical dimension in the units desired, and record it for reference. In this example, the height of the plastic case is 31.9 cm.

m Click the **Adjust** button in the **Vertical** section. The cursor will go to the video image and a line will appear in the color selected.

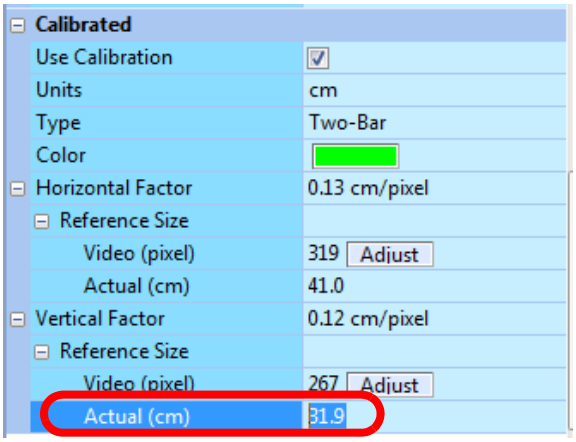
In the image above note the vertical green line with a box at each end. This line is the sizing bar.

n Select the vertical sizing bar and move it horizontally so that it rests on the desired feature in the frame (in this example, it will be moved to the center of the disk). Select one end of the sizing bar and move it over the desired feature in the image. Repeat this process for the other end of the sizing bar. In this example, the ends of the sizing bar will be placed at the edges of the plastic case.



o Right-click to record the length of the bar in pixels.

p In the row labeled **Actual**, double click on the number and enter the actual dimension of the object under the sizing bar (31.9 cm in this example).

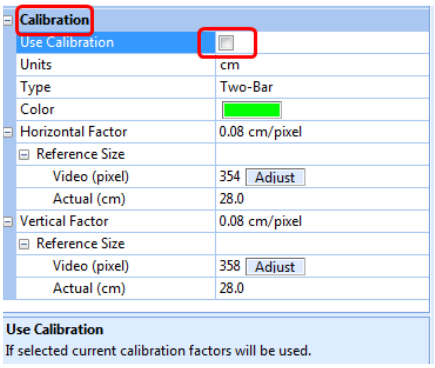


Now the system has been calibrated in both horizontal and vertical directions. Note that the horizontal and vertical factors are slightly different in this example. This situation typically occurs if the camera is not orthogonal to the plane of the object that was measured during the calibration. The system uses the horizontal and vertical factors to provide a more accurate calibration (versus a one-bar calibration).

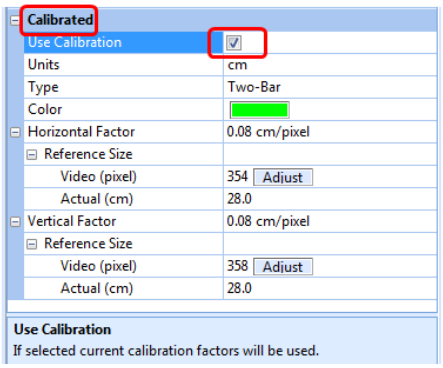
q Click all five "**-**" signs once the adjustments are satisfactory if you want to hide the adjustments.

r If you have not already done so, be sure to click the **Use Calibration** checkbox. Be aware that the positional coordinates will be extracted from the AVI file in pixels if the **Use Calibration** checkbox was not checked during recording. See the examples below.

Use Calibration unchecked
when recording started -
Extracted coordinates will be in pixels



Use Calibration checked
when recording started -
Extracted coordinates will be in cm



6.4 Modifying the Arena and Calibration During an Experiment

The system provides some flexibility in modifying the behavioral arena and calibration parameters in an experiment that already has one or more sessions recorded.
For a description of these options and procedures, see Appendix D, Modifying Arenas and Zones.

6.5 Where to Go Next

Go to the following chapters, as applicable, for further configuration of the behavioral camera:

- Chapter 7, Configuring the Tracking Parameters
- Chapter 8, Behavioral Features and Procedures

After you have completed the configuration of the behavioral camera, go to Chapter 9, Configuring and Viewing Global Events.

Chapter 7
Configuring the Tracking Parameters

7.1 Before You Start

Verify that you have completed the procedures in Chapter 5, Setting Up the Behavioral Camera.

7.2 Introduction to the Tracking Feature

This chapter describes the tracking functions, the experimental applications for which it can be used, and how to use it. The tracking function enables the computation and recording of positional data.

The list below is a summary of the procedures for using the tracking function. The remainder of this chapter provides the detailed procedures:

1. Position the behavioral camera so that the desired physical experiment area is within the field of view of the camera.
2. Set the recording location (directory path) for the Experiment folders that will contain the recorded files.
3. Create a new Experiment folder or select an existing one.
4. Select the tracking mode to be used—**Object Contour**, **LEDs** or **Color Markers**.

IMPORTANT

Once you select a tracking mode for an experiment and record your first session, you cannot change the tracking mode. Furthermore, if you do not select any tracking mode, then you record your first session, you cannot select any tracking mode for that experiment in the future. This restriction ensures the data structure for the experiment will be consistent across all sessions. See Section 3.12, “Ensuring Consistent Parameter Settings in an Experiment” on page 51.

In LED or Color Markers tracking—After you set the tracking mode and configure the tracked objects for an experiment, and then record your first session, you cannot change which objects will be tracked, and you cannot change the setting for the Animals in Area parameter in the Global Config tab. This ensures consistency for all sessions in the experiment. (You can change some of the display options for the tracked objects, but not the number of objects.)

5. Set the parameters in the **Global Config** tab
6. Verify (or set) the basic parameters for the recording (including the AVI video file)
7. Perform a calibration of the dimensions in the arena (See Chapter 6, Calibrating the Arena Dimensions)
8. Define the shape of the arena for the behavioral camera.

9 Set the parameters for the selected tracking mode

10 (If your experiment includes behavioral analysis) Set the parameters for behavioral events as described in Chapter 8, Behavioral Features and Procedures

11 (If your experiment includes photometry) Set the parameters for photometry as described in Chapter 4, Photometry Features and Procedures.

12 Start recording

13 Stop recording

The system default window layout is suitable for most instances. However, to load, change or save a window layout, see Section 2.5, “Navigating the User Interface” on page 26. Additional information is available in Appendix B-Navigating the Plexon User Interface.

7.3 Setting Up the Behavioral Camera and the User Interface

For accurate tracking, you should ensure that the ratio of the object size to the video image size is such that the system will be able to track the object. If the ratio is too small (if the size of the object is less than a few pixels) the system will not be able to distinguish the object from noise. If the ratio is too large (more than 1/4 of the video image) the system will not track the object. A warning message will pop up if this is the case.

The procedure for setting up the camera is described in Chapter 5, Setting Up the Behavioral Camera.

7.4 Creating or Selecting an Experiment

To create a new experiment or select a previously saved experiment, see these procedures, as applicable:

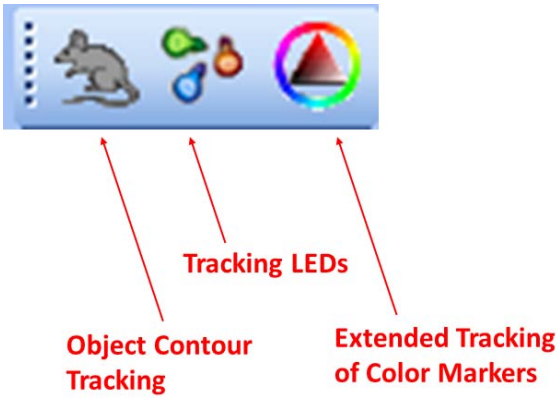
- Section 3.1, “Data Storage and Organization” on page 30
- Section 3.2, “AVI Video Format, Data Rate, Timestamps and Compression” on page 31
- Section 3.3, “Planning the Database” on page 32
- Section 3.4, “Setting Parameters for an Experiment with Multiple Sessions” on page 33
- Section 3.5, “Setting the Recording Folder Location” on page 35
- Section 3.6, “Creating a New Experiment” on page 36
- Section 3.7, “Selecting a Previously Saved Experiment” on page 44
- Section 3.8, “Editing the Experiment Name, Descriptor and Variable Values” on page 45

7.5 Selecting the Tracking Mode and Tracking Settings

There are a number of parameters that need to be configured to enable and manage tracking functions. This section assists you in selecting the tracking mode to use for the experiment. It also explains the purpose of the **Threshold** setting, which is common to all tracking modes. See the following subsections.

7.5.1 Understanding the Tracking Mode Options

The Tracking toolbar (shown below) allows you to select the desired tracking mode.When one of the tracking modes is selected, the system tracks the position of subject(s) in the video images in real time.



The tracking modes are listed below.

Note: Only one tracking mode can be active at a time. Tracking modes cannot be combined.

Note: After you set the tracking mode for an Experiment, and then record your first session, you cannot change it. This ensures that the same tracking mode is used for all sessions in the Experiment.

- **Object Contour Tracking**: In this mode, the system analyzes the image to find a whole-body shape that corresponds to the desired object, and then computes (and tracks) the center of gravity of the shape.
- **Tracking LEDs**: In this mode, the system tracks up to three light emitting diodes (LEDs) on the subject or subjects being tracked.
- **Extended Tracking of Color Markers**: In this mode, the system tracks up to 12 colors (colored tape or paint, for example) on the subject or subjects being

tracked. The actual number of trackable colors depends on the colors themselves, their relative sizes, and the lighting within the arena.

Note: This mode is also referred to simply as **Color Markers** in this user guide.

7.5.2 Guidelines for Selecting the Tracking Mode

This section assists you in selecting the tracking mode to use for the experiment, and optimizing tracking parameters to suit the needs of the experiment.

The table below provides an overview of the recommended tracking mode to use under various conditions.

Tracking Device to be Used on the Animal	Are LEDs the Brightest Spots in Arena?	Recommended Tracking Mode to Use
No LEDs. No colored markers	Not Applicable	Object Contour (See Section 7.12, “Object Contour Tracking” on page 149)
LEDs	Yes	LED (See Section 7.13, “LEDs In Darkness with LED Tracking” on page 161) or Color Markers (See Section 7.14, “LEDs In Darkness or Light with Color Markers Tracking” on page 168 and Section 7.15, “Color Markers Tracking” on page 169)
LEDs	No	Color Markers (See Section 7.14, “LEDs In Darkness or Light with Color Markers Tracking” on page 168 and Section 7.15, “Color Markers Tracking” on page 169)
Colored markers	Not Applicable	Color Markers (See Section 7.15, “Color Markers Tracking” on page 169)

7.5.3 Additional Guidelines for Tracking

In general, for a given experimental setup, only one tracking mode is optimal. The following guidelines help to determine which tracking mode to use.

- In general, the best tracking results occur when light-emitting diodes (LEDs) are attached to the animal. This is due to:
  - The relatively small size of the objects being tracked
  - The high intensity of colors against a dark or partially lit background
- The easiest way to use LEDs is to mount them directly on the animal. This is best done with a small LED/battery assembly strapped or glued to the animal's head or back, in a glove or sleeve, or on an object connected to the animal.
- To track not only position but orientation, use two or three LEDs.
- For LED tracking, the system finds the brightest spots on the image, and determines their positions in the color space. Therefore, LED tracking mode is recommended when the LEDs are the brightest spots on the image and the rest of the image is dark. Check the **Pure Colors** option to cause the system to recognize Plexon® standard red, green, and blue LED colors automatically. Otherwise, select the colors to track.
- Object Contour mode can only track the "center of gravity" of one animal's contour. Therefore, Object Contour mode cannot be used with LEDs or multiple animals.
- If the animals are multicolored, especially with high-contrast colors (for example, Long-Evans rats), Object Contour mode works best with a contrasting background color, such as "salmon pink." Good contrast makes it easier for the system to track the whole body of the animal. It may be necessary to experiment to determine the best background color in individual situations.
- Object Contour mode is more sensitive to changes in background and lighting conditions compared to the other tracking modes.
- In general, more effort is required to configure Object Contour mode to obtain optimal tracking results than is required for either LED or Color Markers modes. This is because slight variations in background and lighting can cause the animal's contour to vary slightly, even if the animal is not moving.

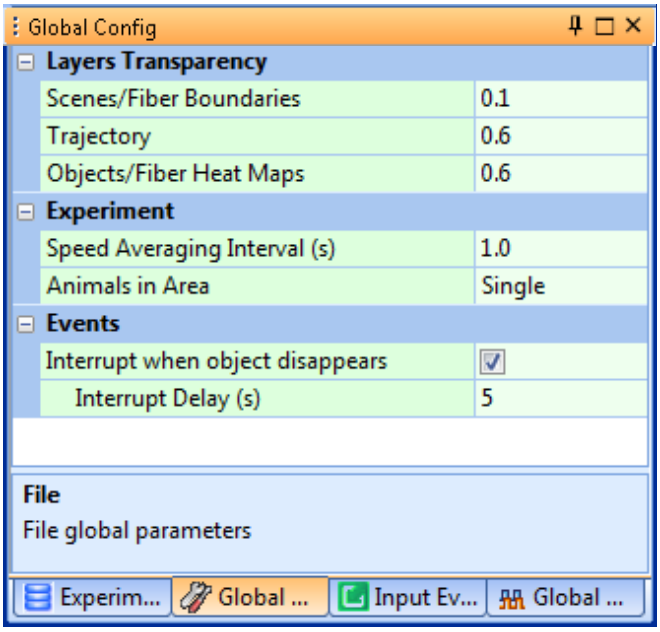
7.5.4 Understanding the Threshold Setting

The **Object Contour** and **LED** tracking modes have a user-configurable **Threshold** parameter that is used in the calculation the system performs to locate and track an object. The result of the calculation depends on [1] the contrast between the tracked object and the background, and [2] the **Threshold** value set by the user. For a general understanding of how the **Threshold** value affects tracking, see Section 7.12.2, “Setting the Threshold” on page 151. That section presents the **Threshold** concept from the point of view of **Object Contour** tracking mode, but the discussion applies to LED tracking mode also.

7.6 Setting Parameters In the Global Config Pane (Tracking Enabled)

If you have selected one of the Tracking modes, you will see additional parameters displayed in the **Global Config** pane. See the image below.

- **Layers Transparency**. This group of settings specifies the transparency of objects displayed over the captured image in the video windows. Each of the transparency is adjustable from 0.0 (opaque) to 0.9 (90% transparent). The three transparency options are as follows:
  - — **Scenes/Fiber Boundaries**—Transparency for user-specified shapes, specifically, arena and zones for the behavioral video, and fiber boundaries for the photometry videos.
  - — **Trajectory**—Transparency for the tracked path of the animal during the session.
  - — **Objects/Fiber Heat Maps**— Transparency for all object layers (center of gravity, tracking window, contour and body) for the behavioral video, and fiber heat maps for the photometry videos.



- **Speed Averaging interval (s)**. This setting specifies the size of the sliding time window, (0 to 1.0 seconds) that the system uses to average the speed values. If this parameter is set to 0 (the default value), the system reports the instantaneous speed value for each video frame.
- **Animals in Area**. This setting specifies whether a single or multiple animal(s) are being tracked; it has dropdown list with the options **Single** and **Multiple**. Use **Single** when all color markers or LEDs are located on the same animal. Use **Multiple** when each marker identifies a different animal, as might be used for social tracking. The **Animals in Area** setting does not

appear in **Object Contour** (whole body) tracking mode because there is only one animal being tracked.

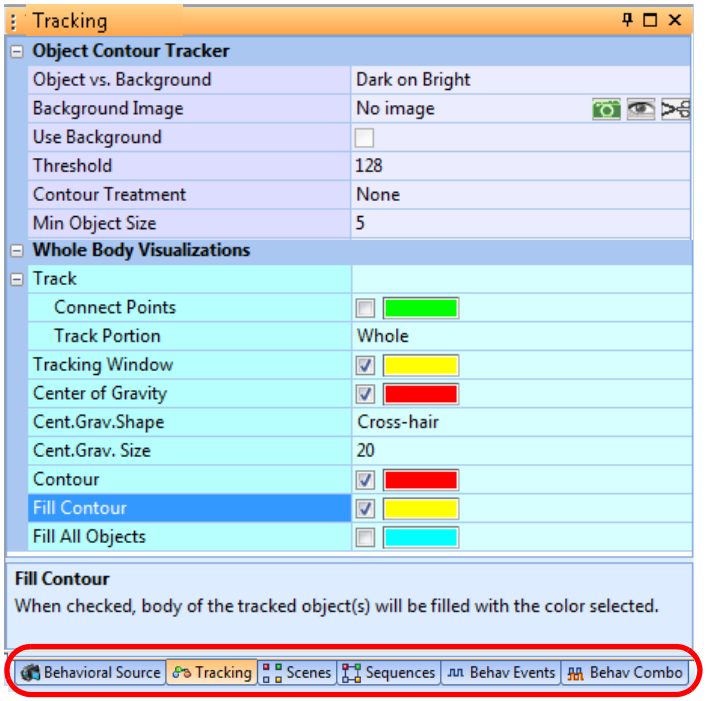
- **Events—Interrupt when object disappears**. This setting specifies how you want the system to react when the object disappears (is not being tracked). If you check this checkbox, the system will not record any events related to tracked objects that have disappeared (are not currently being tracked). If the object reappears and the system begins tracking it again, and events related to the object can once again be recorded.
- **Interrupt Delay (s)**. When **Interrupt when object disappears** is selected, the **Interrupt Delay** defines the waiting time, in seconds, before events will be interrupted after tracking stops finding the object.

7.7 Calibrating the Arena Dimensions

In most experiments, it is useful to calibrate the video images from the behavioral camera so the ratio of centimeters to pixels (or inches to pixels) is known. This allows the system to report animal locations and speed in physically meaningful units. For the procedure, see Chapter 6, Calibrating the Arena Dimensions. Once you set a calibration, it will automatically be applied to all future sessions. You can make individual session adjustments after recording.

7.8 Recording Parameters—Source, Tracking and Scenes

Each video window has an associated set of parameters. The parameters are accessed by clicking the appropriate tabs (see the image below).



- **Source**—Used to set parameters for the video stream, either from camera or from existing files. This tab contains parameters that relate to optical settings for the behavioral camera (such as gain, brightness, white balance and shutter), labeling/timestamping of frames and calibration.
- **Tracking**—Used to set parameters associated with the specific type of tracking that has been chosen (**Object Contour**, **LEDs** or **Color Markers** mode). The image above is the Tracking tab applicable to the **Object Contour** tracking mode.
- **Scenes**—Used to set the shape of the experimental arena so the system can ignore any parts of the image that are outside the user-designated area of interest for the experiment.

Note: The **Sequences**, **Events** and **Behav Combo** tabs contain parameters applicable to the behavioral features. See Chapter 8, Behavioral Features and Procedures

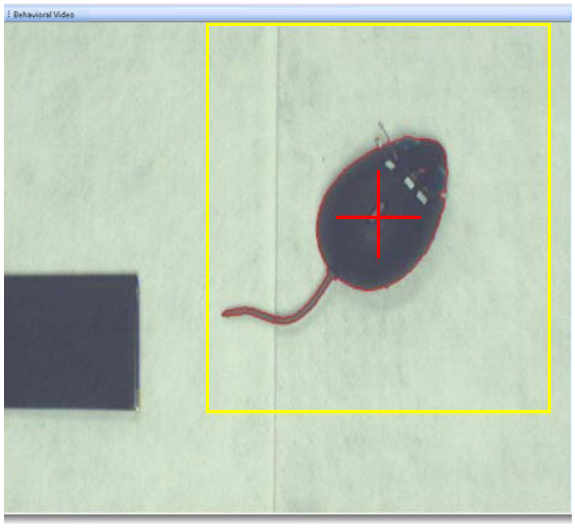
7.9 Understanding Tracking Windows and Arenas

This section explains how the system manages the video processing load by using system-generated tracking windows and user-defined arenas.

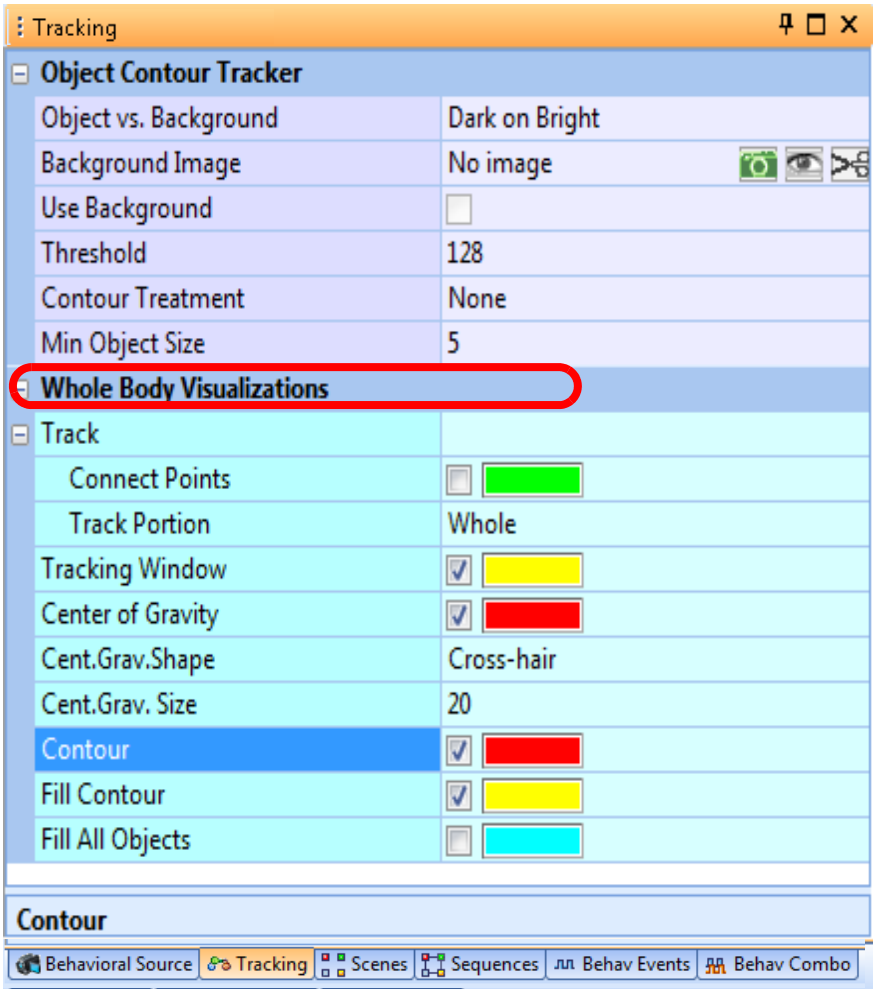
7.9.1 System-generated Tracking Windows

The system generates tracking windows to limit the processing and analysis of video data to a small portion of the video image, typically to the body contour of the animal or the color markers or LEDs mounted on the animal. This reduces the overall CPU load and allows tracking more colors. Tracking windows also reduce problems with unwanted parasite objects (for example, reflections).

Tracking windows are displayed as rectangles in the video display, as shown in the example below (the yellow rectangle). You can change the colors of the rectangle and crosshairs (and show or hide the rectangle and crosshairs) with the **Visualizations** parameters in any of the tracking modes, as described later in this chapter.



Note: In **Object Contour** mode, the area will be labeled **Whole Body Visualizations**. In **LED** mode, it is labeled **LED Visualizations**, and in **Color Markers** mode it is **Marker Visualizations**.



The system automatically sets the size of the tracking window to cover the whole object. Each object (whole animal, LED, or marker) has its own tracking window. The tracking window is repositioned automatically. A history of an object's movement is used to predict its next position. If the object disappears because of occlusion by a cable or other means, its tracking window increases in size. If the system is tracking multiple LEDs or markers in single animal mode, the system will use the positions of the found LEDs or markers to predict the position of the one that disappeared. If the object is not found after a certain number of frames, the size of the tracking window will be increased. If the object is not found within two seconds, the system switches to search in the whole frame (or within the arena, if an arena shape has been defined).

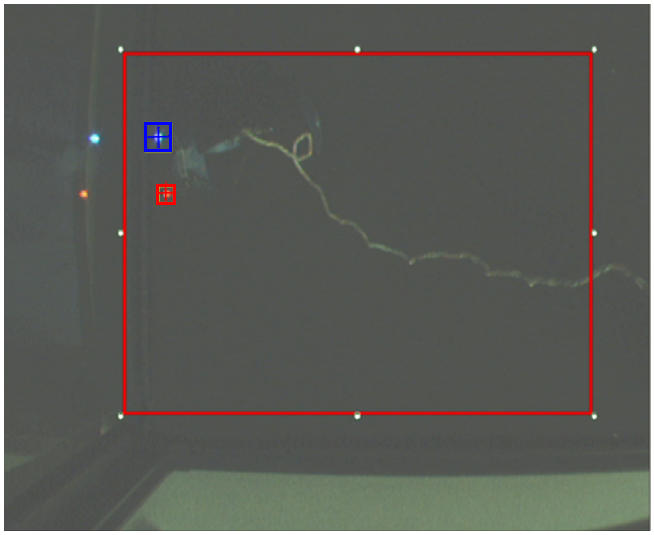
7.9.2 User-defined Arenas

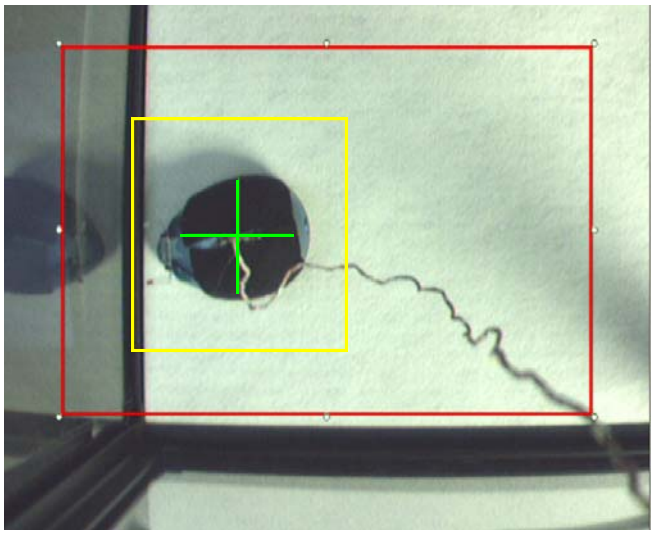
You can define an arena (or area of interest) for the behavioral camera image. The arena reduces problems with unwanted reflections and parasite objects outside of the working area.

Using the tools provided by the system, you can draw an outline of the experimental arena over the video image. Once this outline is drawn, the system ignores objects outside of the arena when processing the images. This results in lower CPU usage and reduces false object detections due to reflections and shadows. It also allows the experimenter to exclude areas of the image that have no relevance to the experiment. Arena tools allow drawing circles, ellipses, rectangles, and freehand objects. Arena operators (Union shapes, Intersect shapes, Subtract shapes, XOR shapes) allow combining multiple arena shapes to produce a single complex arena shape.

Example - Using Arenas to Exclude Reflections

The following drawings show two examples of arenas (red rectangles) - one for LED tracking mode and one for Object Contour tracking mode. In each case, the reflections outside the arena are ignored by the system.

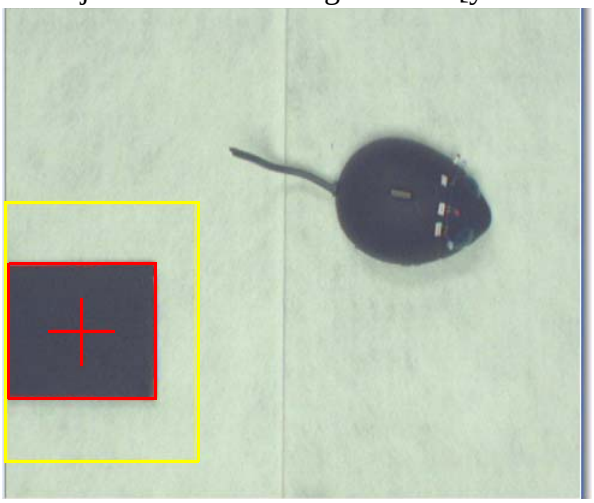




Example - Using Arenas to Exclude Background Objects or Colors

If a background object (or background color) might interfere with the tracking function, the object (or color) can be removed from the arena. This will allow the system to track the animal correctly.

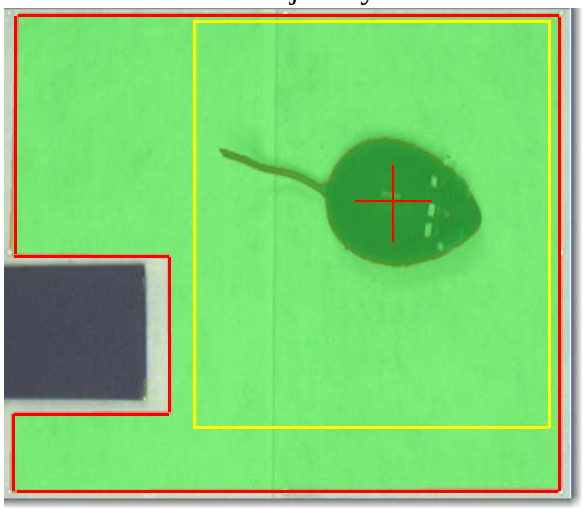
This image shows an animal to be tracked along with a background object of approximately the same color. The background object is larger than the animal and the system is tracking the background object (the red cross hair is centered on the object and the tracking window [yellow rectangle] is surrounding the object).



To avoid this situation, the experimenter can use one or both of the following tools:

- Define a specific arena shape in which to track
- Use background subtraction (discussed in Section 7.12.5, “Background Subtraction” on page 156)

This image shows a defined arena (in green) that the system will use to track the animal. The arena excludes the background object, therefore the system will never lock onto the object by mistake.



Modifying Arenas In Offline Mode

If you transfer an AVI file to an offline analysis computer on which the Plexon® photometry software is installed, that file can be retracked with a new or changed arena.

Note: The standalone computer must have minimum memory, processor and instruction set to run software efficiently. The Plexon "PHT + BEHV" license must be plugged into the computer.

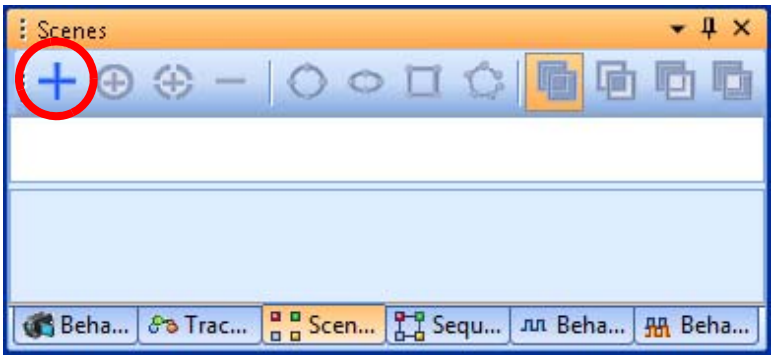
It is also important to determine that the computer on which the installation will be done meets the minimum system requirements. The requirements are summarized in Section 1.8, "Computer to Run Photometry Software" on page 11.
Requirements that are more current are available from Plexon Support (support@plexon.com).

7.10 Defining the Arenas

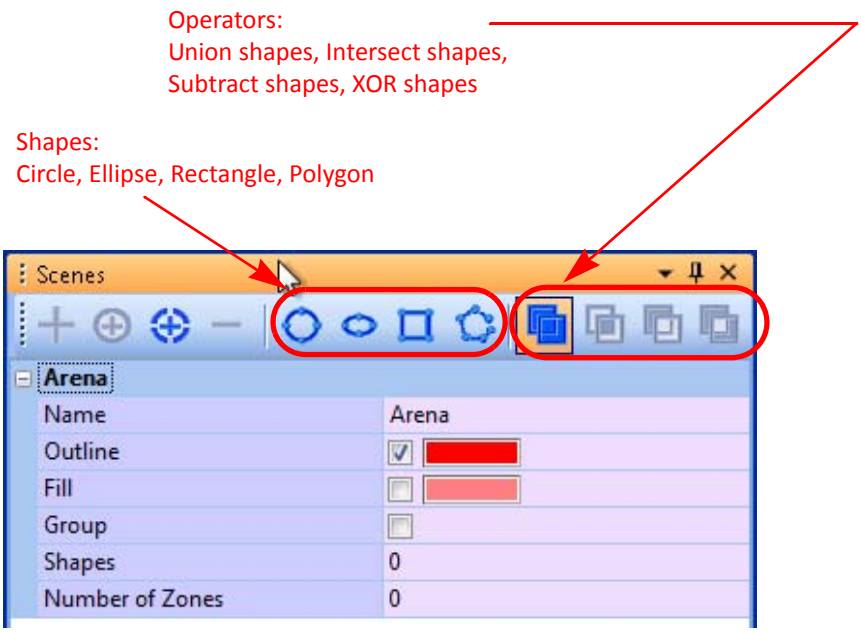
This section explains how to define an arena.

Adding the Arena

1 In the Scenes tab, click the **Add arena (+)** icon.

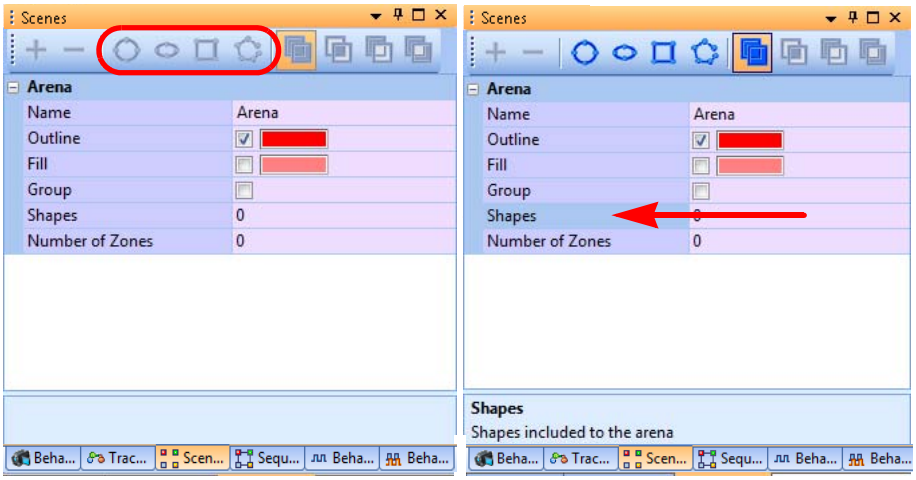


A Scenes settings tab is displayed, and the toolbar now displays active icons for shapes and operators.



2 In some situations, typically when you return to an existing file in Files mode, the **Shapes** icons might appear inactive (grayed out). In that case, simply

click on the **Shapes** row and the **Shapes** icons will be active again. See the images below.



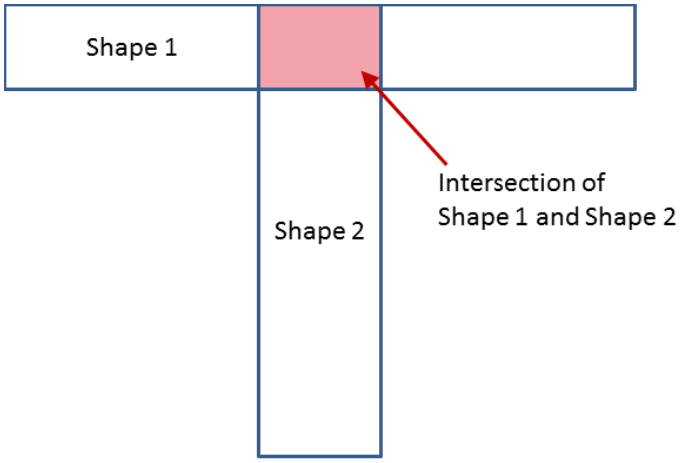
3 One or more shapes can be added to the arena. To draw a shape, click the desired shape on the toolbar (**Circle**, **Ellipse**, **Rectangle**, or **Polygon**). For a circle, ellipse or rectangle, left-click and hold the mouse on a desired point on the image and move the mouse to size the arena.

Note: For a freehand polygon, click the left mouse button over desired points that will be the nodes of the polygon, and use a right-click to close the polygon. The shape can be moved or resized by left-clicking and dragging.

4 Multiple shapes can be drawn and logically combined to create an arena with a complex shape. After the first shape is drawn, draw a second shape as follows:

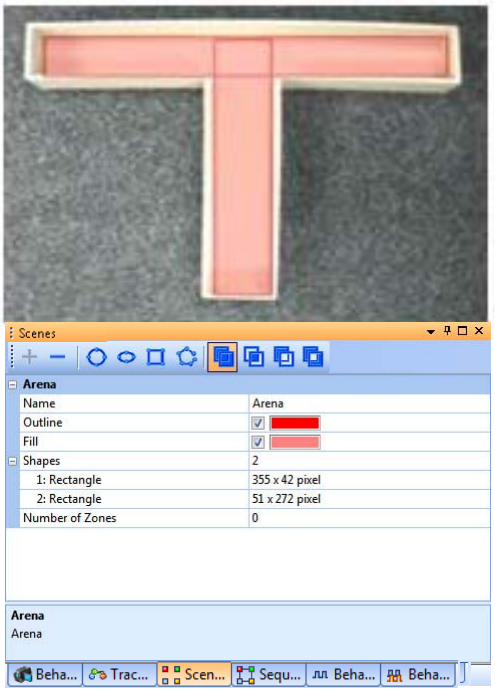
- Click on the operator that will combine the first and second shape.
- Then click on the icon for the second shape and draw the second shape.
- To view the combined shape more clearly, click on the **Fill** checkbox to select it.

The example below shows how to combine shapes. The process begins with the definition of the first shape, then the selection of the operator and then the definition of the second shape. In this example, Shape 1 is a horizontally oriented rectangle and Shape 2 is a vertically oriented rectangle. For example, if the **Intersect shapes** operator is selected, the result is the shaded area where the two shapes overlap as shown in the illustration below.

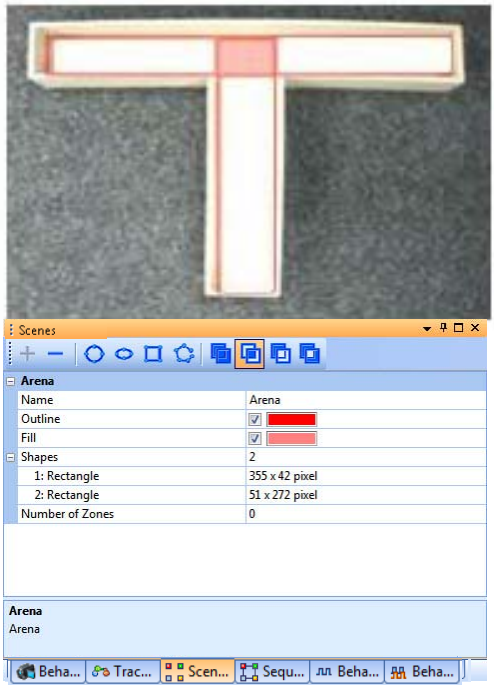

Shape 1
Shape 2
Intersection of
Shape 1 and Shape 2

The images below show examples of combined arena shapes. Tracking will only occur in the shaded regions.

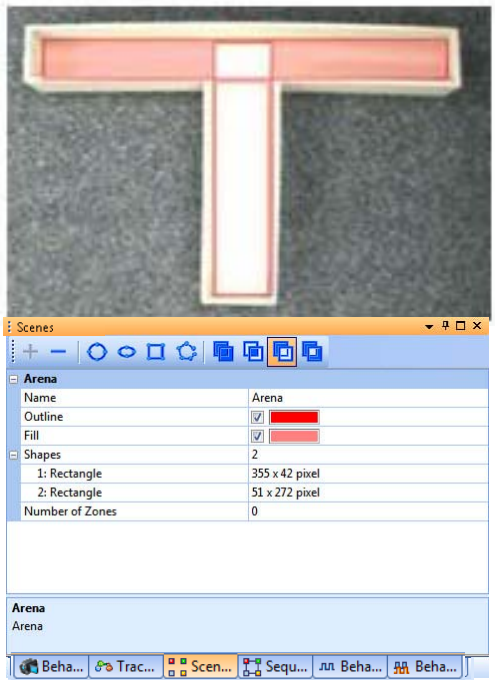
Union shapes



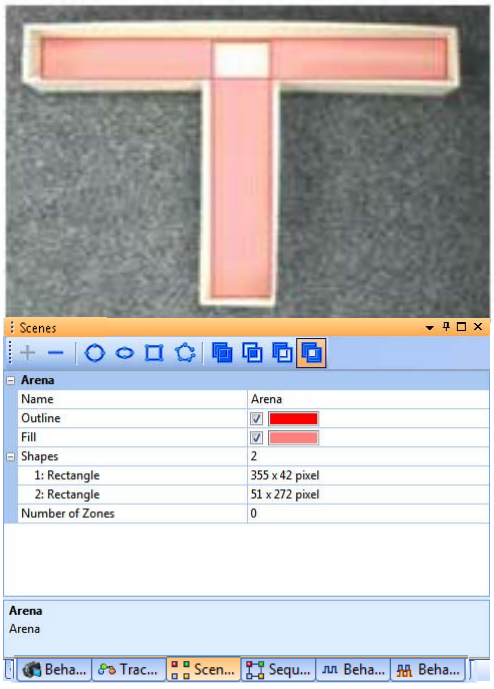
Intersect shapes



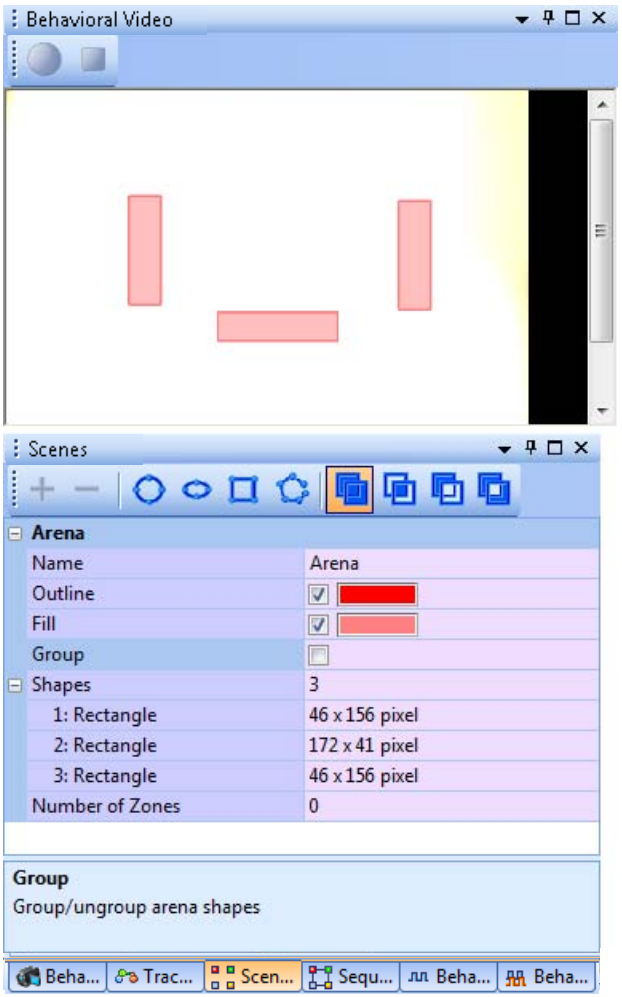
Subtract shapes



XOR shapes



Arenas drawn from several shapes do not need to be contiguous (as was the case in the examples above). The image below shows an arena that contains noncontiguous shapes. Tracking will only occur in the shaded regions.

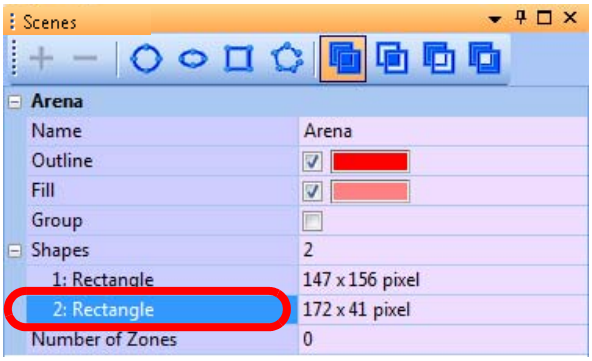


5 If desired, use the operator and shape icons multiple times to add and join several shapes to accurately define the arena.

6 After you have finished drawing the arena areas, you can check the **Group** checkbox (see the image above) to combine all of the shapes into a single object. This allows you to move the entire combined arena object as a unit.

7 To delete a shape, select the desired shape in the Arena area of the Source tab (or the desired shape in the Video window) and press the **Delete** key on

the computer keyboard. In the images below note that the rectangle is selected for deletion.



Note: The system provides some flexibility in modifying arenas in an experiment that already has one or more sessions recorded. For a description of these options and procedures, see these sections:
— Section 11.13, “Using the Overlay Feature during Analysis” on page 290
— Appendix D, Modifying Arenas and Zones

7.11 Tracking Parameters - List of Configuration Procedures

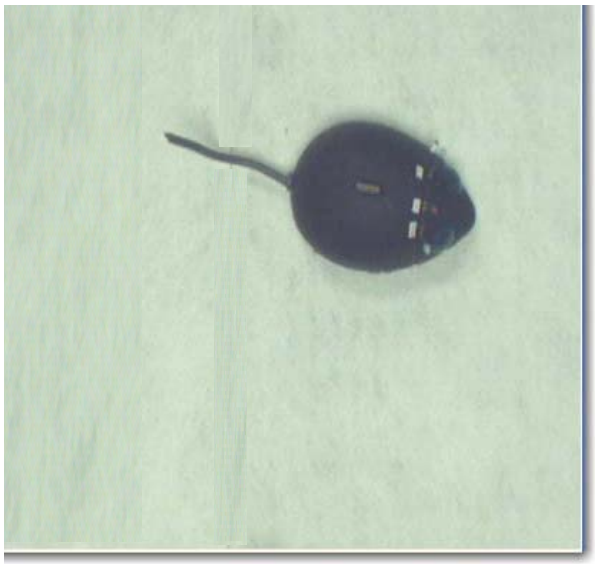
The following sections contain procedures that configure the tracking parameters for specific experimental applications. Select the appropriate procedure for your experiment:

- Section 7.12, “Object Contour Tracking” on page 149
- Section 7.13, “LEDs In Darkness with LED Tracking” on page 161
- Section 7.14, “LEDs In Darkness or Light with Color Markers Tracking” on page 168
- Section 7.15, “Color Markers Tracking” on page 169

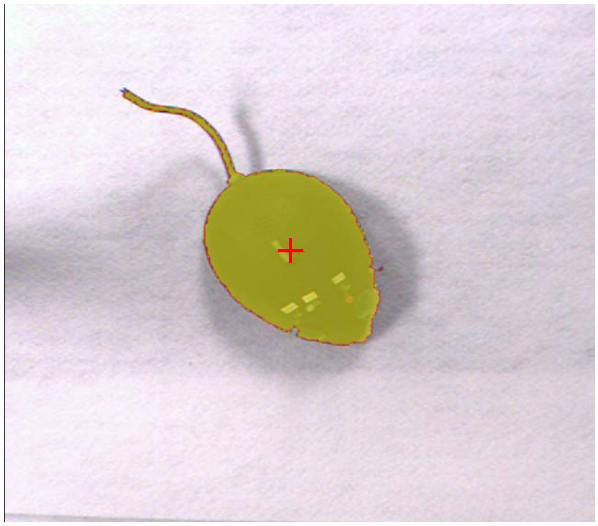
7.12 Object Contour Tracking

Object Contour tracking records coordinate data for the movement of an animal within an experimental arena without requiring LEDs or color markers to be attached to the animal.

In Object Contour tracking mode, the system compares the color of the animal with the background color and any background objects. The image below shows a dark colored animal to be tracked on a light colored background and without any background object in the window. The background's light color is uniform and provides a good contrast to the animal's color.



The image below shows an example of a well defined animal contour generated by the system.

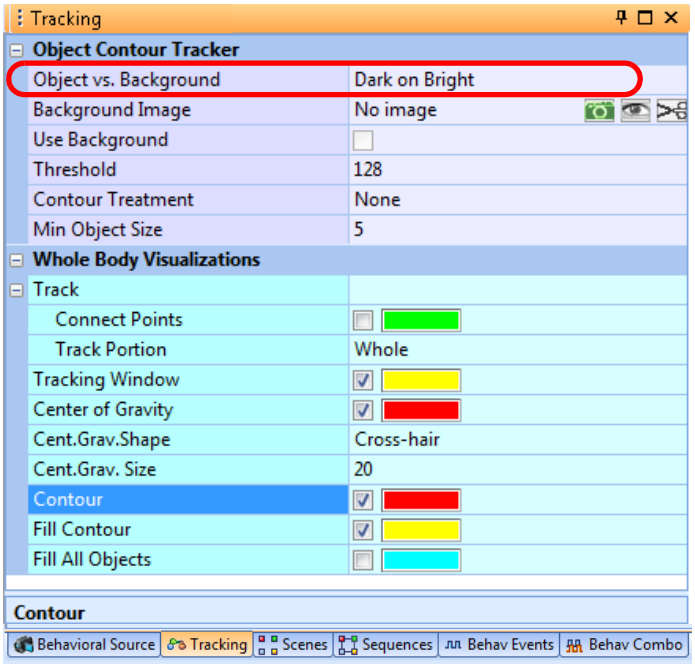


7.12.1 Configuring Parameters for Object Contour Tracking

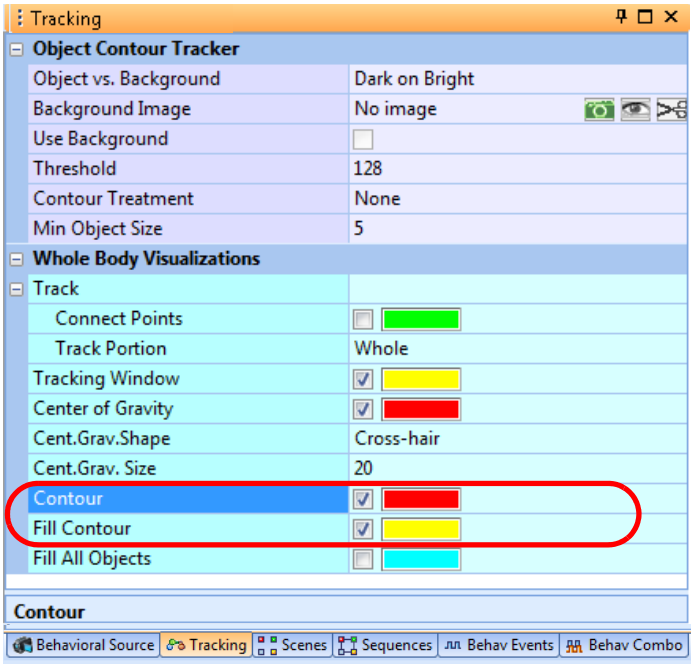
Perform these steps to select and configure Object Contour tracking.

1. Place the animal in the camera field of view.
2. At the camera lens, adjust the zoom, focus, and iris settings for the best picture quality. For additional guidance on camera setup and video quality, see Chapter 5, Setting Up the Behavioral Camera.
3. Click the Tracking tab to display the tracking parameters.
4. To select the Object Contour tracking mode, click the appropriate icon on the Tracking toolbar.

5. Identify whether your test subject is darker or lighter than the background, and select **Dark on Bright** or **Bright on Dark** from the **Object vs. Background** dropdown list.



6 In the **Whole Body Visualizations** group, check **Contour** and **Fill Contour** so that the objects found by the system are visible.



7 (Optional) To change the colors of any of the parameters in the **Whole Body Visualizations** section of the Tracking tab, click on the color. The system displays a dialog box in which you can select another color.

8 In the video image, view the contour(s) of the object(s) that the system is tracking. If the contour of the tracked object is not well defined, follow the instructions in one or both of the following sections to obtain good tracking results:

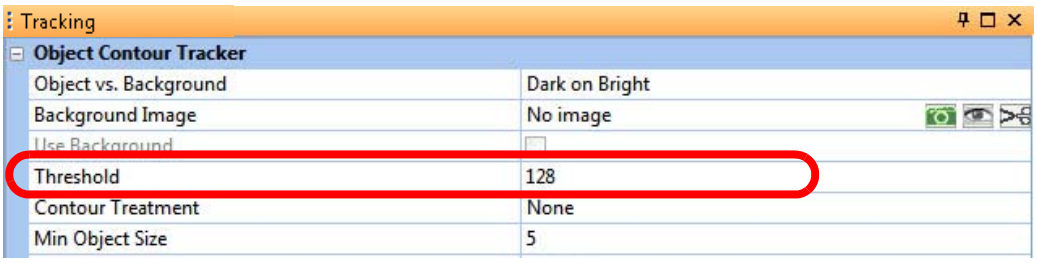
- Section 7.12.2, “Setting the Threshold” on page 151
- Section 7.12.3, “Object Contour Mode Advanced Functions—Overview” on page 153

Note: The system automatically saves the parameter values you set.

9 Now you may record sessions as explained in Chapter 10, Recording and Monitoring.

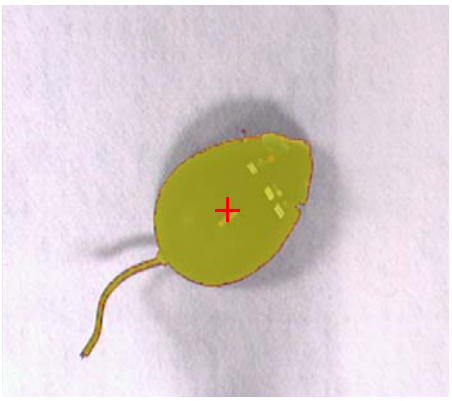
7.12.2 Setting the Threshold

The system can locate an object of a specified color by the color contrast between the desired color and the threshold setting. If the animal has good contrast relative to the arena image, and the background is uniform (that is, there are no other objects with similar contrast), adjust the **Threshold** setting in the **Object Contour Tracker** area to fill the whole image of the animal.



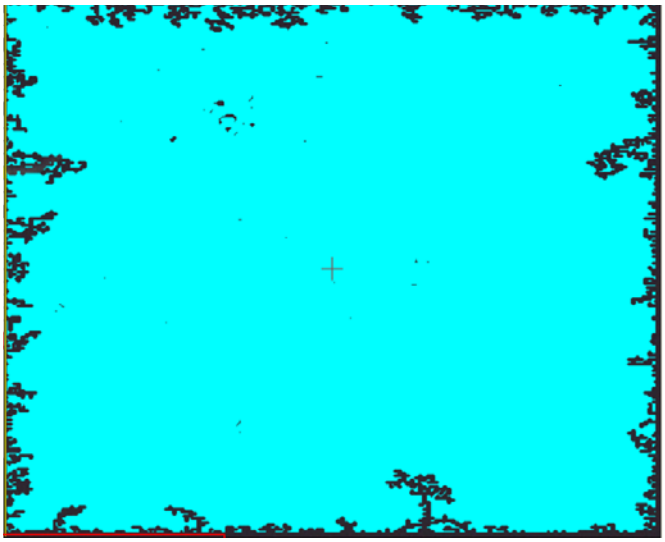
Note: If the background is not uniform or does not have good contrast with the animal, setting the **Threshold** parameter might not be sufficient for tracking. If this condition exists, perform the procedure in Section 7.12.5, “Background Subtraction” on page 156 to remove the background from the video.

The image below shows an example of a well defined animal contour.

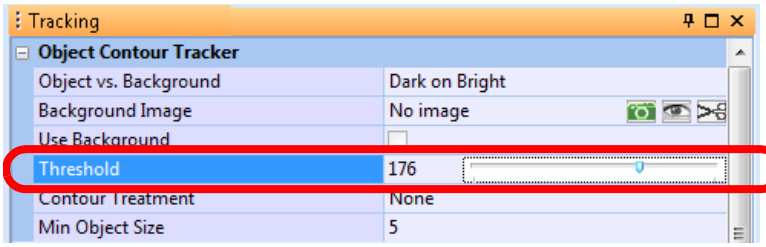


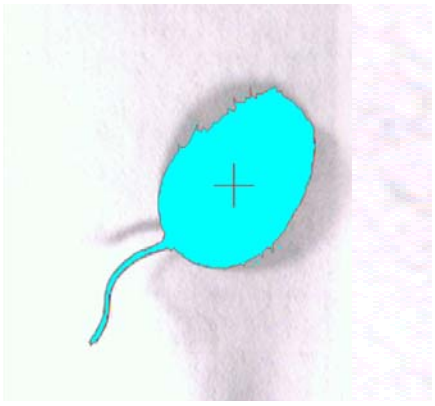
Use the guidelines below to adjust the **Threshold** setting.

1 If the threshold setting is too low, the image could be similar to the one shown below. It will probably be necessary to experiment with the setting to obtain the optimal value.



2 Adjust the **Threshold** bar by clicking on the bar slider, and then make fine adjustments with the left and right arrow keys on the keyboard. You should see a red plus sign in the middle of your object when you have found the appropriate threshold. Verify your settings by clicking on the **Contour** and **Fill Contour** checkboxes in the **Whole Body Visualizations** area. This will [1] draw an outline of the object, as detected by the computer algorithms and [2] fill in the entire area identified as an object for easy visualization.





Note: If the size of the found target object on the camera is larger than 1/4 of the frame area, there will be a red blinking message on the bottom of the screen notifying that the threshold is probably too low and both the **Arm** and **Record** icons will be disabled.

Note: The system automatically saves the parameter values you set.

3 Now you may record sessions as explained in Chapter 10, Recording and Monitoring.

7.12.3 Object Contour Mode Advanced Functions—Overview

For some experimental arrangements, adjusting the **Threshold** setting is not, by itself, sufficient to obtain the desired tracking results. Additional adjustments may be needed. Object Contour mode includes optional advanced functions to handle special circumstances in the experimental environment. These functions are accessed in the Tracking tab.

Use one or more of the following procedures to improve tracking:

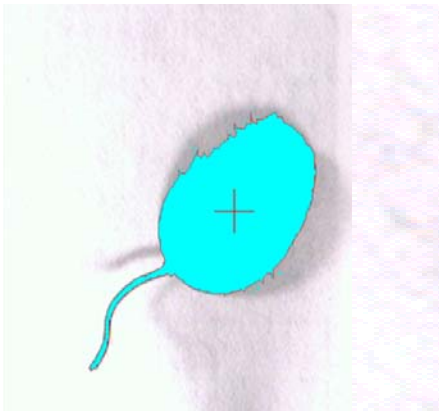
- Section 7.12.4, “Detail Filtering Adjustment” on page 154
- Section 7.12.5, “Background Subtraction” on page 156
- Section 7.12.6, “Close Contour Option” on page 160

7.12.4 Detail Filtering Adjustment

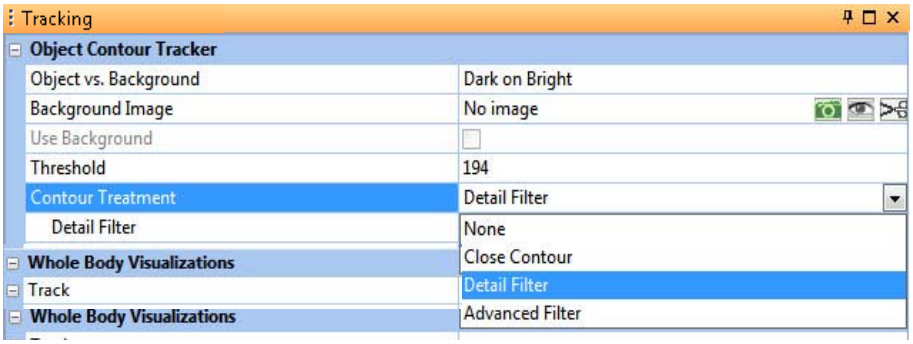
Use Detail Filtering to remove small features of the image that distort the tracking results. For example, a long tail on a target animal can skew the centroid calculation; cables attached to the target can also skew the results. The Detail Filtering adjustment parameter, which has a range of 1 - 10, can remove progressively larger features. Use the lowest setting that provides adequate results for the experiment.

To set the Detail Filtering Adjustment value

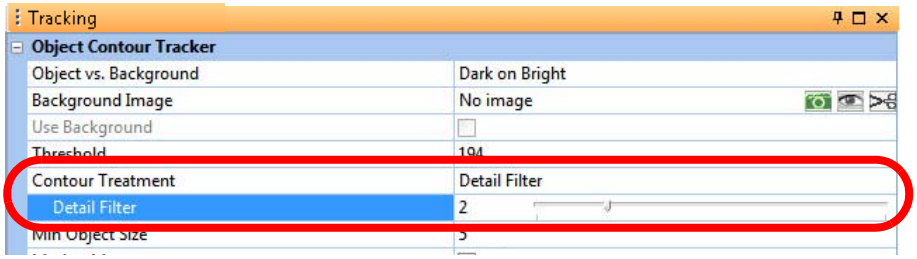
1 In the Properties window **Object Contour Tracker** area, click **Show Contour**, **Fill Contour** and/or **Find All Objects** checkboxes, and view the video image.



2 Select **Detail Filter** in the **Contour Treatment** dropdown list.



3 Click **Detail Filter** and set the slider to 2.



4 Observe the effect on the image. Set the **Detail Filter** slider to lowest setting that removes the undesirable features from the target image.

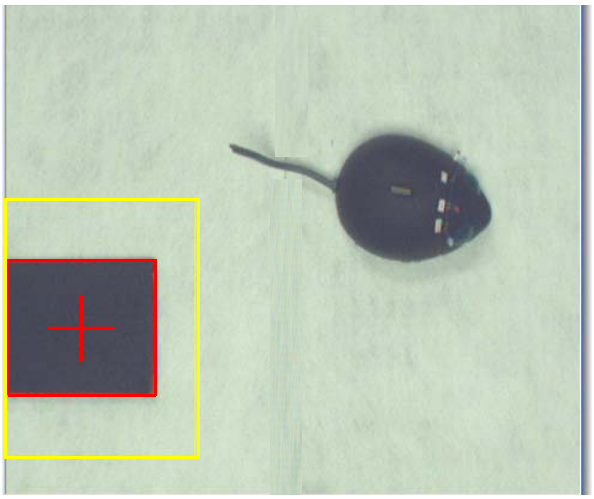
Note: The system automatically saves the parameter values you set.

5 Now you may record sessions as explained in Chapter 10, Recording and Monitoring.

7.12.5 Background Subtraction

If a background object (or background color) might interfere with the tracking function, the system can be configured to ignore the object (or colored area). This will allow the system to track the animal correctly.

This image shows an animal to be tracked along with a background object of approximately the same color. The background object is larger than the animal and the system is tracking the background object (the red cross hair is centered on the object and the tracking window [yellow rectangle] is surrounding the object).

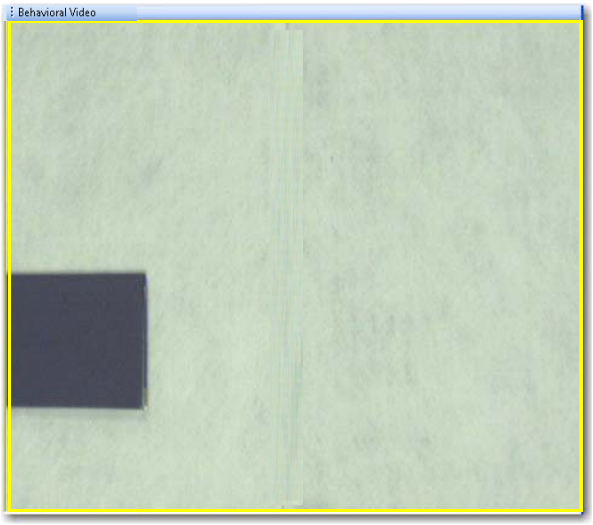


To avoid this situation, you can use one or both of the following tools:

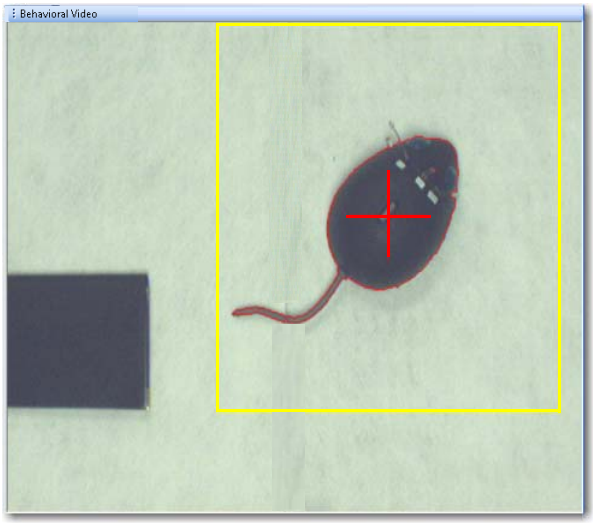
- Use background subtraction (discussed in this section)
- Define a specific arena shape in which to track (discussed in Section 7.9.2, “User-defined Arenas” on page 140)

Example - Using Background Subtraction to Exclude Background Objects

This image shows the background object with nothing else in the window. You can capture the image of this window to use for background subtraction.

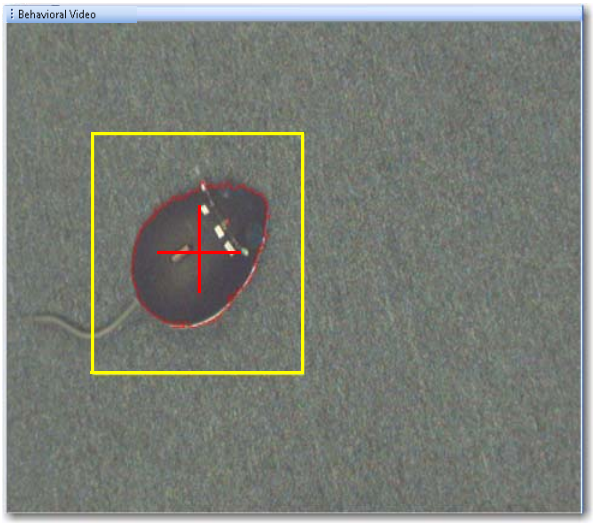


This image shows the background object and the animal together after the user has configured the background subtraction function. Note that the system is tracking the animal, as indicated by the positions of the red cross hair and yellow rectangle and the contour around the animal.



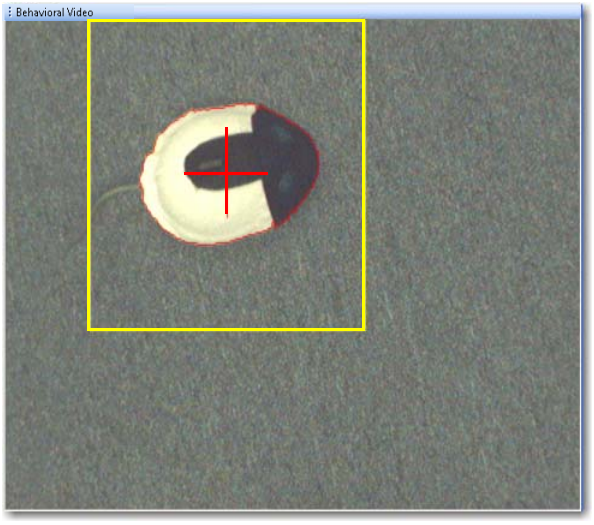
Example - Using Background Subtraction to Exclude a Low-Contrast Background Color

Background subtraction will allow you to do body tracking even when there is a low color contrast between the animal and background, a condition which is seen in the following image.



Example - Using Background Subtraction with a Multicolored Animal

You can track multicolored animals by using background subtraction. In this case be sure that all colors contrast with the background color. You can use different background colors to try to improve the quality. For example, a “salmon pink” background works well for Long-Evans rats. Note that the contour encloses the complete animal.



TIP

Shelf-lining paper can be used to alter background color

Contact paper for lining shelves is an economical method for temporarily altering the background color of an arena. This material can be cleaned easily between sessions to remove the scent of the previous subject.

To apply the Background Subtraction option

Use this procedure to obtain results similar to those shown in the sample images above.

1. In the Object Contour Tracker area, ensure that the **Contour** and **Fill Contour** checkboxes are selected (so the object contour can be viewed in the Video window, as described later in this procedure).
2. Remove the animal from the tracking area.
3. Capture background image by clicking the **Camera** icon. The **Use Background** control will activate so that it may be clicked.

4. Check the **Use Background** checkbox (see diagram above). Checking this box configures the system to subtract the background image from the video.

   Note: Pressing the eye icon brings up a window containing the latest background image. Pressing the scissors icon deletes the latest background image.

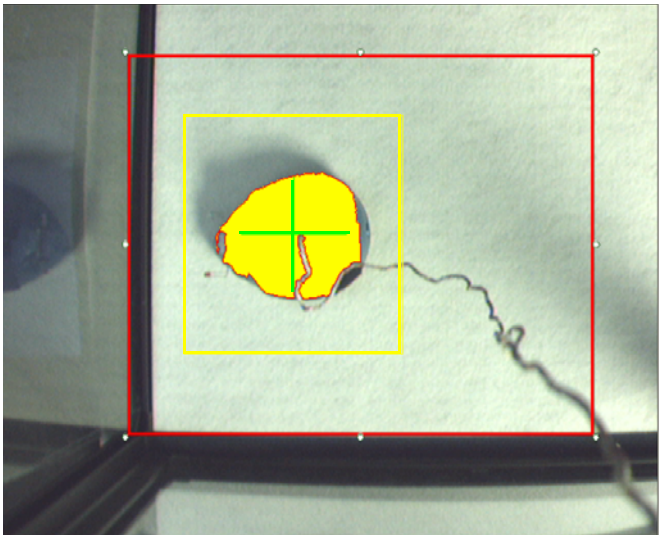
5. Replace the animal in the tracking area.
6. Adjust the Threshold (see diagram above) so the animal is detected and the animal's outline is filled in the Video window (see Section 7.12.2, "Setting the Threshold" on page 151).

   Note: The system automatically saves the parameter values you set.

7. Now you may record sessions as explained in Chapter 10, Recording and Monitoring.

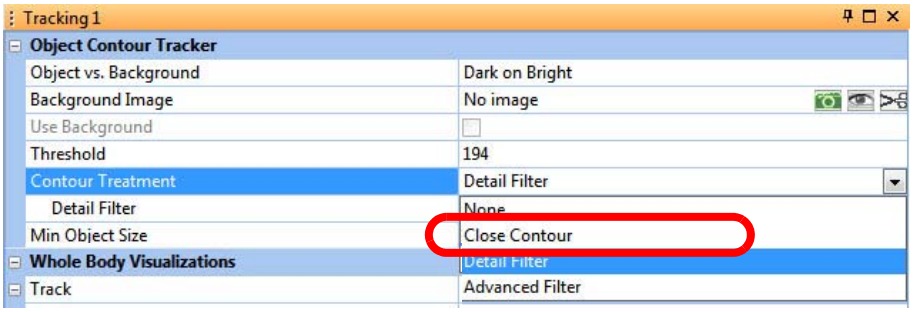
7.12.6 Close Contour Option

The Close Contour capability causes the tracker to merge multiple objects in near proximity to each other into a single object. This can occur, for example, when a cable passes between the animal and the camera. Adjust the setting so that a single object is displayed. The image below is taken before the Close Contour setting is applied.

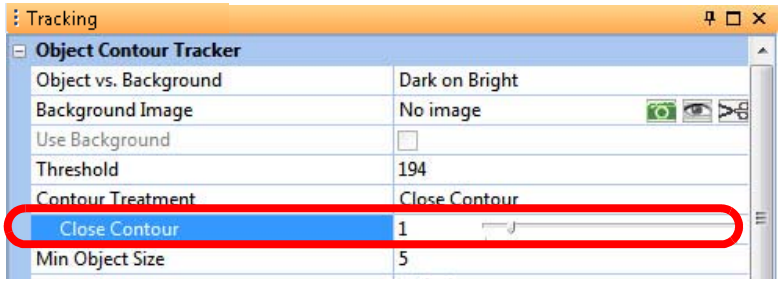


To apply the Close Contour option

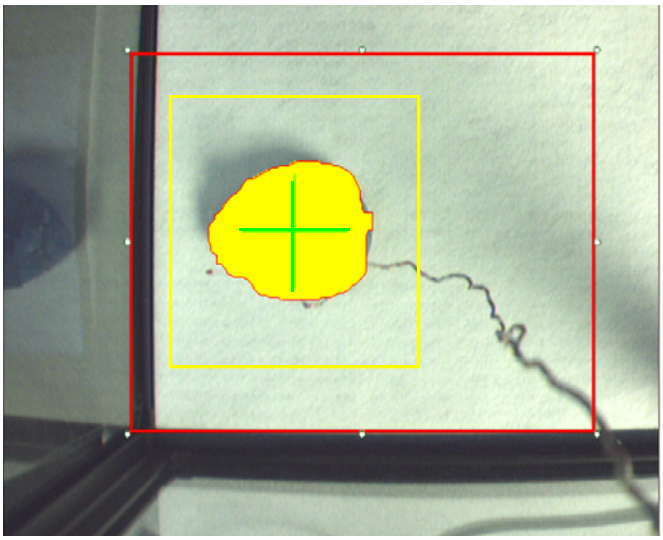
1 Select **Close Contour** in the **Contour Treatment** dropdown list.



2 Adjust the **Close Contour** setting until the image contour is closed.



The image below is taken after the **Close Contour** setting is applied.



Note: The system automatically saves the parameter values you set.

3 Now you may record sessions as explained in Chapter 10, Recording and Monitoring.

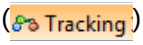
7.13 LEDs In Darkness with LED Tracking

This section configures the system to track colored LEDs in darkness with the LED tracking mode selected. Up to three different colored LEDs can be tracked.

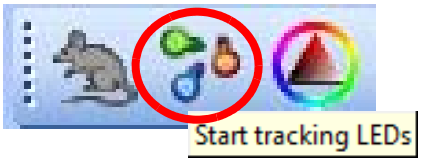
In darkness, the LEDs are the brightest spots in the window, therefore, the LED tracking mode is excellent for this condition. The image below shows three LEDs being tracked in darkness.

7.13.1 Configuring Parameters for LED Tracking

Perform these steps to select and configure LED tracking. (Some of the selections shown in this procedure are examples. You should select parameter values that work best for your particular experiment.)

1. Place powered LEDs in the camera field of view.
2. At the camera lens, adjust the zoom, focus, and iris settings for the best picture quality. For additional guidance on camera setup and video quality, see Chapter 5, Setting Up the Behavioral Camera.
3. Click on the Tracking tab (

) to view the Tracking parameters.
4. To select the LED tracking mode, click the appropriate icon on the Tracking toolbar.

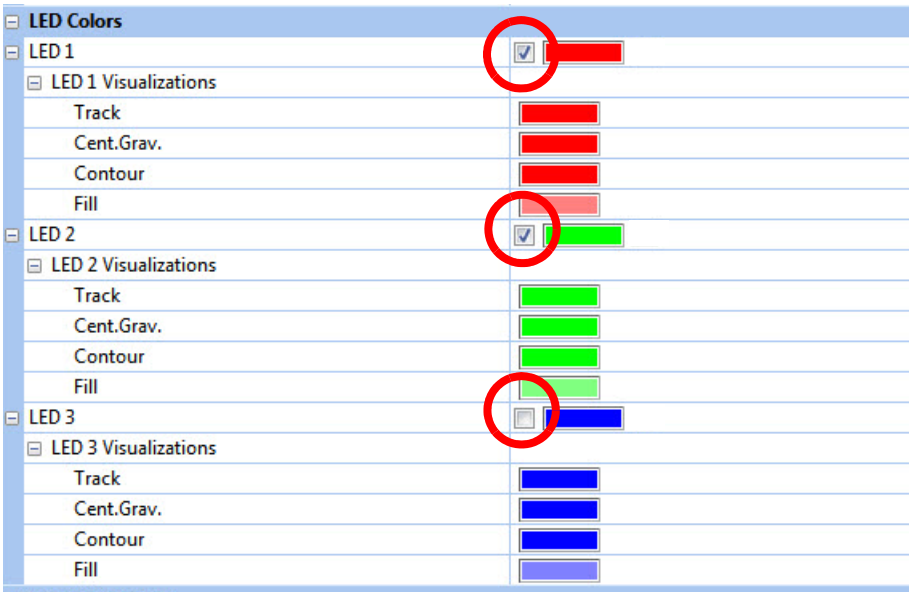


5. In the **LED Visualization** group check **Contour** and **Fill Contour** so that the objects found by the system are visible. These selections are common to all LEDs used in the experiment.

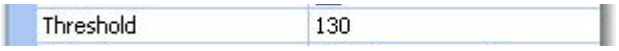
6. Check the **Pure Colors** checkbox in the LED Tracker area. Checking this box causes the standard red, green and blue LED colors to appear in the **LED Colors** area in the Tracking configuration tab.

Note: In **Pure Colors** mode, software is optimized for the red, green and/or blue LEDs supplied by Plexon.

7 Check each color to track in the LED properties area (LED 1, LED 2 and/or LED 3).



8 Change the **Threshold** (common to all LEDs) so that the correct objects are filled and outlined on the Video window. Their color crosses (representing the centers of gravity) should be centered on the correct objects.



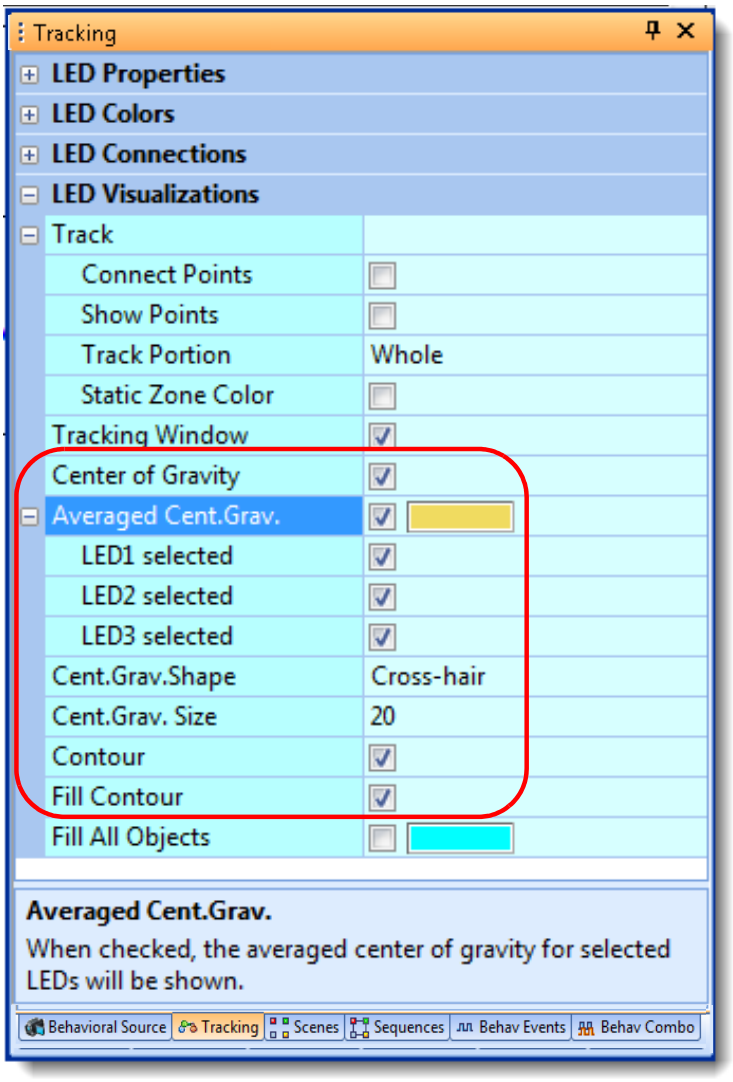
9 If the **Pure Colors** option does not give good results, use the pick-up tool to select the color from the video (see "Using the Color Pick-up Tool with LED Tracking" below). Adjust the **Threshold** as needed.

Note: The system automatically saves the parameter values you set.

10 The **LED Visualizations** area (below) contains a number of parameters that allow you to customize the display of LEDs in the Video window. These selections are common to all LEDs used in the experiment. You can adjust these values in a manner that gives you the best visualization for your application. For example, it is useful to select the checkboxes for **Contour** and **Fill Contour** so the objects found by the system are visible.

It can also be useful to configure values for **Cent.Grav.Shape** and **Cent.Grav.Size** to optimize visualization of the center of gravity for each of the LEDs. These visualization parameters are discussed in more detail in this chapter and in Chapter 8, Behavioral Features and Procedures based on their typical usage.

The **Averaged Cent.Grav.** is another useful parameter. You can select which LEDs will be included in the average. See the description in Section 8.8.5, “Events Based on Head Direction To Object” on page 209.



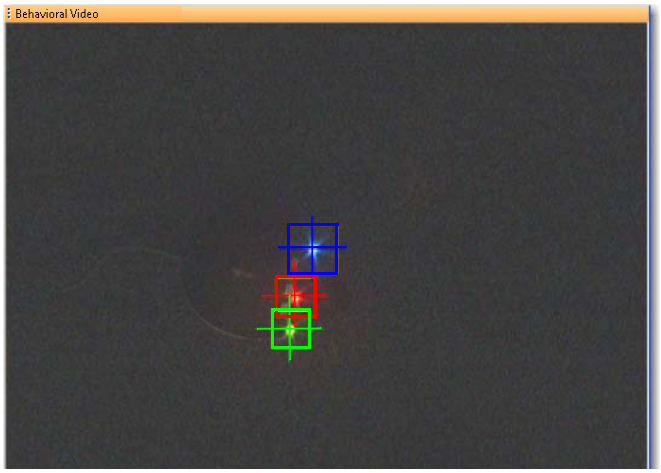
11 Now you may record sessions as explained in Chapter 10, Recording and Monitoring.

7.13.2 Using the Color Pick-up Tool with LED Tracking

This procedure explains how to use the color pick-up tool to adjust Color 1, Color 2, or Color 3 to match an existing color of an LED on the animal.

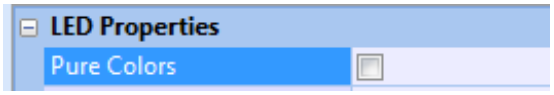
LED color shade selection is possible, but requires precision in the pixel selection process. This is because an LED image is a hot white center surrounded by pixels of varying shades of color. You must click on one of the pixels of the desired color, but not white.

1 Ensure that the LEDs are visible in the Video window.

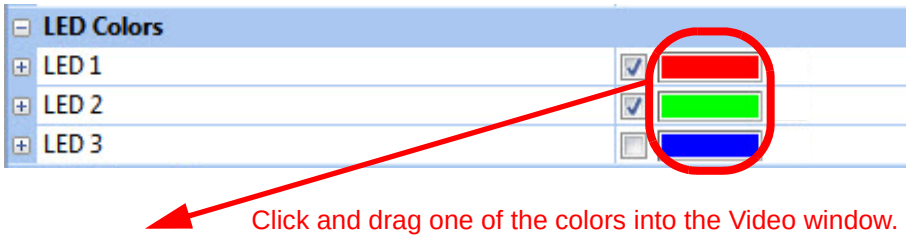


2 At the camera lens, adjust the zoom, focus, and iris settings for the best picture quality. For additional guidance on camera setup and video quality, see Chapter 5, Setting Up the Behavioral Camera.

3 Deselect the **Pure Colors** option.

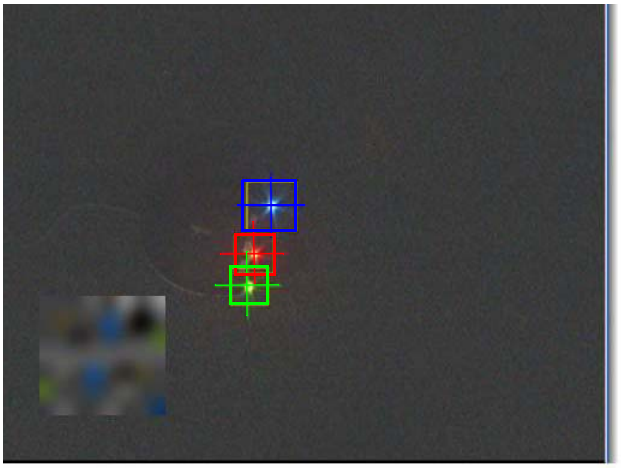


4 To use the color pick-up tool on a specific **LED**, click on the **Color Selection** box for the color that is to be adjusted, and drag it into the Video window.



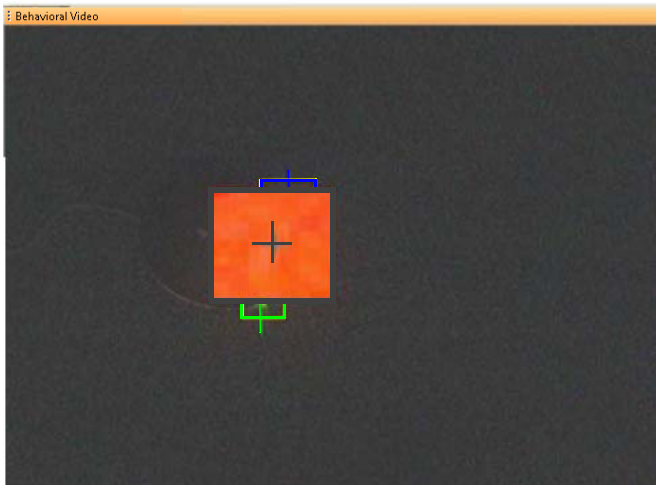
5 In the Video window a square area surrounding the cursor displays as a magnified window. Move the magnified window over the area to be shown in

the **Color Selection** box. The image below shows the magnified cursor window in the lower left of the diagram - note the digitization of the area.



6 Position the crosshair over a representatively colored pixel. The **Color Selection** box displays the color of the pixel directly under the **crosshair**. The image below shows a red pixel under the magnified area.

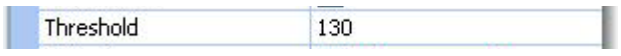
Note: Be sure that the pixel is the dominant color of the LED, and not the white center of the LED.



7 Click the pixel.

8 The color of the corresponding color box in the properties window will be changed to that of the clicked pixel.

9 Change the **Threshold** (common to all LEDs) so that the correct objects are filled and outlined on the Video window. Their color crosses (representing the centers of gravity) should be centered on the correct objects.



Note: For a general discussion of Threshold values, see Section 7.12.2, “Setting the Threshold” on page 151.

10 If the cursor does not track accurately with the threshold set to optimum, you can restart the color pickup procedure beginning with Step 4. If the image in the Video window needs improvement, repeat the procedure in Chapter 5, Setting Up the Behavioral Camera.

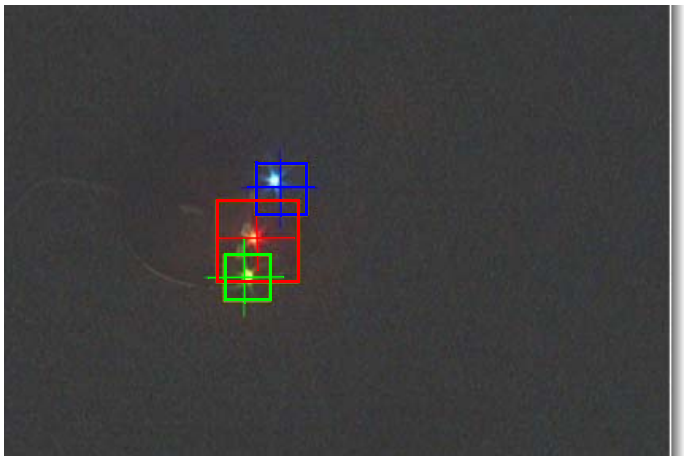
Note: The system automatically saves the parameter values you set.

11 Now you may record sessions as explained in Chapter 10, Recording and Monitoring.

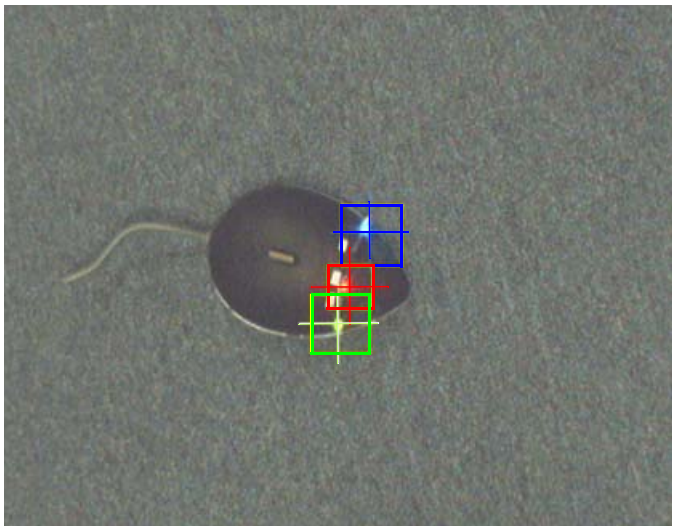
7.14 LEDs In Darkness or Light with Color Markers Tracking

The system can track colored LEDs in darkness or light with the **Color Markers** tracking mode selected.

In darkness, the LEDs are the brightest spots in the window, therefore, the **Color Markers** mode works well for this condition. The system treats the LEDs as if they were color markers. The image below shows three LEDs being tracked in darkness.



In light, the LEDs might (or might not) be the brightest spots in the window. If the LED colors have sufficient brightness and contrast with respect to the background, **Color Markers** works well for this condition. The image below shows three LEDs being tracked in light.



Note regarding use of the color pick-up tool with LEDs

With the color pick-up tool, LED color shade selection is possible, but requires precision in the pixel selection process. This is because an LED image is a hot white center surrounded by pixels of varying shades of color. You must click on one of the pixels of the desired color, but not white.

To configure tracking of LEDs with Color Markers tracking

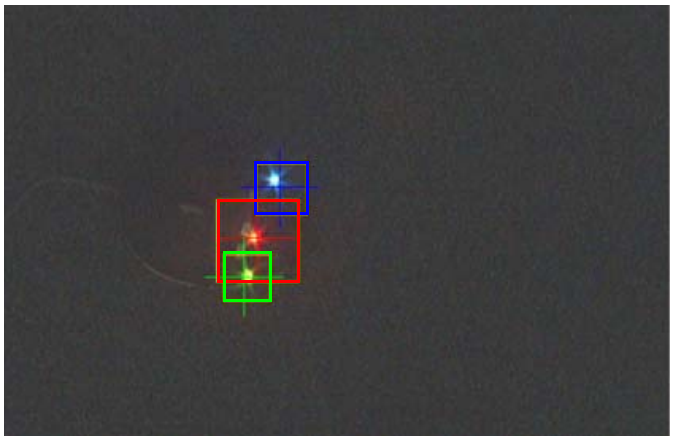
Follow the same procedure as with Color Markers Tracking (below).

7.15 Color Markers Tracking

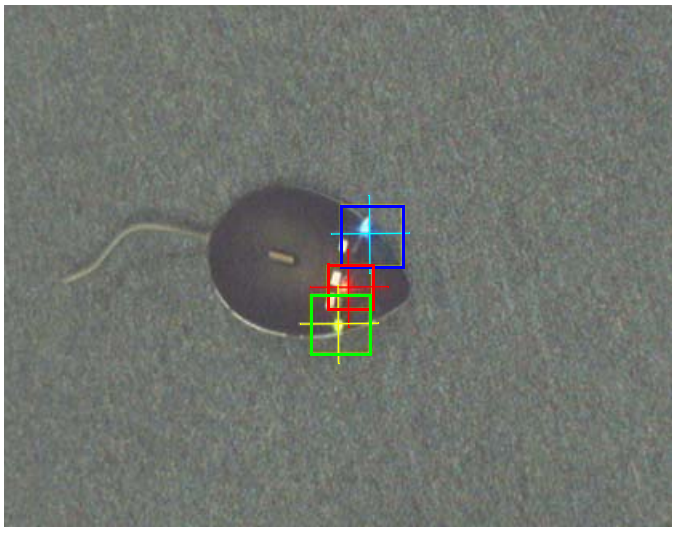
This section configures the system to track an animal with **Color Markers** mode selected. Up to 12 different colored markers can be tracked. It is applicable to any of the following experimental configurations:

- LEDs in darkness
- LEDs in light
- Color markers in light

The images below show these objects being tracked.



LEDs in darkness



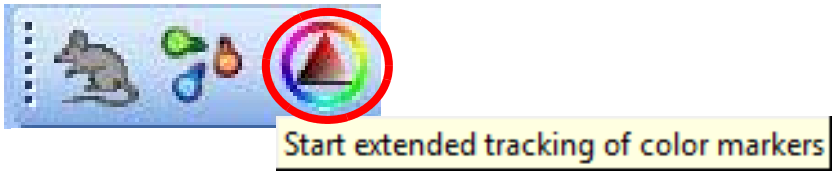
LEDs in light

Color markers

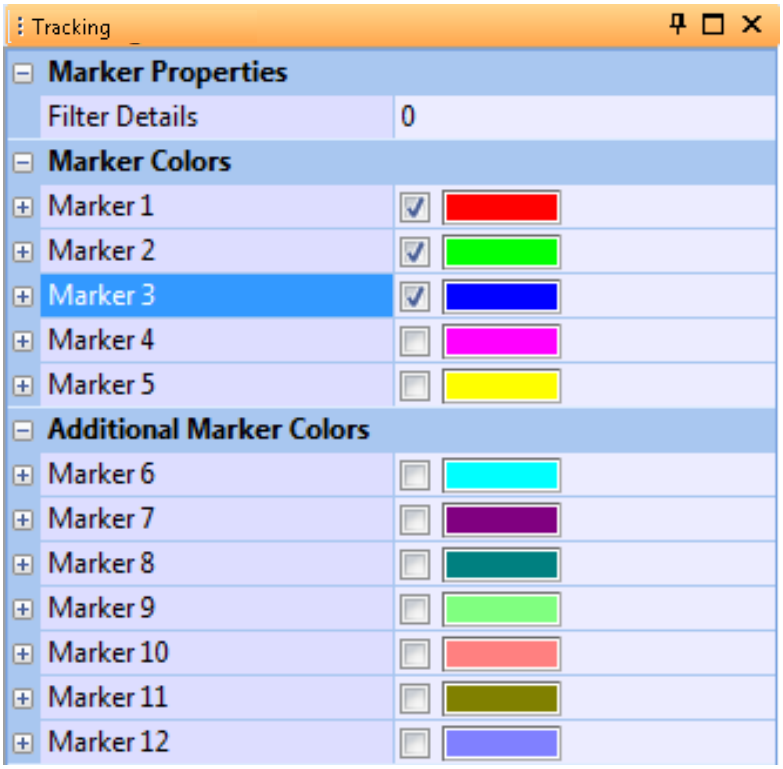
7.15.1 Enabling Color Markers Tracking

Perform these steps to select and configure **Color Markers**.

1. Place the colored targets in the camera field of view.
2. At the camera lens, adjust the zoom, focus, and iris settings for the best picture quality. For additional guidance on camera setup and video quality, see Chapter 5, Setting Up the Behavioral Camera.
3. Click on the Tracking tab (Tracking) to view the Tracking parameters.
4. To select the **Color Markers** mode, click the appropriate icon on the Tracking toolbar.

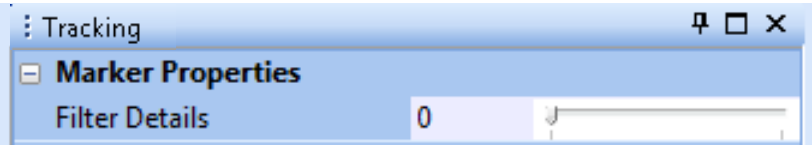


The system displays the Marker parameters.



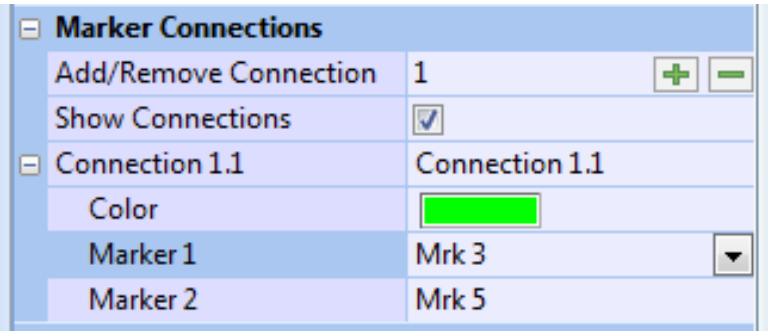
Note: The adjustment of the color for each Color Marker will be discussed in Section 7.15.2, “Using the Color Pick-up Tool with Color Marker Tracking” on page 174

5 In the **Marker Properties** area, use the slider to set the value of the **Filter Details** parameter. This parameter specifies the fineness of detail removed from the tracked objects. It also helps to remove parasite objects. As you view the subjects in the arena, you can adjust the value of this parameter to obtain the best results.



6 In the **Marker Colors and Additional Marker Colors** areas, click the checkboxes to select the colors to be tracked.

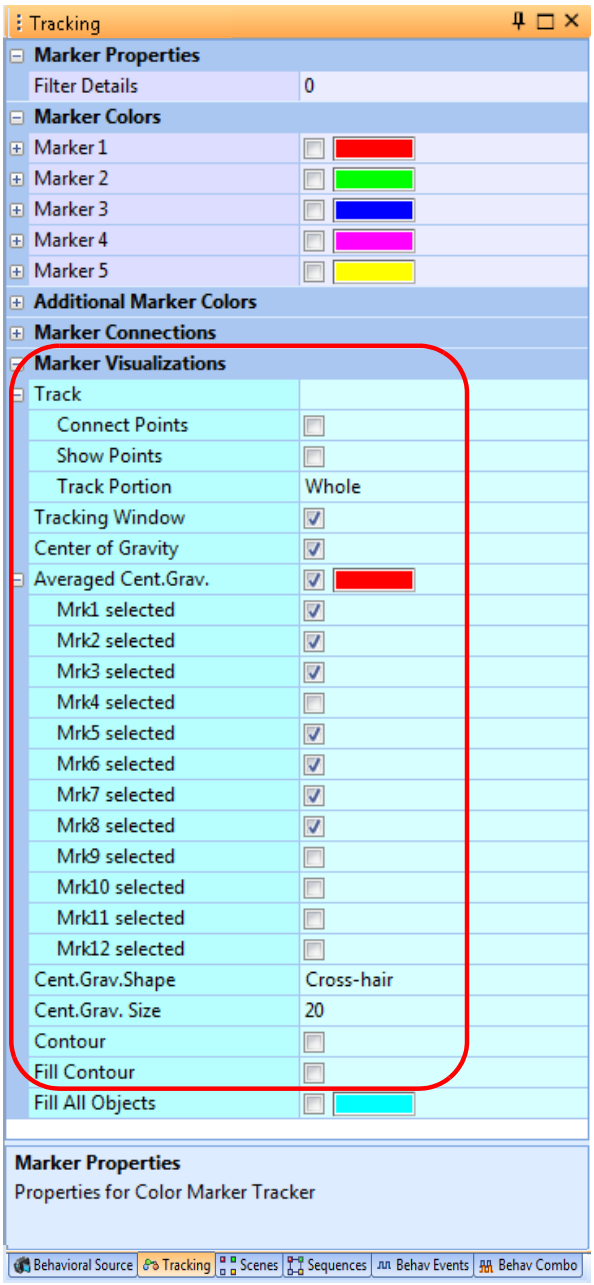
7 If desired, in the **Marker Connections** area, add connections between pairs of markers, and select the **Show Connections** checkbox if you want to display these connections.



The **Marker Visualizations** area (below) contains a number of parameters that allow you to customize the display of color markers in the Behavioral Video window. These selections are common to all color markers used in the experiment. You can adjust these values in a manner that gives you the best visualization for your application. For example, it is useful to select the checkboxes for **Contour** and **Fill Contour** so the objects found by the system are visible.

It can also be useful to configure values for **Cent.Grav.Shape** and **Cent.Grav.Size** to optimize visualization of the center of gravity for each of the color markers. These visualization parameters are discussed in more detail in this chapter and in Chapter 8, Behavioral Features and Procedures based on their typical usage.

The **Averaged Cent.Grav.** is another useful parameter. You can select which LEDs will be included in the average. See the description in Section 8.8.5, “Events Based on Head Direction To Object” on page 209.


Tracking
Marker Properties
Filter Details
0
Marker Colors
Marker 1
Marker 2
Marker 3
Marker 4
Marker 5
Additional Marker Colors
Marker Connections
Marker Visualizations
Track
Connect Points
Show Points
Track Portion
Whole
Tracking Window
Center of Gravity
Averaged Cent.Grav.
Mrk1 selected
Mrk2 selected
Mrk3 selected
Mrk4 selected
Mrk5 selected
Mrk6 selected
Mrk7 selected
Mrk8 selected
Mrk9 selected
Mrk10 selected
Mrk11 selected
Mrk12 selected
Cent.Grav.Shape
Cross-hair
Cent.Grav. Size
20
Contour
Fill Contour
Fill All Objects
Marker Properties
Properties for Color Marker Tracker
Behavioral Source
Tracking
Scenes
Sequences
Behav Events
Behav Combo

7.15.2 Using the Color Pick-up Tool with Color Marker Tracking

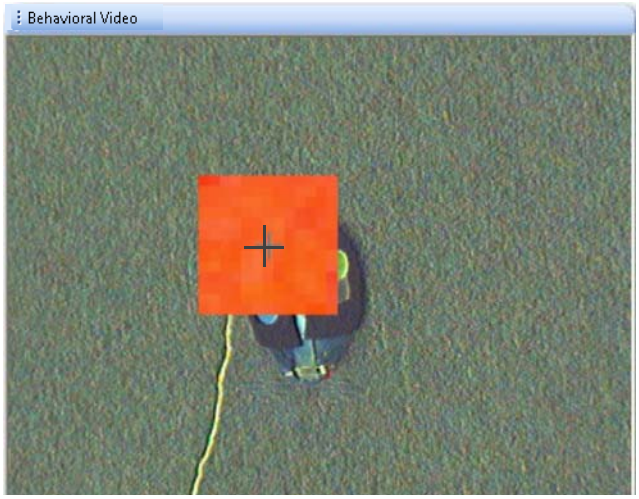
This procedure explains how to use the color pick-up tool to adjust any of the tracked colors (Color 1 through Color 12) to match an existing color of a color marker on the animal.

1 To use the color pick-up tool on a specific **color**, click on the **Color Selection** box for the color that is to be adjusted.

2 The image below shows a sample video window before clicking the **Color Selection** box.

3 In the Video window a square area surrounding the cursor displays as a magnified window. Move the magnified window over the area to be shown in the **Color Selection** box. The image below shows the magnified cursor window in the lower left of the diagram - note the digitization of the area.

4 Position the crosshair over a representatively colored pixel. The **Color Selection** box displays the color of the pixel directly under the crosshair. The image below shows a red pixel under the magnified area.



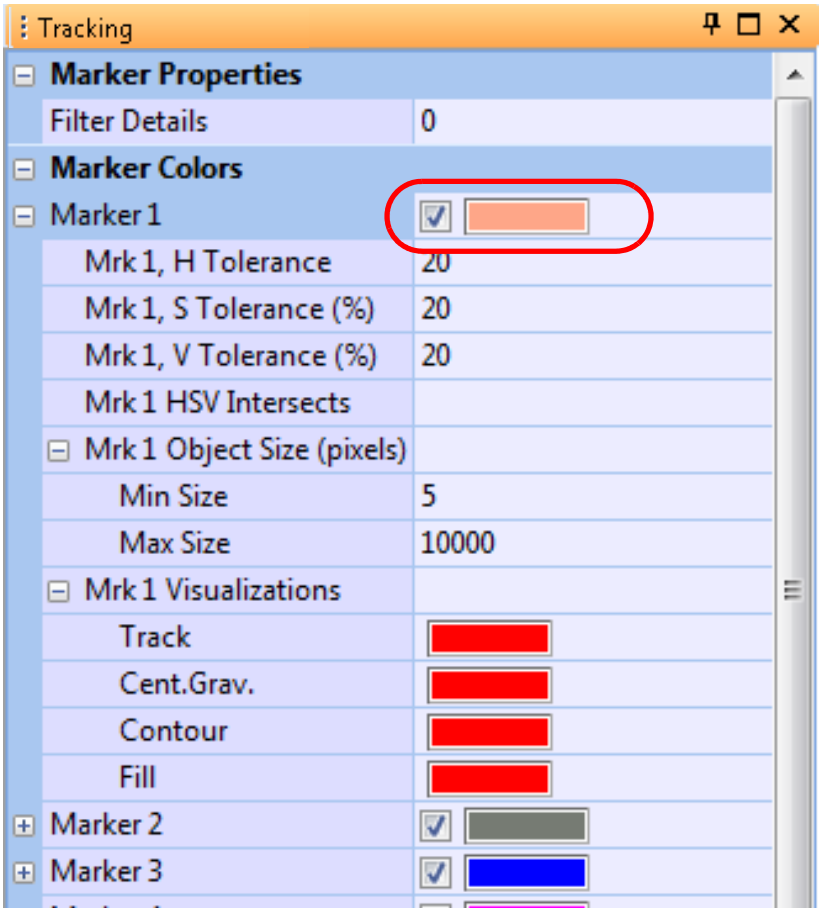
5 Click the pixel.

TIP

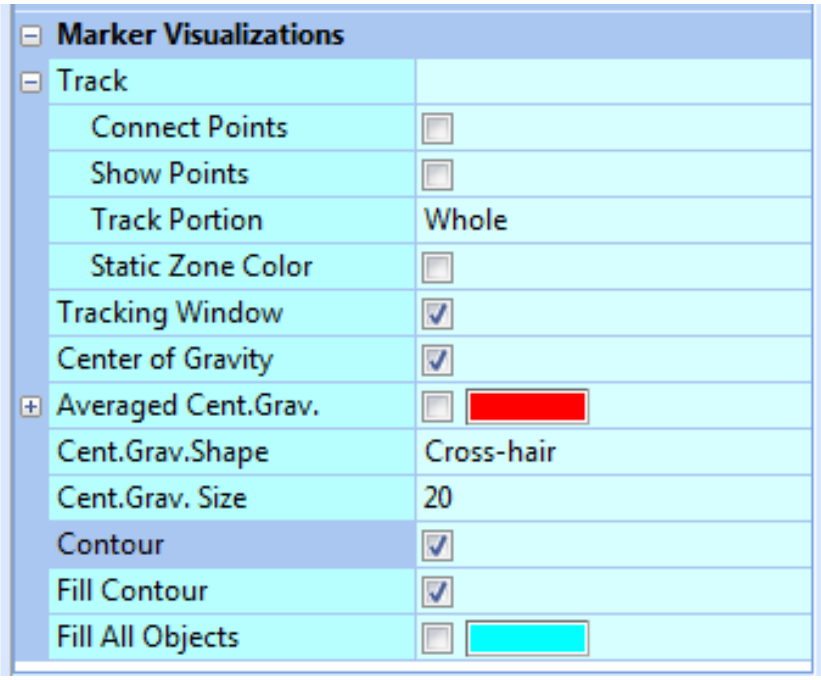
Select the pixel that gives the best tracking

If the color shades from lighter to darker across the magnified window, pick a shade in between the extremes. Then observe the tracking, and, if the tracking is not satisfactory, try a darker or lighter shade until tracking works well.

6 The color of the corresponding color box in the properties window will be changed to that of the clicked pixel.



7 Click the checkboxes for **Contour** and **Fill Contour** in the **Marker Visualizations** area (if not already selected) so that the image of the selected object is seen in the video image.



8 View the Behavioral Video window. If the cursor does not track the moving color object accurately, you can redo this color pickup procedure. If the image in the window needs improvement, repeat the procedure in Chapter 5, Setting Up the Behavioral Camera.

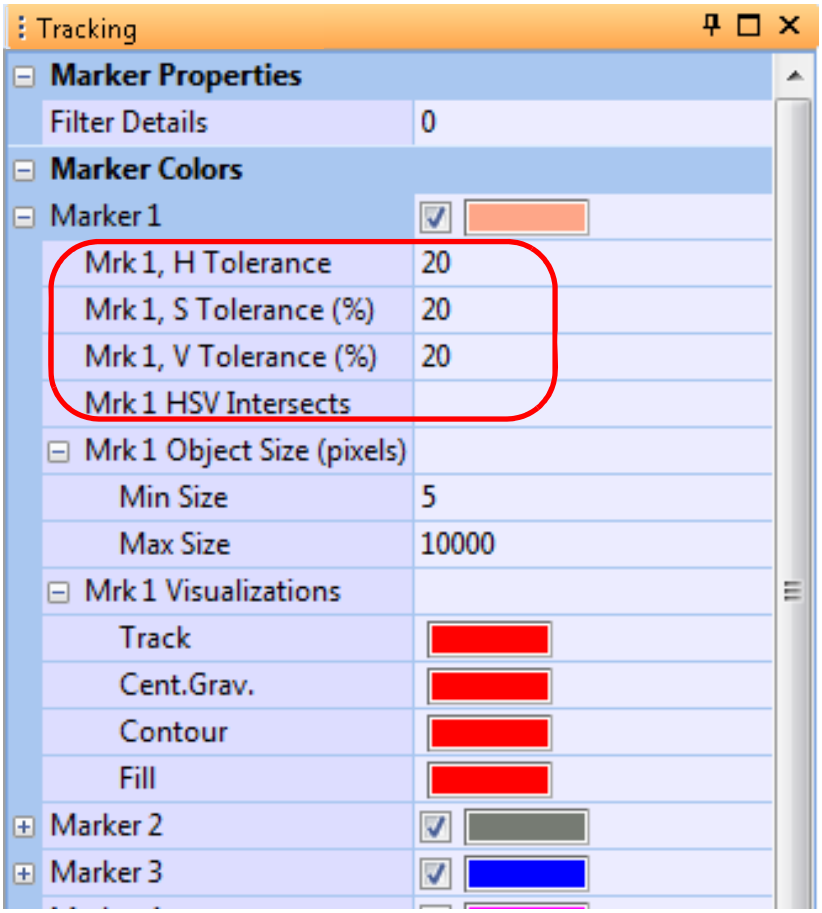
9 Repeat Step 1 through Step 8 for the other colors that will be tracked in the experiment.

Note: Once sessions have been recorded for an experiment, you cannot select additional colors or uncheck any that have already been selected. This ensures that all sessions in the experiment have the same tracking objects, and ensures that the analysis is consistent across all sessions.

10 Work with the **Marker Colors** tolerance, size and visualization parameters (in the following section) to improve tracking efficiency.

7.15.3 Improve Tracking Efficiency with Tolerance Parameters

In some cases, you can improve tracking efficiency by making adjustments to the color marker tolerances. These adjustments are available in both **Cameras** mode and Files **mode** (except in **Files/View Sessions As Recorded** submode).



1 If necessary to improve tracking, adjust the **H**, **S** and **V** tolerances so there are no **Intersects** among the various colors.

2 Change the **H**, **S** and **V Tolerance** setting so that the correct object is filled and outlined on the Video window. The crosshair should be centered on the correct object.

Note: In normal situations, you should *not* need to change the **HSV** settings manually. These settings were adjusted automatically by the system when you clicked the colored pixel in the earlier step. Instead, follow the steps below.

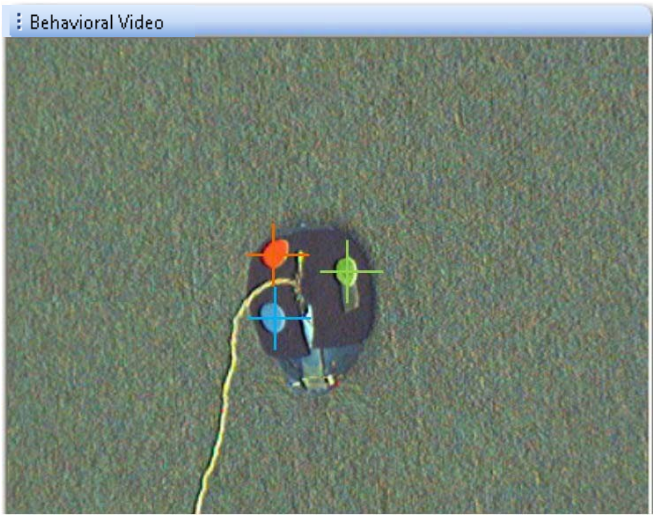
3 If the above steps do not provide accurate visualization of the objects that need to be tracked, or do not eliminate all the **Intersects**, consider making some or all of the following additional adjustments and changes:

- Use different marker colors, especially if two of the colors being used in the experiment are similar to each other.
- Increase the lighting level in the experimental area, if possible.

- Reduce the number of color markers being used in the experiment.
- Increase the gain on the camera (although this will also increase noise).

Note: The above list is intended as a general guide. If you need additional information on these adjustments, contact Plexon® Support.

4 After completing the previous steps, verify that your video looks similar to the following image, in which the system is set up to track three colors.



5 To improve visualization, you can also adjust **Object Size** and **Visualizations** for the marker.

Note: The system automatically saves the parameter values you set.

7.16 Modifying Arenas and Calibration During an Experiment

The system provides some flexibility in modifying arenas in an experiment that already has one or more sessions recorded. For a description of these options and procedures, see these sections:

- Section 11.13, “Using the Overlay Feature during Analysis” on page 290
- Appendix D, Modifying Arenas and Zones

7.17 Where to Go Next

If you would like to configure basic behavior parameters, go to Chapter 8, Behavioral Features and Procedures.

After you have completed the configuration of the behavioral camera, go to Chapter 9, Configuring and Viewing Global Events.

Chapter 8
Behavioral Features and Procedures

8.1 Before You Start

Verify that you have completed the procedures in Chapter 7, Configuring the Tracking Parameters.

8.2 Introduction to the Basic Behavior Functions

This chapter describes the behavioral analysis functions, the experimental applications for which they can be used, and how to use them.

8.2.1 Procedures for Behavioral Events and Data Analysis

Following are the procedures for behavioral events and analysis the system provides in addition to the tracking features described in Chapter 7, Configuring the Tracking Parameters.

- Defining static and dynamic zones of interest within an experimental arena
- Defining zone sequences
- Monitoring objects traversing zones and sequences and generating logical and digital events
- Defining behavioral events
- Recording and viewing real-time and offline information about behavioral events and tracked objects, including such attributes as speed, direction (vector), limb angles, presence in particular zones in the arena, proximity to other objects, and sequence of zones visited
- Monitoring vectors between objects and creating behavioral events when the objects are within/outside of a customer-defined angle and tolerance
- Monitoring animal head direction based on markers or LEDs
- Monitoring animal speeds to create digital events when the speed is over/under a user-specified threshold
- Accumulating and displaying behavioral event statistics
- Grouping event results in the behavioral video and in combination with events occurring in the photometry videos
- Visualizing the animal’s movements around the arena during user-specified time intervals
- Computing and displaying analysis results in graphs, and exporting data to comma separated values (CSV) files for further calculations and analysis
- Generating digital output signals when behavioral events occur

8.2.2 Terminology for the Behavioral Video

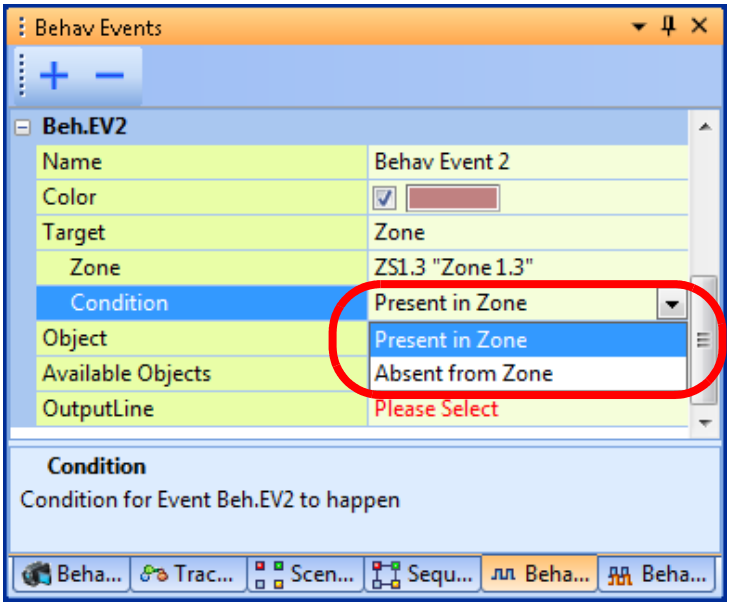
The following terminology is useful in discussing behavior recording, tracking and analysis:

- **Zones**: A zone is a defined portion of the arena that has significance in an experiment. Many zones can be defined simultaneously. The system provides tools that allow you to draw zone outlines on top of the video image. These tools operate in the same way as the arena tools, allowing complex shapes to be created by means of operations (Union shapes, Intersect shapes, Subtract shapes and XOR shapes).

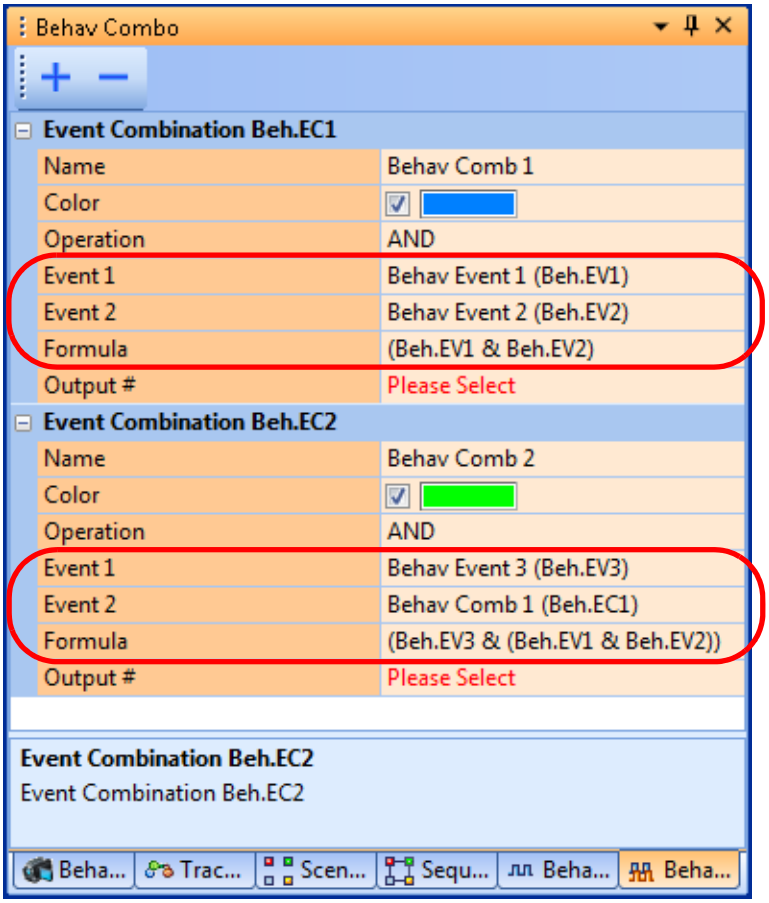
Note: Complex zones can be created for static zones only; see the definitions of static zones and dynamic zones, below.

- **Static Zones**: Zones that do not move with respect to the image or the arena are referred to as static zones. Often they are used in object recognition, place preference or conditioning experiments. For example, a static zone could be an area the animal should be present in or avoid to receive a reward or it could be an area the animal must traverse to receive the reward. It could even be one of the zones in a sequence of zones the animal should traverse.
- **Dynamic Zones**: A dynamic zone is a circular area around a marker or LED. In general, it is used to detect social interactions among animals. Dynamic zones are available for **LED** tracking mode and **Color Markers** mode. They are not available in **Object Contour** tracking mode.
- **Sequences**: A sequence is an ordered list of zones. Many different sequences can be defined.

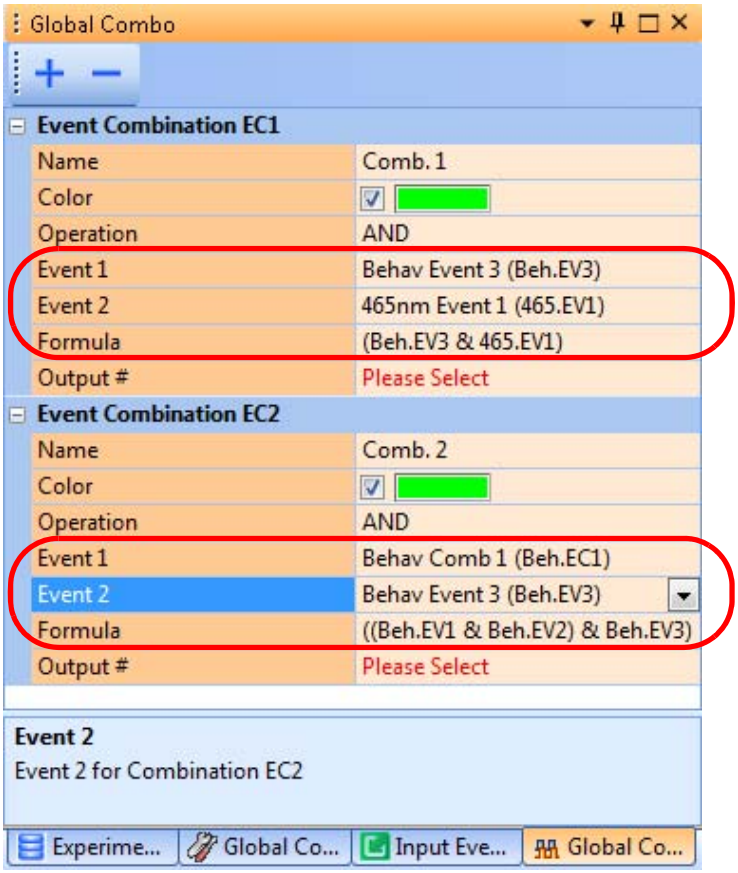
- **Events**: Logical events can be defined so that, when an animal enters or leaves a zone, or completes a sequence, the event becomes true. Many other events can be specified, including speed, angle and direction. Their current states are dynamically displayed in the Event Statistics window while the experiment is running. An example is shown below.



- **Combination Events in the behavioral video (Behav Combo):**
  A combination event is an event that becomes true when two or more other events are true. You can combine combinations of single events or combinations of combination events that occur within an individual video source, as shown in the example below.

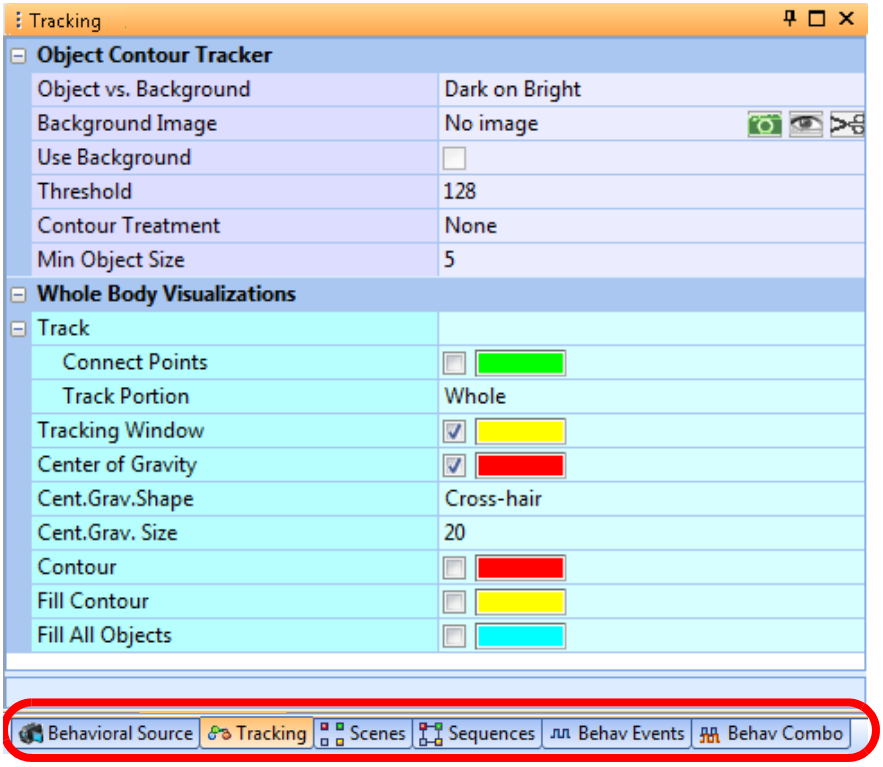


- **Global Combination Events (Global Combo):** You can combine global combination events, that is, combinations across multiple video sources, as shown in the example below. This tab allows you to combine events from any of the three photometry sources and behavioral camera. For example, you could combine a behavioral event with an event from the 465nm source. You can also create combination events that contain other (existing) combination events. This procedure is described in more detail in Section 9.3, “Creating Global Combination Output Events” on page 228.



8.3 Source, Tracking, Scenes, Sequences, Events and Combination Events

The Behavioral Video window has an associated set of tabs with configurable parameters.



These tabs are accessed by clicking the appropriate tabs (see the diagram above), and include:

- **Behavioral Source**—Used to set parameters for the video stream, either from the behavioral camera or from existing files. This tab contains parameters that relate to optical settings such as gain, brightness, white balance and shutter, labeling/timestamping of frames and calibration.
- **Tracking**—Used to set parameters associated with the specific type of tracking that has been chosen (**Object Contour**, **LEDs** or **Color Markers** mode).
- **Scenes**—Used to set the shape of the experimental arena so the system can ignore any portion(s) of the image which are outside the user-designated area of interest for the experiment. Also used to set static and dynamic zones of interest for behavioral events that will occur in the experimental arena.
- **Sequences**—Used to specify sequences of zones for a series of behavioral events that may occur in the experimental arena.

- **Behav Events**—Used to specify the conditions for behavioral events. When the user-specified conditions become true, the event has occurred.
- **Behav Combo**—Used to specify events that are combinations of one or more existing events on which logical operations are performed. The existing events can be any single or combination event. The four logical operators are **NOT**, **AND**, **OR** and **XOR**. For definitions and use of these operators, see Section 8.9, "Creating Behavioral Combination Events" on page 217.

8.4 Configuring Zones, Sequences and Behavioral Events

This section explains how to create and configure zones, sequences of zones, events and combination events.

You can specify values for behavioral events for tracked objects, including such attributes as speed, direction (vector), limb angles, presence in particular zones in the arena, proximity to other objects and sequence of zones visited. For example, the system can create a digital event when the animal's speed is over/under a user-specified threshold, or the animal is within a user-specified distance of a certain point in the arena.

8.4.1 Before You Start

The procedures in Chapter 7, Configuring the Tracking Parameters are required for configuring the arena. Arena and tracking parameters must be configured before zones and behavioral events can be created.

8.4.2 Overview of Procedure

The procedure consists of the following tasks:

- Section 8.5, "Adding Zones (Static and Dynamic)" on page 188
- Section 8.6, "Defining Sequences of Zones" on page 197
- Section 8.7, "Modifying Arenas, Zones and Calibration During an Experiment" on page 199
- Section 8.8, "Creating Behavioral Events" on page 200

8.5 Adding Zones (Static and Dynamic)

There are two types of zones, static and dynamic. Static zones are created by clicking the **Static Zone** icon and defining the shape, size and location of the zone in the video image. Dynamic zones are created by clicking the **Dynamic Zone** icon and selecting the LED or color marker around which to draw it. (Dynamic zones are not applicable to Object Contour tracking.)

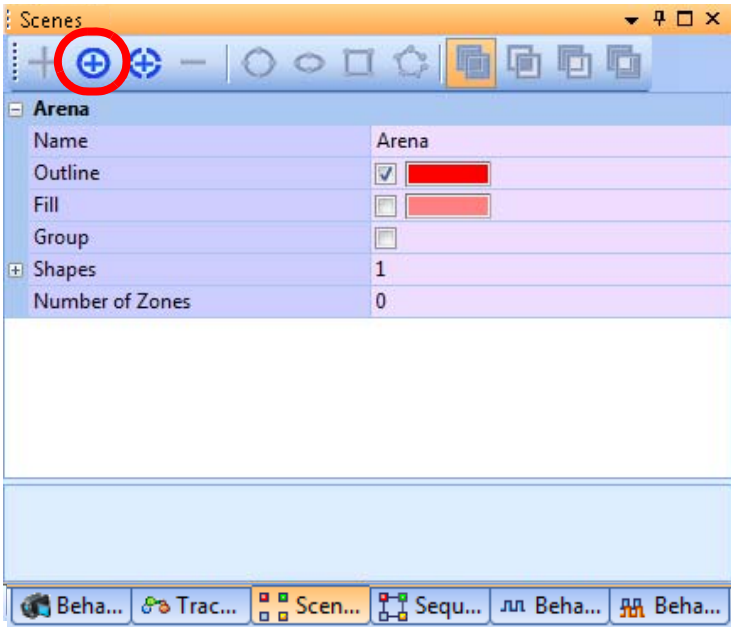
Note: See Section 8.2.2, "Terminology for the Behavioral Video" on page 183 for definitions of static zone and dynamic zone. In general, static zones are associated with static objects in the arena and dynamic zones are associated with objects attached to the animal.

8.5.1 Adding a Static Zone

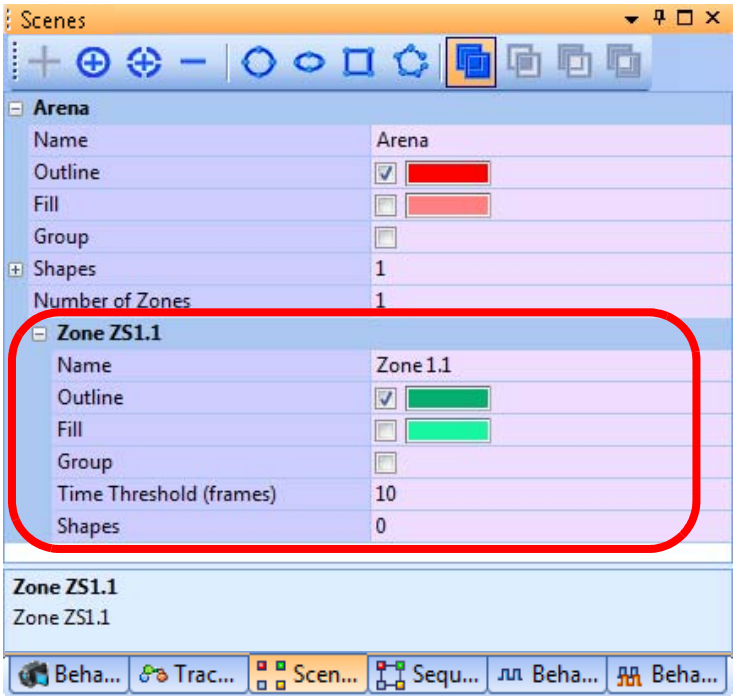
This section explains how to add a static zone in an arena.

In this example, we use a red circle for the arena.

1 On the **Scenes** tab click the **Add static zone to current arena** icon.

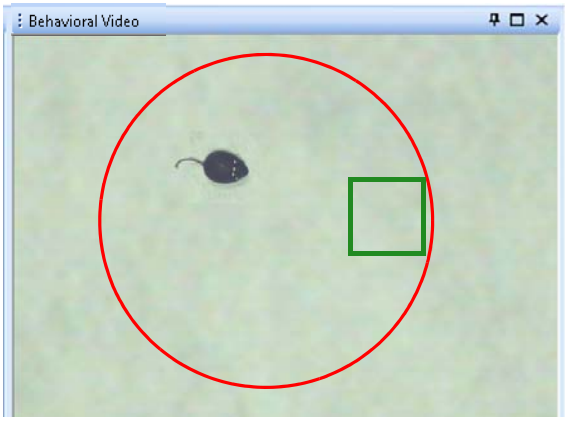


The parameters are displayed for the new Static Zone.

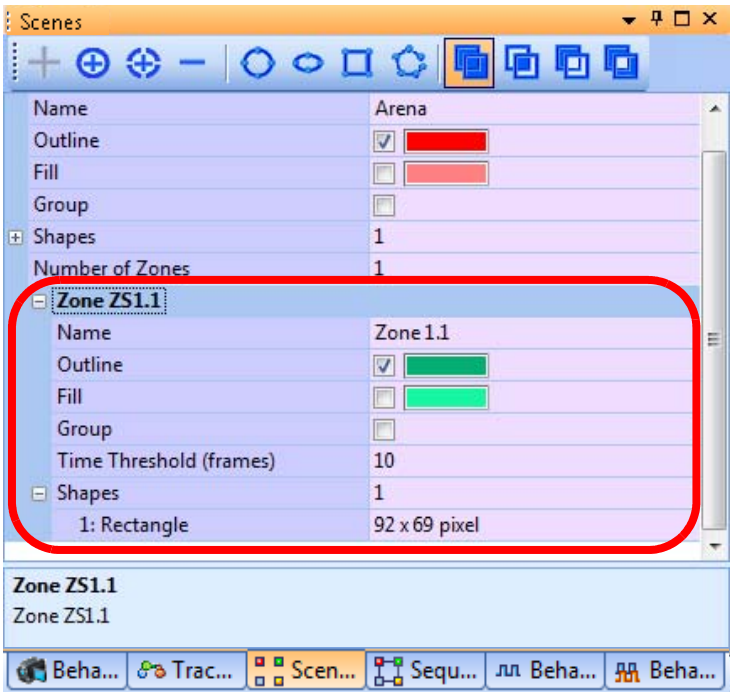


2 Select the drawing tool that makes the most sense for the zone that needs to be created. In the example that follows, the rectangle shape will be selected.

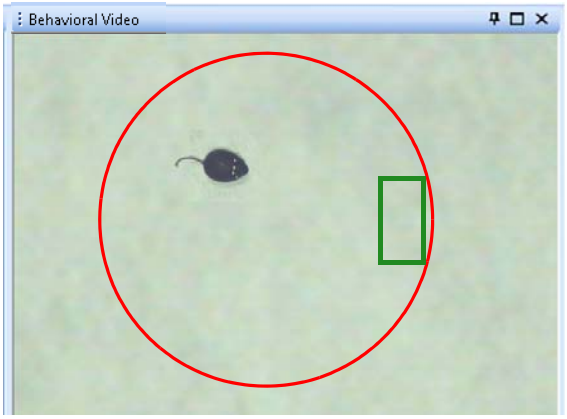
3 Draw the zone outline over the image corresponding to the first physical zone of interest. For the image below, the existing arena is a red circle, and a green rectangle has been added as the zone. This rectangle might represent, for example, a novel object the subject can view.



The Scenes tab shows the parameters for the zone shape.



4 If desired, adjust size and shape of the zone shape. For the image below, the rectangle was adjusted to better fit the actual object of interest.

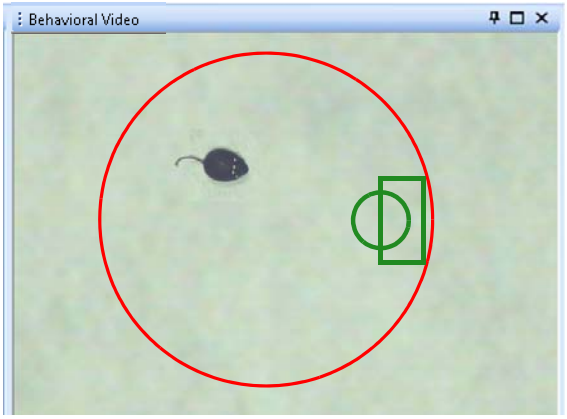


5 If a complex zone is desired, select a different drawing tool if needed, and the operator to be applied.

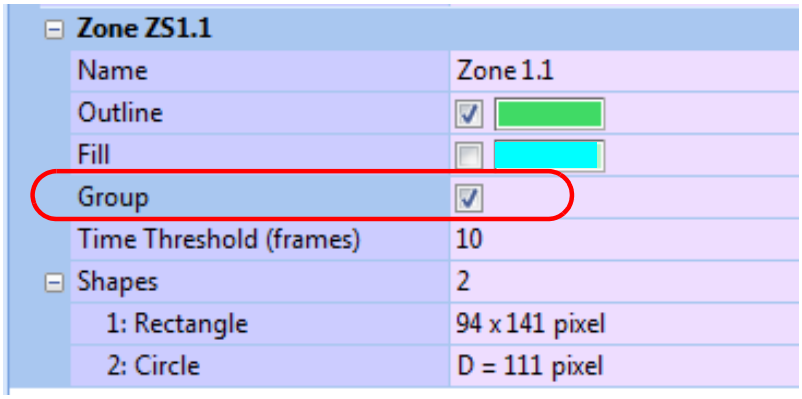
Note: Remember that complex zones can be created by applying operations to additional shapes as they are added to the zone. These operations are selected from the Scenes toolbar:

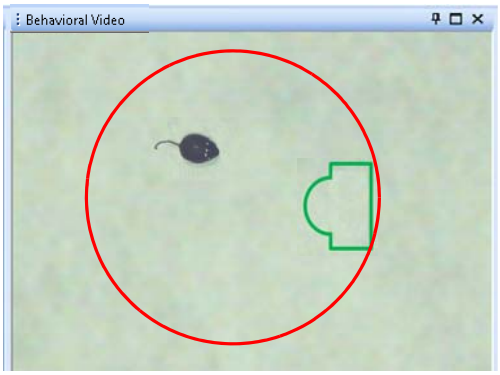
For a detailed description of the operators, see Section 7.10, “Defining the Arenas” on page 143.

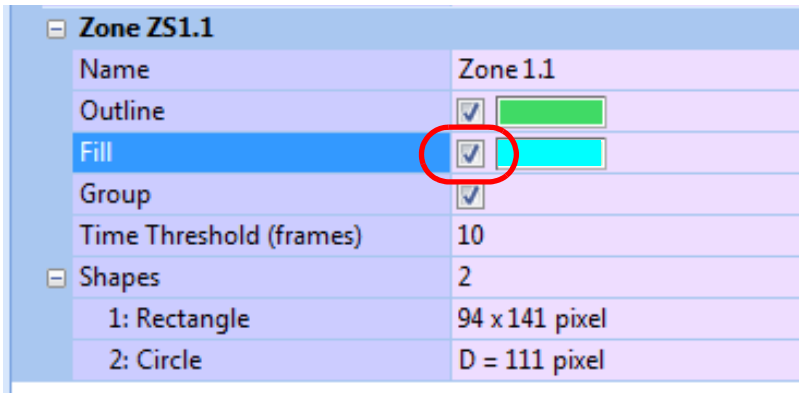
The image below shows an example of a complex static zone obtained using the **Union shapes** operator on round and rectangular shapes.

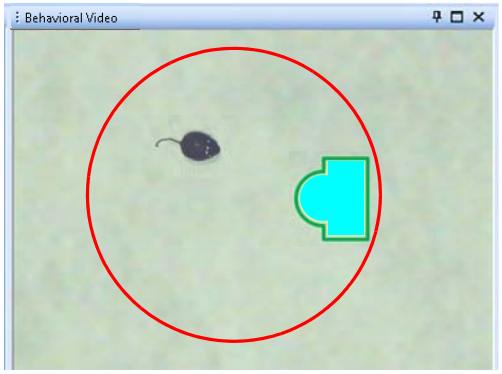


6 If desired you can group the shapes, so the system treats the combined shape as a single entity. You can also fill the shape with a color. See the examples below.






Zone ZS1.1
Name
Zone 1.1
Outline
Fill
Group
Time Threshold (frames)
10
Shapes
2
1: Rectangle
94 x 141 pixel
2: Circle
D = 111 pixel

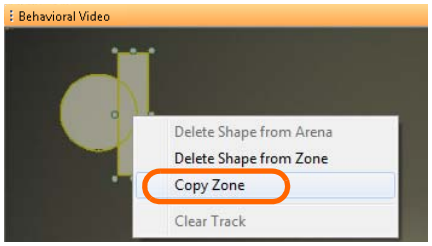

Behavioral Video

Note: If you have created a group shape (a combined zone object) you will be able to move the entire combined zone object as a unit.

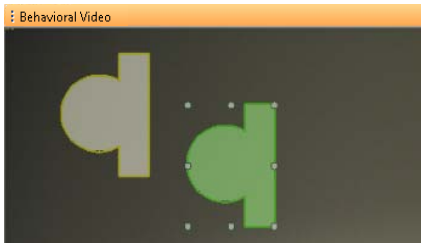
TIP

Copying a static zone

If you have one static zone and you want to create another identical static zone (whether it is has a single or multiple shapes), you can do so easily with the copy function. For example, if you have a zone like the one shown below, you can copy it as follows. Left click inside the zone to select it. Then right click to display the **Copy Zone** option. Click on **Copy Zone.**



The system will create a second zone of the same shape. You will see the second zone displayed in the Video window and also in the zones list in the **Scenes** tab.



If your initial zone was composed of multiple shapes, the system automatically groups both the initial and the new zones. You can observe this effect in the example above.

Note: This copying method works only for static zones, not dynamic zones.

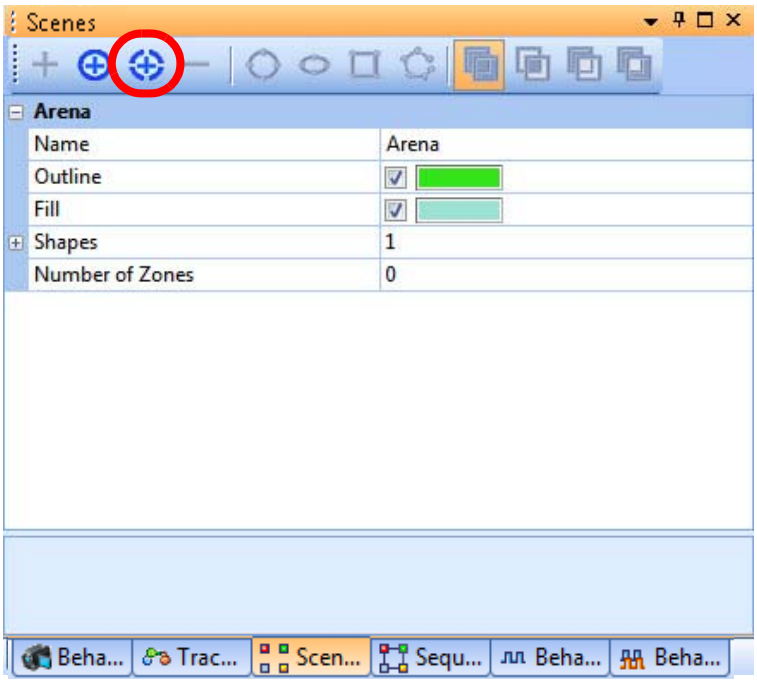
7 If necessary, adjust the value for the **Time Threshold (frames)** parameter. This parameter specifies the number of frames that an object must be detected in the zone before it is considered “present.” Whether an object is “present” or not can be used to determine that the zone-related event has become true. You can adjust this value at any time before you start recording. For a further description of this parameter, see Section 8.8.1, “Events Based on a Zone” on page 8-201.

8 Repeat Step 1 through Step 7 for each additional zone to be defined.

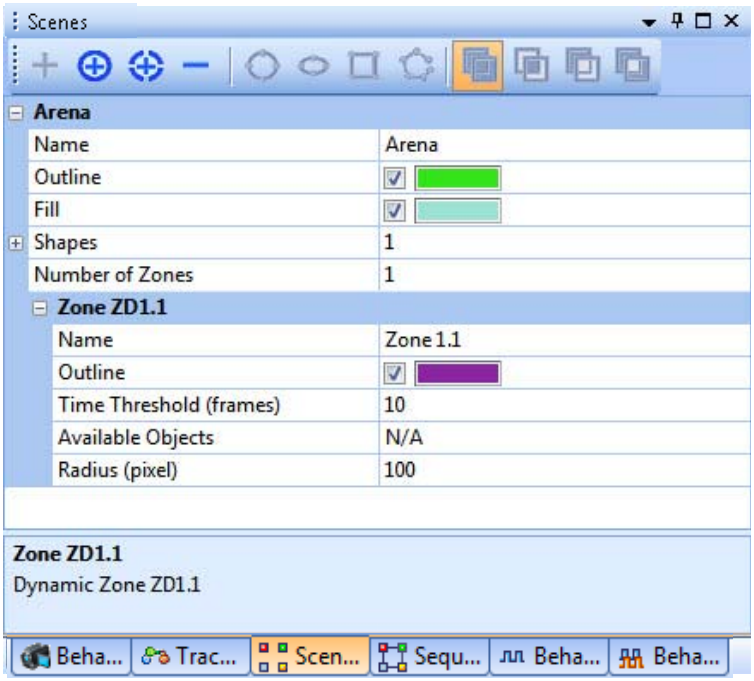
8.5.2 Adding a Dynamic Zone

Note: Dynamic Zones are not available for the Object Contour tracking mode.

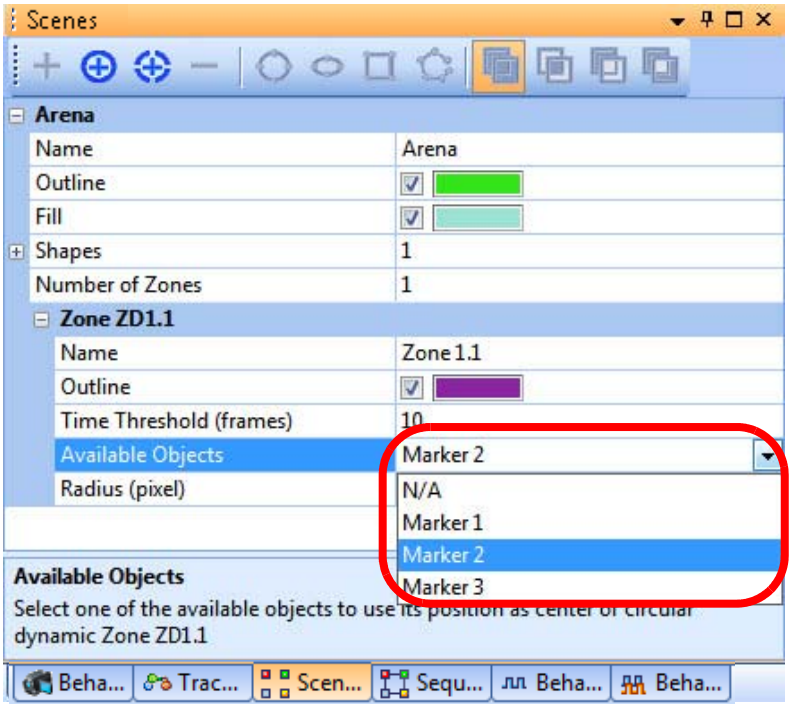
1 On the Scenes tab click the **Add dynamic zone to current arena** icon.



The parameters are displayed for the new Dynamic Zone.



2 In the **Available Objects** row, select the specific marker or LED on which the dynamic zone will be centered.



3 If necessary, adjust the values for the **Time Threshold (frames)** and **Radius** parameters.

The **Time Threshold (frames)** parameter specifies the number of frames that an object must be detected in the zone before it is considered “present.” Whether an object is “present” or not can be used to determine that the zone-related event has become true. You can adjust this value at any time before you start recording. For a further description of this parameter, see Section 8.8.1, “Events Based on a Zone” on page 8-201.

The **Radius** parameter specifies the radius of the dynamic zone from the geometric center of the LED or color marker.

4 Repeat Step 1 through Step 3 for each additional zone to be defined.

8.6 Defining Sequences of Zones

Note: Before defining sequences of zones, two or more zones must be defined.

TIP

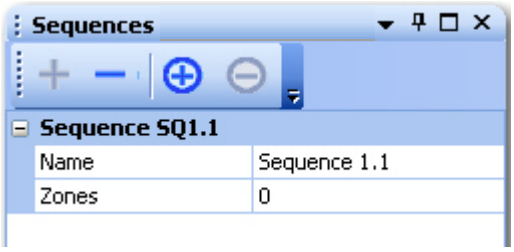
To view all windows in the user interface, reset to default layout

If some of the windows are not visible in the user interface, it may be helpful to restore the default screen layout. From the **Window** dropdown list, select **Layout**, then **Reset to Default Layout**.

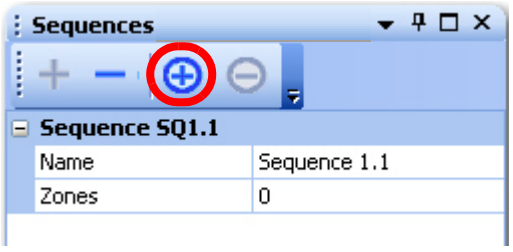
1 After two or more zones have been added, use the Sequences tab.

2 Click the **Add new sequence** icon to add a sequence.

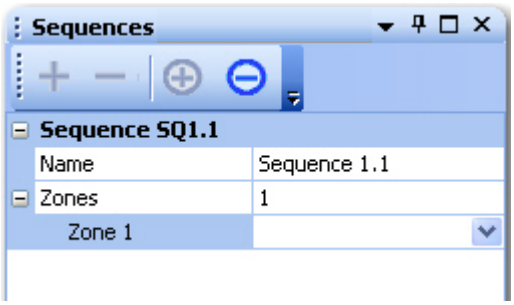
A new sequence is added to the tab.



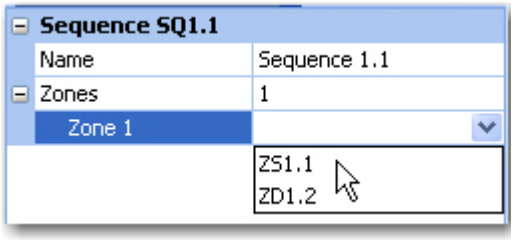
3 Click the **Add zone to selected sequence** icon to add a zone to the sequence.



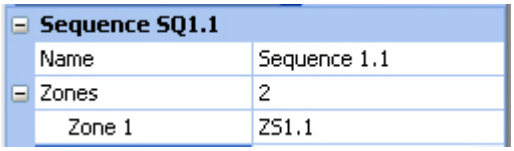
A zone is added to the sequence.



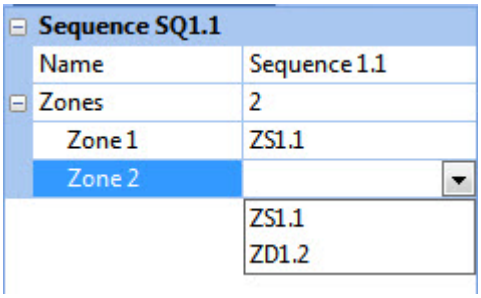
4 Select available zones from the drop down list for the new sequence.



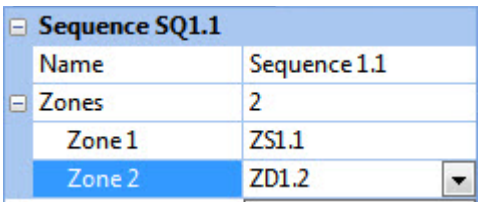
The selected zone is displayed in the **Zone 1** setting.



5 To add a second zone, repeat Step 3 and Step 4.



The selected zone is displayed in the **Zone 1** setting.



6 Repeat these steps for each additional sequence to be defined.

TIP

Understanding how sequences of zones are used

The purpose of defining a sequence of zones is to use this sequence to define a behavioral event. For example, you might configure an event in which the animal enters Zone 1 and receives a stimulus, then moves to Zone 2 to obtain a reward, then moves to Zone 3.

When you are configuring a sequence on zones, keep in mind how the system detects events based on these sequences, as described in Section 8.8.2, “Events Based on a Sequence of Zones” on page 203.

8.7 Modifying Arenas, Zones and Calibration During an Experiment

The system provides some flexibility in modifying arenas and zones in an experiment that already has one or more sessions recorded. For a description of these options and procedures, see these sections:

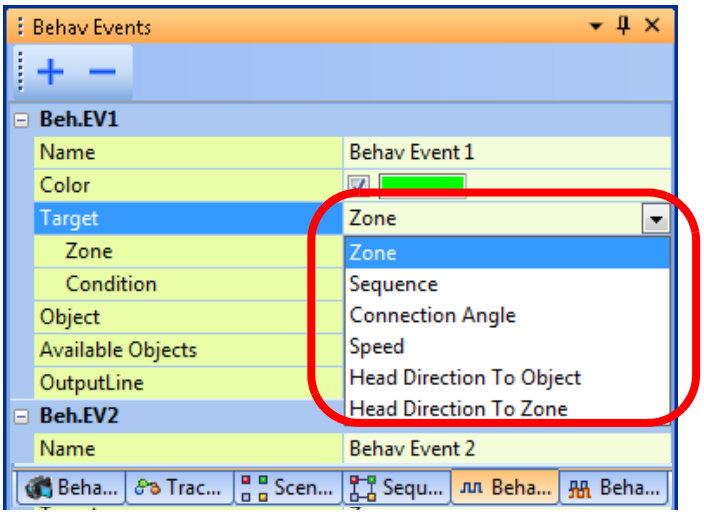
- Section 11.13, “Using the Overlay Feature during Analysis” on page 290
- Appendix D, Modifying Arenas and Zones

8.8 Creating Behavioral Events

The system can generate logical events based on images in the behavioral video stream. Logical events can be defined as TRUE when an animal enters or leaves a zone or completes a sequence, or when a specified parameter (the angle between two vectors, the animal's speed or the animal's head direction) is within a specified range.

1 In the **Events** tab, select the **Add new tracking event** icon to create an Event.

2 In the **Target** row, select the type of behavior to be associated with the event.



The available parameters vary according to the type of target selected.

The available targets depend on the type of tracking:

- For Object Contour tracking, the targets listed in the dropdown are **Zone**, **Sequence** and **Speed**.
- For LEDs and Color Markers, the targets listed in the dropdown are **Zone**, **Sequence**, **Angle**, **Speed**, **Head Direction To Object**, and **Head Direction To Zone**.

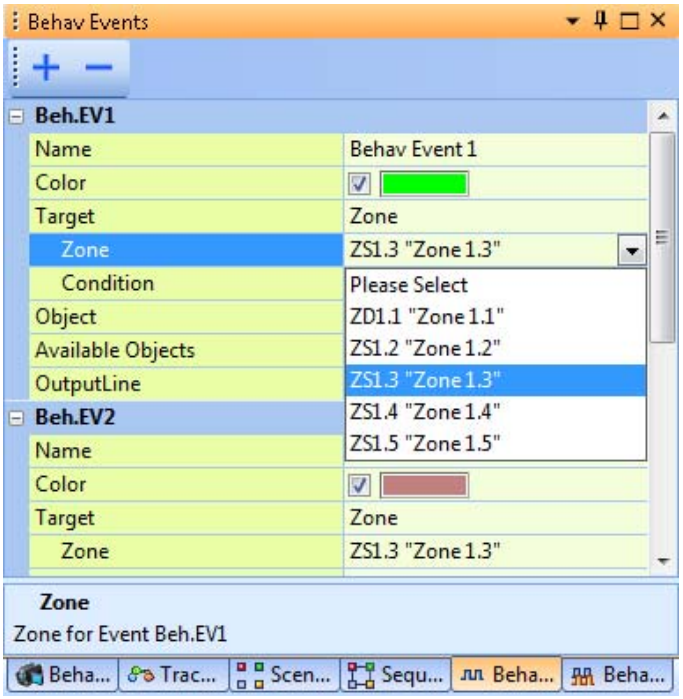
See the specific procedure for each type of target:

- Section 8.8.1, "Events Based on a Zone" on page 201
- Section 8.8.2, "Events Based on a Sequence of Zones" on page 203
- Section 8.8.3, "Events Based on a Connection Angle" on page 205
- Section 8.8.4, "Events Based on Speed" on page 208
- Section 8.8.5, "Events Based on Head Direction To Object" on page 211
- Section 8.8.6, "Events Based on Head Direction To Zone" on page 214

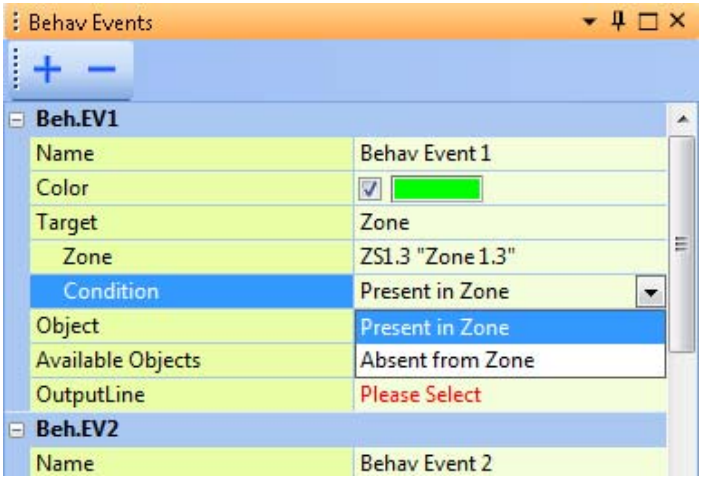
Note: In the sections that follow, you will see that there is a parameter in every Events tab—**Output Line** or **Output #**—to specify an output line. For information on this parameter, see Section 4.12, “Specifying Digital Outputs when Events Occur” on page 85. This parameter is optional—use it only if you want to transmit digital outputs from the DIGITAL OUTPUT port on the Trigger Box.

8.8.1 Events Based on a Zone

1. Select **Zone** as the **Target**.
2. Select a **Zone**.



3. Define the **Condition**.



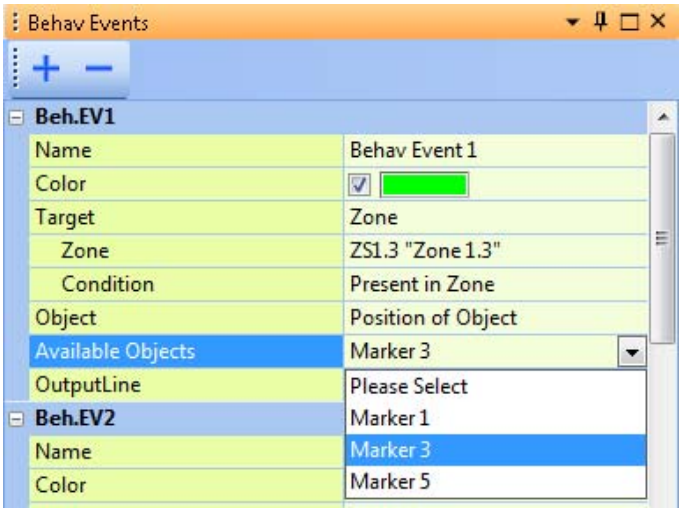
The **Present in Zone** condition is dependent on the **Time Threshold (frames)** parameter that was set in the Scenes tab (see Section 8.5.1, “Adding a Static Zone” on page 189 or Section 8.5.2, “Adding a Dynamic

Zone" on page 195 as applicable). Specifically:

- An object is considered present in a zone only when the zone wait time (the value of the **Time Threshold (frames)** parameter in the Scenes tab), in consecutive frames, has been satisfied. The Event is considered to be true on the first frame that the transition to "present" is detected.
- An object is considered absent from a zone in the first frame its coordinates are not in the zone.

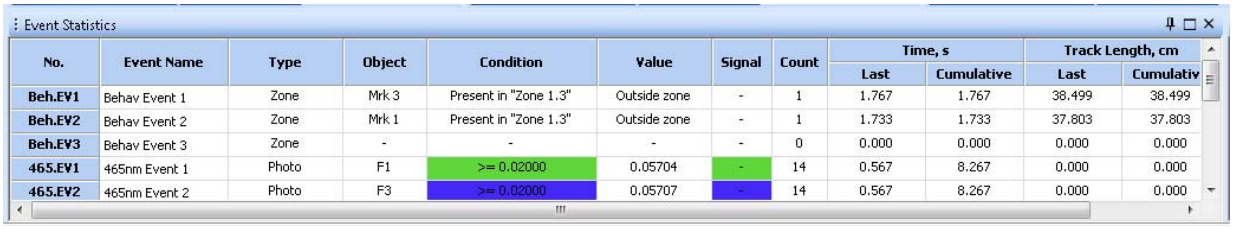
Note: (The meaning of "object" is explained in the **Available Objects** discussion in the next step.)

4 In the **Available Objects** row, select a tracked object to traverse the specified zone.



The event will become TRUE when the conditions are satisfied. At that point, the event count will be incremented and times and track lengths in the Event Statistics will be extended.

5 Event statistics can be viewed in the Event Statistics window of the user interface while the experiment is running.



No.	Event Name	Type	Object	Condition	Value	Signal	Count	Time, s		Track Length, cm	
								Last	Cumulative	Last	Cumulativ
Beh.EV1	Behav Event 1	Zone	Mrk 3	Present in "Zone 1.3"	Outside zone	-	1	1.767	1.767	38.499	38.499
Beh.EV2	Behav Event 2	Zone	Mrk 1	Present in "Zone 1.3"	Outside zone	-	1	1.733	1.733	37.803	37.803
Beh.EV3	Behav Event 3	Zone	-	-	-	-	0	0.000	0.000	0.000	0.000
465.EV1	465nm Event 1	Photo	F1	>= 0.02000	0.05704	-	14	0.567	8.267	0.000	0.000
465.EV2	465nm Event 2	Photo	F3	>= 0.02000	0.05707	-	14	0.567	8.267	0.000	0.000

6 (Optional) If you want to send a signal to an external device, configure the line number in the **Output Line** row. Follow the procedure in Section 4.12, "Specifying Digital Outputs when Events Occur" on page 85. This parameter is optional—use it only if you want to transmit digital outputs from the DIGITAL OUTPUT port on the Trigger Box.

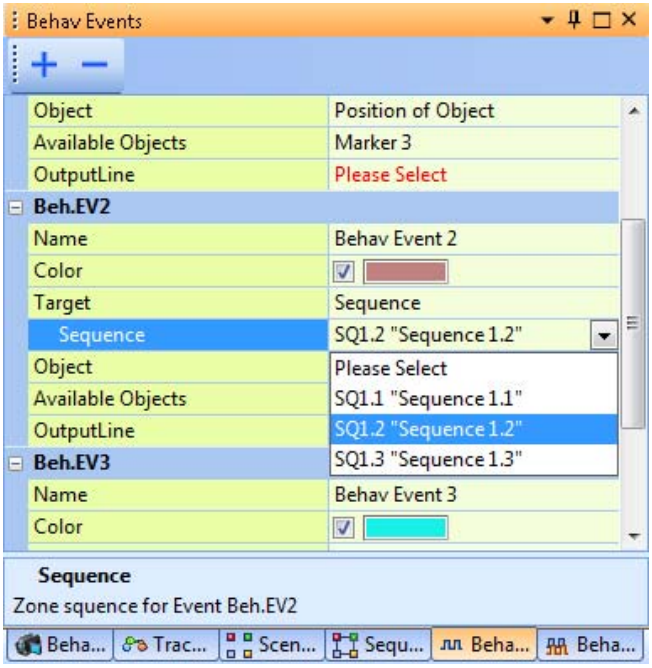
8.8.2 Events Based on a Sequence of Zones

This section explains how to configure events based on a sequence of zones and how the system detects and reports these events. It is recommended that you have a thorough understanding of the information in this section when you are defining sequences (as described in Section 8.6, “Defining Sequences of Zones” on page 197) and when you are creating events based on those sequences (as discussed below).

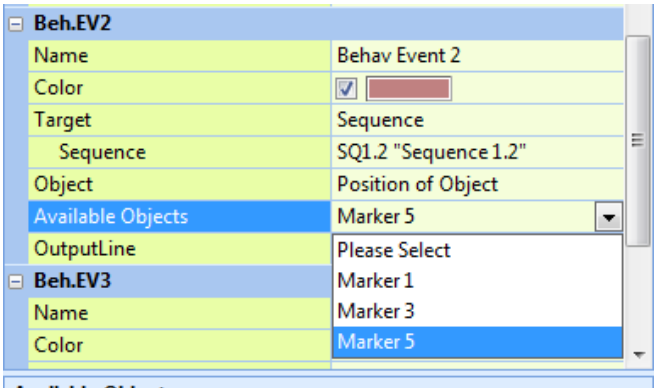
Procedure

1 Select **Sequence** as the **Target**.

2 Select a **Sequence**.

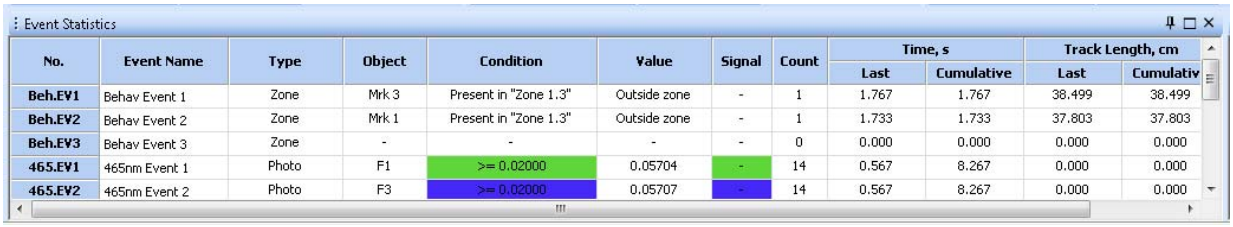


3 In the **Available Objects** row, select a tracked object to traverse the specified sequence of zones. In the example below, the tracked object is Marker 5.



The event will become TRUE when the conditions are satisfied. At that point, the event count will be incremented and times and track lengths in the Event Statistics will be extended.

4 Event statistics can be viewed in the Event Statistics window of the user interface while the experiment is running.



No.	Event Name	Type	Object	Condition	Value	Signal	Count	Time, s		Track Length, cm	
								Last	Cumulative	Last	Cumulativ
Beh.EV1	Behav Event 1	Zone	Mrk 3	Present in "Zone 1.3"	Outside zone	-	1	1.767	1.767	38.499	38.499
Beh.EV2	Behav Event 2	Zone	Mrk 1	Present in "Zone 1.3"	Outside zone	-	1	1.733	1.733	37.803	37.803
Beh.EV3	Behav Event 3	Zone	-	-	-	-	0	0.000	0.000	0.000	0.000
465.EV1	465nm Event 1	Photo	F1	>= 0.02000	0.05704	-	14	0.567	8.267	0.000	0.000
465.EV2	465nm Event 2	Photo	F3	>= 0.02000	0.05707	-	14	0.567	8.267	0.000	0.000

5 (Optional) If you want to send a signal to an external device, configure the line number in the **Output Line** row. Follow the procedure in Section 4.12, “Specifying Digital Outputs when Events Occur” on page 85. This parameter is optional—use it only if you want to transmit digital outputs from the DIGITAL OUTPUT port on the Trigger Box.

Understanding how the system interprets events based on sequences

The system treats sequence-based events “strictly.” Any interruption in the traversing of a sequence results in the system restarting the entire sequence from the beginning. The following conditions can cause a sequence to restart:

- Loss of tracking of the object fulfilling the sequence for even one frame
- Exit from the arena by the object fulfilling the sequence for even one frame
- Entry into any zone not in the sequence
- Entry into a zone that is not the next zone specified in the sequence (for example, a sequence is configured as Zone1 > Zone2 > Zone3, and the object enters and exits Zone1 then enters Zone3)
- Exit from any zone in the sequence followed by re-entry into that zone before the sequence is completed

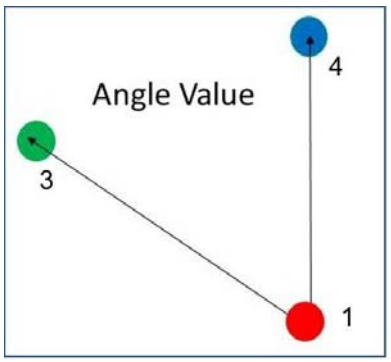
If a sequence restarts, it means that the event has not occurred. In that case, the system does not increment any counters and it resets the timer.

The system computes the track length and time for a sequence event as follows. Consider a sequence that consists of an object entering Zone1, then exiting Zone1, and some time later entering Zone2 and then exiting Zone2. The system starts measuring track length and time when the object enters Zone1 and it stops measuring track length and time when the object exits Zone2. The track length and time that the object spends between Zone1 and Zone2 are included in the total measurements.

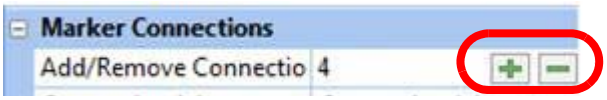
8.8.3 Events Based on a Connection Angle

This section explains how to define and specify values for behavioral events based on the angle between two vectors. The event is generated and recorded when the objects are within or outside of a user-defined angle and tolerance. For example, an event could be generated when the animal's front leg is bent more than a certain angle.

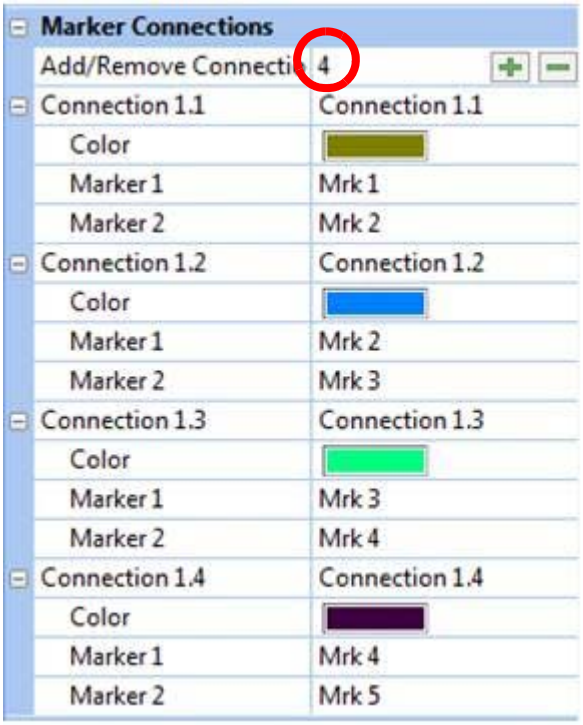
The procedure for creating an angle-based event involves defining *connections* between pairs of markers (or LEDs) and then defining an *angle* between a pair of connections. In the example shown below, one connection is defined from the red marker to the green marker, and a second connection is defined from the red marker to the blue marker. Then the angle of interest (Angle Value) is specified as the angle those two connections, in degrees.



1 Add connections by clicking on the **+** icon in the **Add/Remove Connections** row in the Tracking tab.



2 Define two or more connections. In the example below, the user is defining four connections.



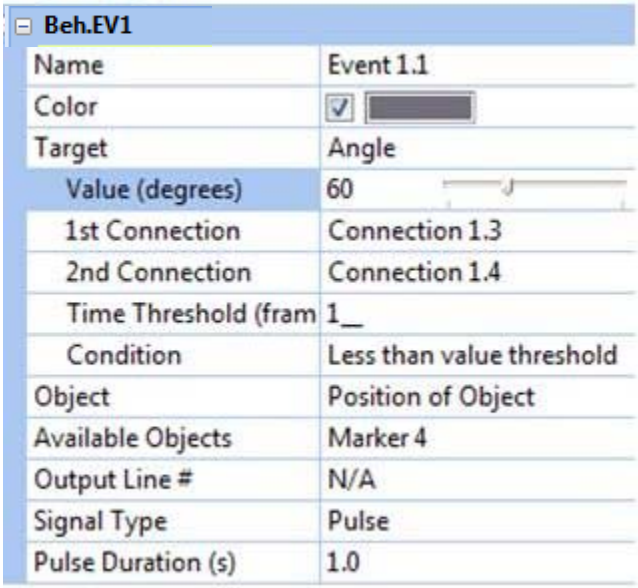
3 In the Behavioral Events tab, select **Angle** as the **Target**.

4 Specify the threshold angle **Value (degrees)** and the additional parameters in the **Event** group. In the example below, if the **Angle** is less than 60 degrees for at least one frame (the **Time Threshold**), the event becomes TRUE.

Note: **Time Threshold (frames)** specifies the number of frames for which the **Condition** must be met for the event to be considered true, 0 to 999 (default 10).

The **Color** selection (optional) defines the color the system will use to highlight the event in the Event Statistics frame, provided that the event becomes TRUE.

The **Available Objects** selection (optional) instructs the Event Statistics to display the track length of the selected object during the time that the event is TRUE.



When the event becomes TRUE, the event count will be incremented and times and track lengths in the Event Statistics will be recomputed and updated.

5 Event statistics can be viewed in the Event Statistics window of the user interface while the experiment is running.

6 (Optional) If you want to send a signal to an external device, configure the line number in the **Output Line** row. Follow the procedure in Section 4.12, “Specifying Digital Outputs when Events Occur” on page 85. This parameter is optional—use it only if you want to transmit digital outputs from the DIGITAL OUTPUT port on the Trigger Box.

8.8.4 Events Based on Speed

This section explains the settings for the calculation of the animal's speed. It is not necessary to adjust any of these settings if you do not need data on the animal's speed (or if you are satisfied with the default values).

Speed Averaging Interval (s) in the Global Config tab

The **Speed Averaging Interval (s)** specifies the time period or window (in seconds) over which the average speed is calculated. This setting is used to minimize jitter in the calculation of the speed of a tracked object. The default value is 0, which means no averaging. You can adjust this setting to any value from 0 to 1.0 in increments of 0.1s (0.0, 0.1, 0.2, ... 0.9, 1.0).

When this parameter is set to 0, the speed is computed for two sequential frames. No averaging is performed. If you see that the jitter or rapid variation in the speed is causing false detection of speed events, consider setting the **Speed Averaging Interval (s)** to a non-zero value.

For example:

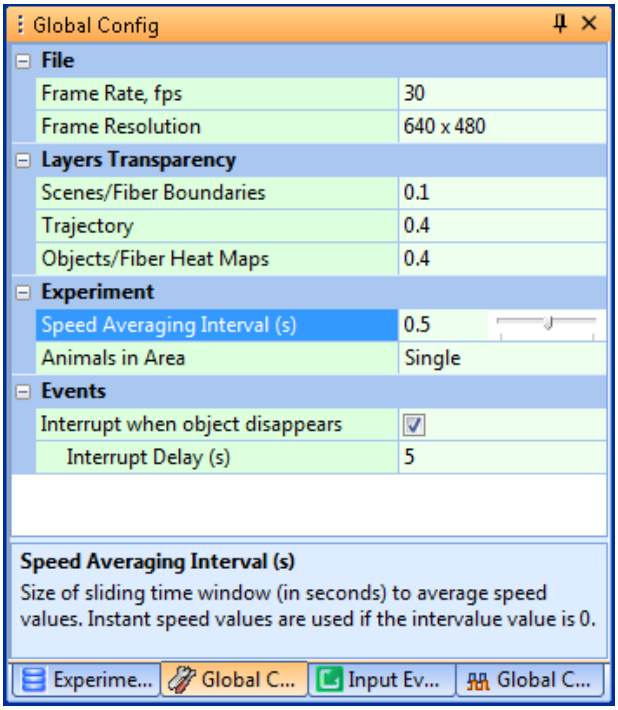
- Assuming the frame rate of the camera is 30 frames per second (fps), a **Speed Averaging Interval (s)** setting of 0 means that the animal's speed would be calculated between sequential frames (30 times per second). Therefore, the speed may jitter.
- Assuming a camera frame rate of 30 fps and a **Speed Averaging Interval (s)** setting of 0.5 (0.5 second), the average speed would be calculated as follows:

  Average speed for the 16th frame =
  { (animal's location in the 16th frame) –
  (animal's location in the 1st frame) } ***/*** (0.5 second)

  Average speed for the 17th frame =
  { (animal's location in the 17th frame) –
  (animal's location in the 2nd frame) } ***/*** (0.5 second)

Procedure

1 Ensure that the **Speed Averaging Interval (s)** setting is configured in the Global Config tab according to the needs of your experiment.



2 In the Events tab, specify the **Target** as **Speed**.

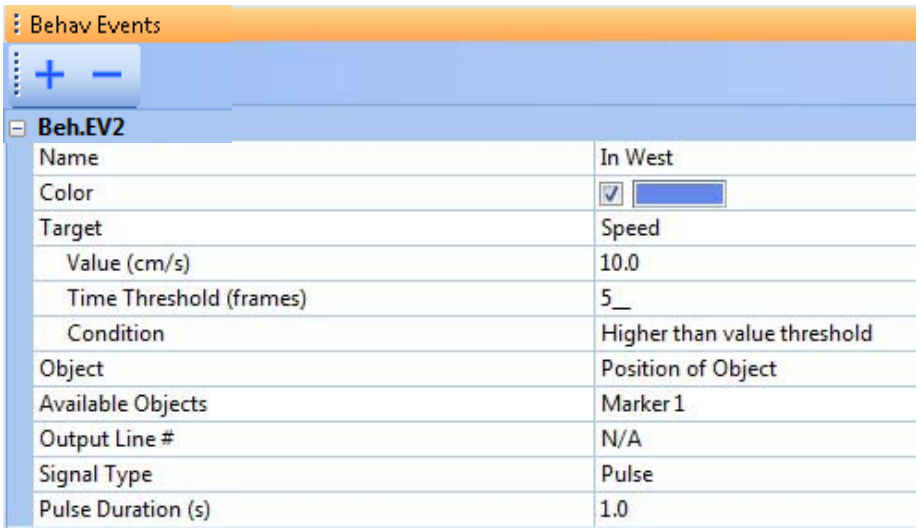
3 Specify the speed (**Value**), **Time Threshold** and **Condition**, plus any additional parameters as needed in the **Event** group. In the example below, if Marker 1 moves faster than 10.0 cm/s for 5 frames the event becomes TRUE.

Note: **Time Threshold (frames)** specifies the number of frames for which the **Condition** must be met for the event to be considered true, 0 to 999 (default 10).

Speed is recorded in pixels/second unless the camera is calibrated.

The **Color** selection (optional) defines the color the system will use to highlight the event in the Event Statistics frame, provided that the event becomes TRUE.

The **Available Objects** selection specifies the object to be tracked. The Event Statistics window displays the track length of the selected object during the time that the event is TRUE.



When the event becomes TRUE, the event count will be incremented and times and track lengths in the Event Statistics will be recomputed and updated.

4 Event statistics can be viewed in the Event Statistics window of the user interface while the experiment is running.

5 (Optional) If you want to send a signal to an external device, configure the line number in the **Output Line** row. Follow the procedure in Section 4.12, “Specifying Digital Outputs when Events Occur” on page 85. This parameter is optional—use it only if you want to transmit digital outputs from the DIGITAL OUTPUT port on the Trigger Box.

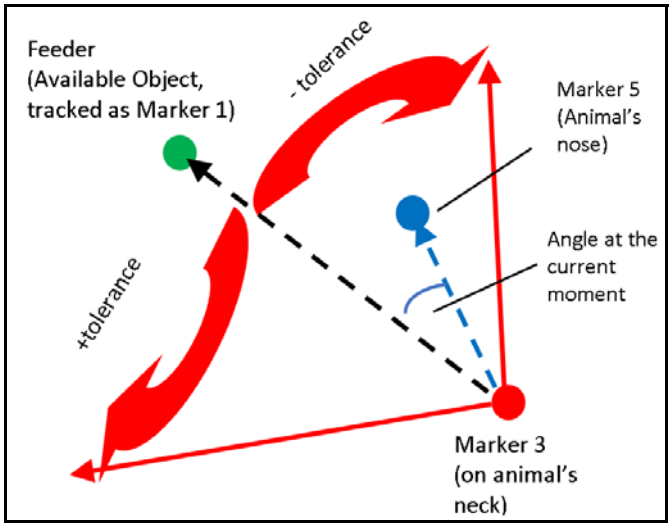
8.8.5 Events Based on Head Direction To Object

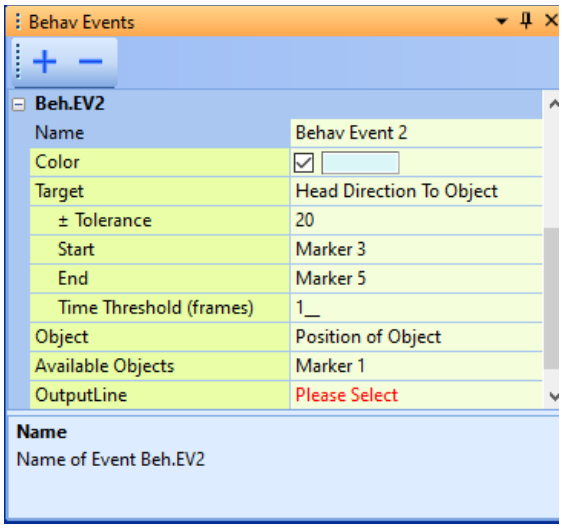
This section explains how to specify values for behavioral events based on the animal's head direction with respect to an object in the arena. The head direction is determined by markers or LEDs on the animal's head or headstage.

The system requires two vectors:

- The head-direction vector, which points from the **Start** LED or marker to the **End** LED or marker
- The target vector points from the **Start** LED or marker to the **Available Object** in the arena. This object could be, for example, a lever or some moving object of interest. Such object should have a marker or LED on it. The marker or LED used as an **Available Object** has to be tracked as well, so the system will know its position.

  For example:





This event will be triggered when the angle between vectors Marker 3 – Marker 5 and Marker 3 – Marker 1 is within ± 20 degrees and stays within this diapason for at least one frame.

Event statistics can be viewed in the Event Statistics window of the user interface while the experiment is running. The trajectory length for this event is computed using the Start point (Marker 3 in our example).

(Optional) If you want to send a signal to an external device, configure the line number in the **Output Line** row. Follow the procedure in Section 4.12, "Specifying Digital Outputs when Events Occur" on page 85. This parameter is optional—use it only if you want to transmit digital outputs from the DIGITAL OUTPUT port on the Trigger Box.

Head direction with respect to average center of gravity

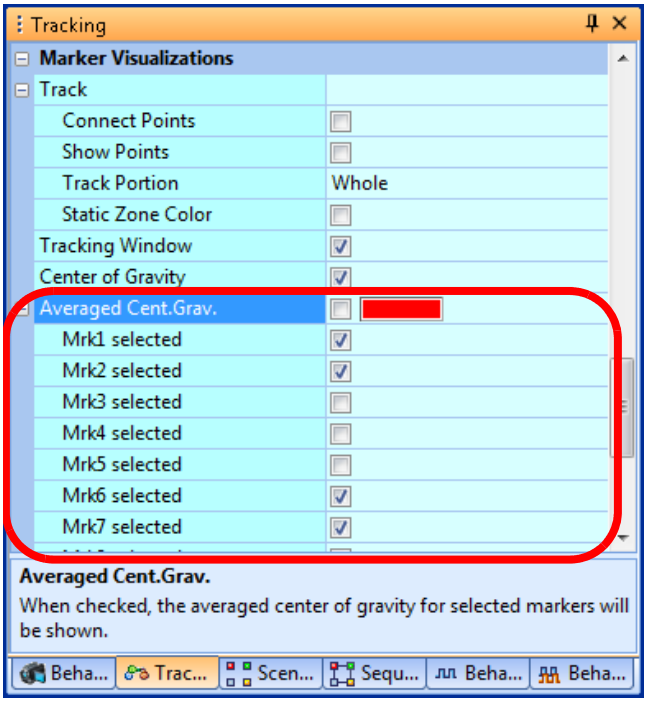
You can specify the head direction with respect to the averaged center of gravity of a group of LEDs or markers. The specific LEDs or markers to be included in the averaged center of gravity determination are set in the **Marker Visualizations** or **LED Visualizations** section of the Tracking tab.

The system determines two vectors:

- The head-direction vector, which points from the **Start** LED or marker to the **End** LED or marker
- The target vector, which points from the **Start** LED or marker to the averaged center of gravity (**Averaged Cent.Grav.**) of all specified LEDs or markers

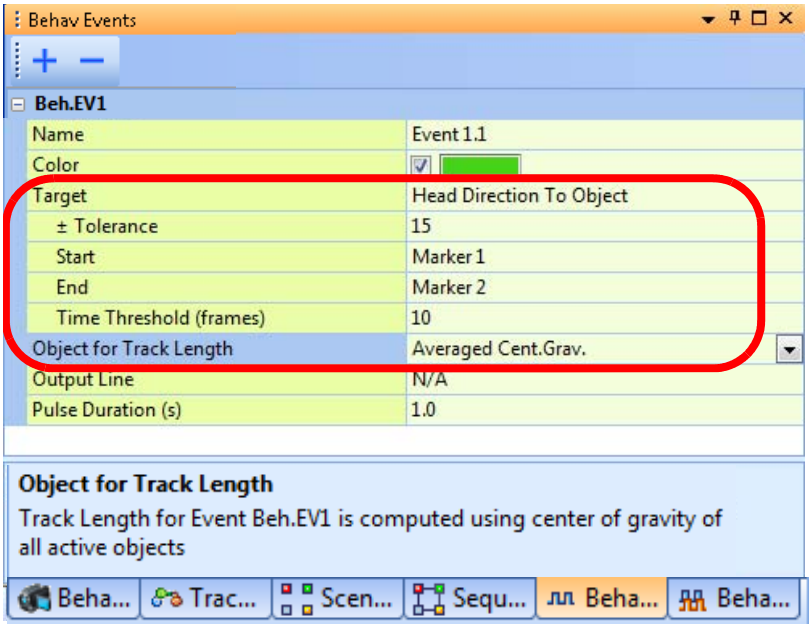
When the head-direction vector points toward the target vector within a customer specified tolerance and duration, the event is considered true.

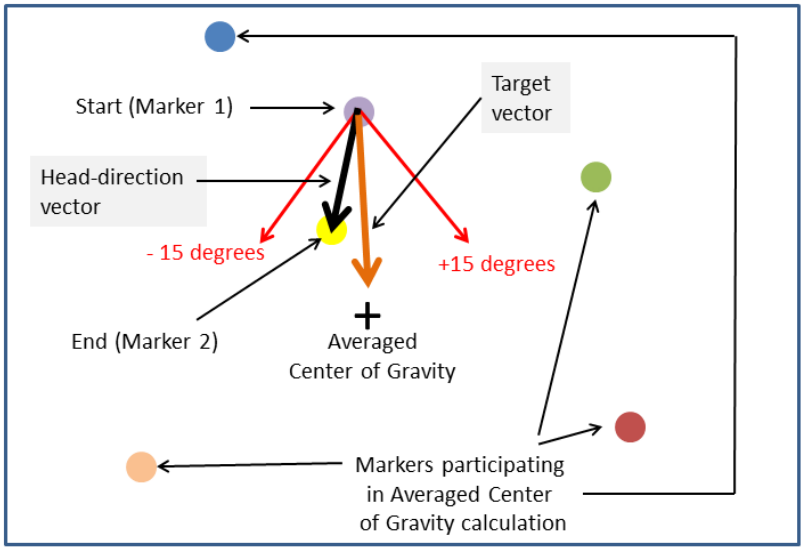
1 In the Tracking tab, specify the LEDs or markers to be included in the **Averaged Cent.Grav.** determination.



2 In the Events tab, specify the **Target** as **Head Direction To Object** and specify the **Object for Track Length** as **Averaged Cent.Grav.** Also specify the value of the **± Tolerance** (in degrees) with respect to the reference vector,

and the **Time Threshold (frames)**, which is the minimum number of frames the condition must be true to cause an event to be considered true.





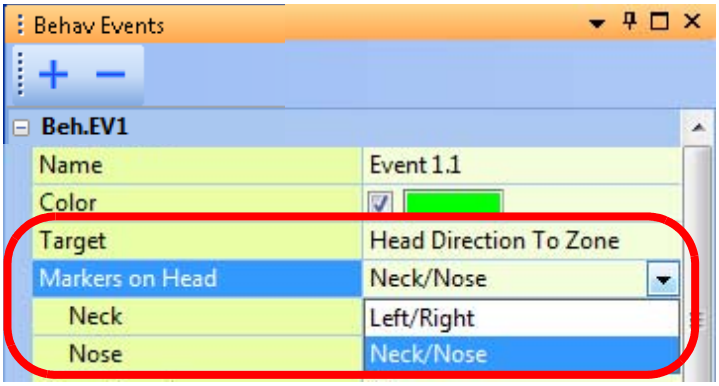
3 Event statistics can be viewed in the Event Statistics window of the user interface while the experiment is running.

4 (Optional) If you want to send a signal to an external device, configure the line number in the **Output Line** row. Follow the procedure in Section 4.12, “Specifying Digital Outputs when Events Occur” on page 85. This parameter is optional—use it only if you want to transmit digital outputs from the DIGITAL OUTPUT port on the Trigger Box.

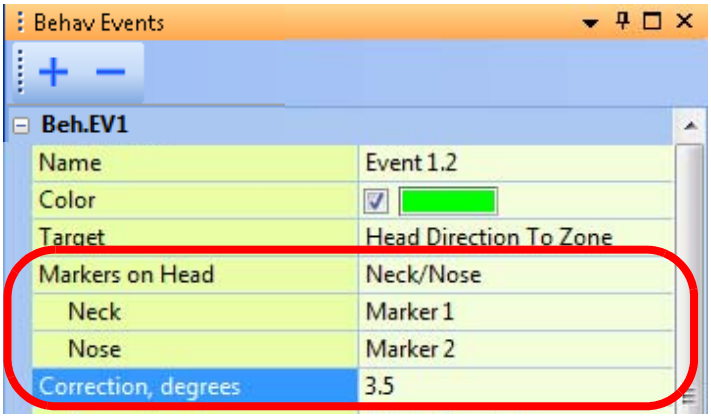
8.8.6 Events Based on Head Direction To Zone

This section explains how to specify values for behavioral events based on the animal's head direction with respect to a zone in the arena. The head direction is determined by markers or LEDs on the animal's head or headstage. For example, an event could be generated when the animal's head faces toward any portion of a feeding zone.

1 In the Event tab, specify the **Target** (**Head Direction To Zone**) and the location of LEDs or markers on the animal's head (either **Left/Right** or **Neck/Nose**).

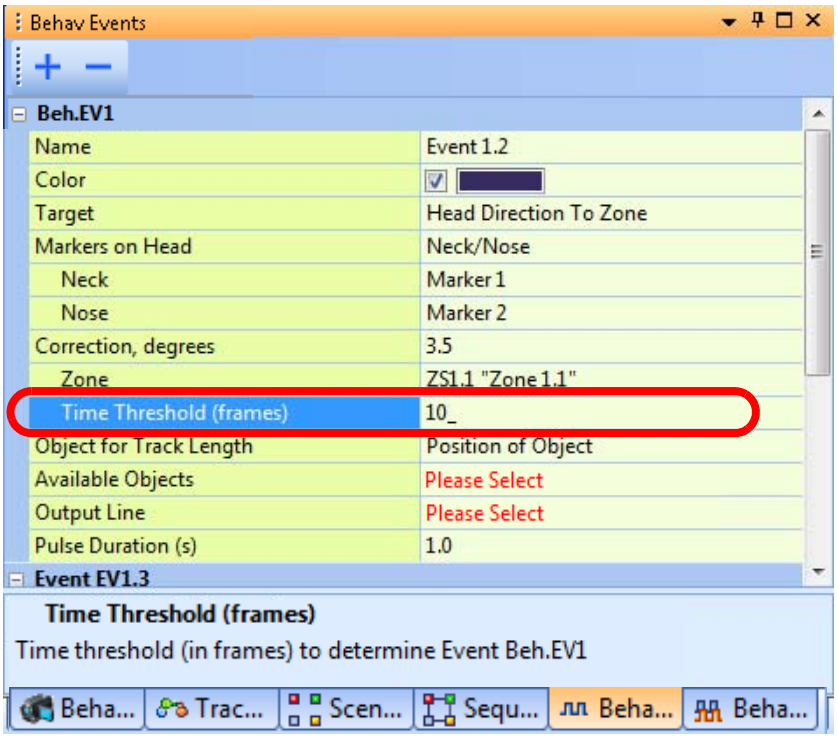


2 Specify which LEDs or markers are being tracked and a correction value (if any) to compensate for the possible offset of the LEDs or markers from their intended mounting positions on the animal's head.



3 In the **Zone** row, specify the zone of interest.

4 Specify the value of **Time Threshold (frames)**, which is the minimum number of frames the condition must be true to cause an event to be considered true.

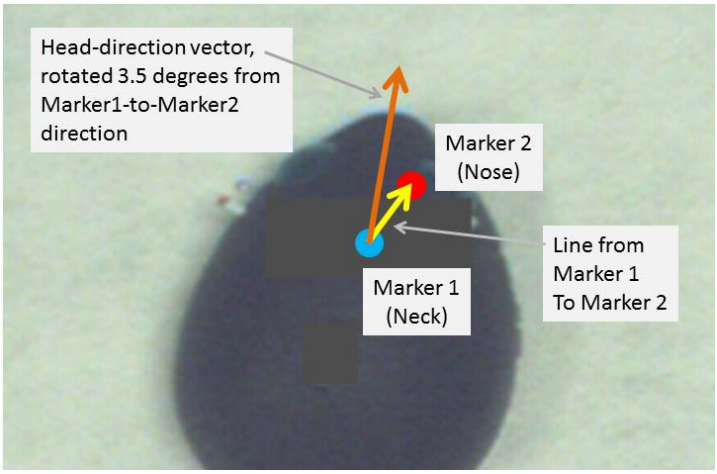


5 Specify the **Object for Track Length** as either **Position of Object** or **Averaged Cent.Grav.**

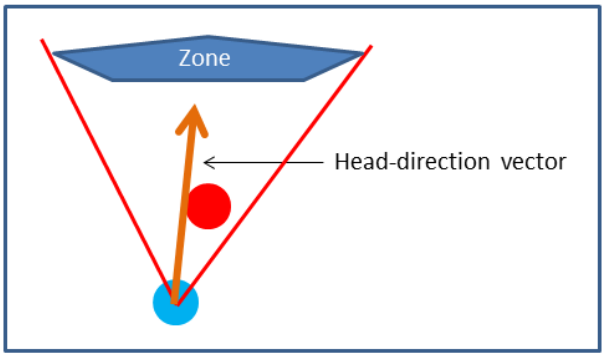
- If you specify **Position of Object**, also specify a particular LED or marker from the **Available Objects** dropdown list.

- If you specify **Averaged Cent.Grav.** ensure that you have selected LEDs or markers to be included in the **Averaged Cent.Grav.** in the **LED Visualizations** or the **Marker Visualizations** section of the Tracking tab.

The diagram below shows the head-direction vector rotated from the Marker1-to-Marker2 direction by an amount equal to the **Correction, degrees** setting (3.5 degrees in the above configuration example).



This diagram shows the head-direction vector pointing toward the selected zone.



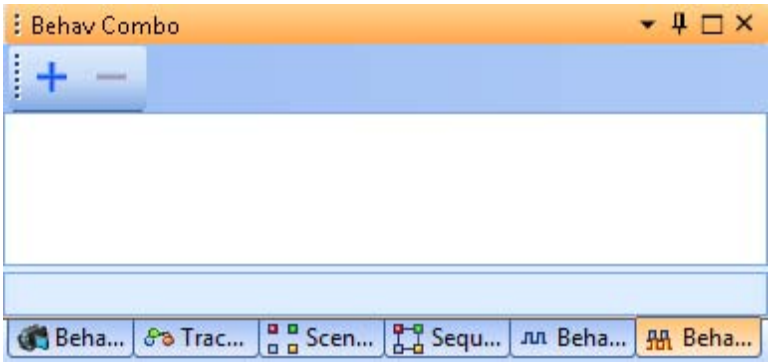
6 Event statistics can be viewed in the Event Statistics window of the user interface while the experiment is running.

7 (Optional) If you want to send a signal to an external device, configure the line number in the **Output Line** row. Follow the procedure in Section 4.12, “Specifying Digital Outputs when Events Occur” on page 85. This parameter is optional—use it only if you want to transmit digital outputs from the DIGITAL OUTPUT port on the Trigger Box.

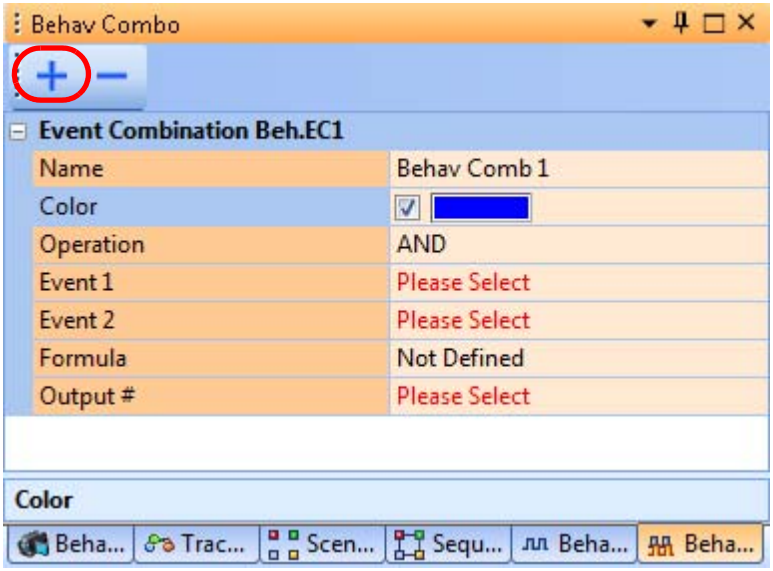
8.9 Creating Behavioral Combination Events

After you create individual events, you have the option of creating combination events. For example you might want to create a combination behavioral event that becomes true when a subject [1] enters a particular zone AND [2] turns its head toward a certain object. Use the following procedure.

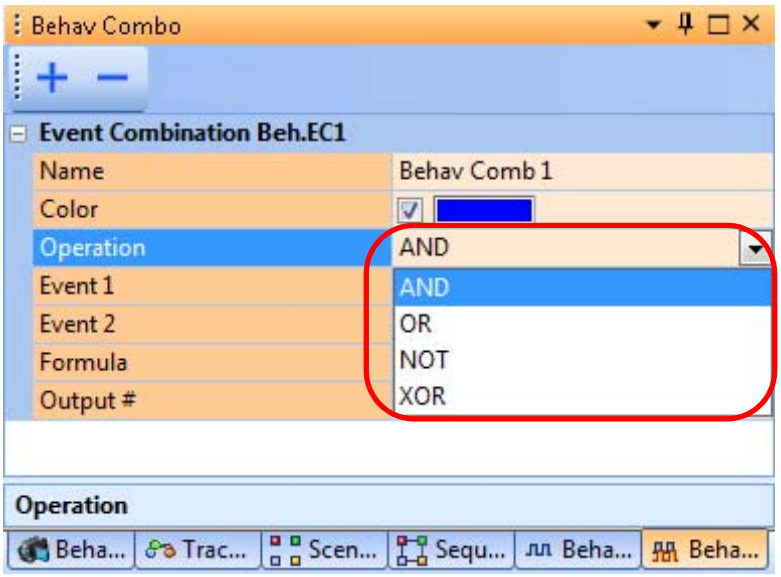
1 Click the **Combination Events** tab.



2 Click the **Add new combination of tracking events** icon.



3 Select the logical operation to use.



Operation (see the image above)—Used to specify events that are combinations of one or more existing events on which logical operations are performed. The existing events can be any single or combination event. The four logical operators are **NOT**, **AND**, **OR** and **XOR**:

NOT—For single events, an event can be defined which is the opposite of it by using the **NOT** operator. The new event is true when the original event is not true and vice versa (the new event is not true when the original event is true). For example, **NOT**[animal is inside feeding zone] means the animal is not inside the feeding zone.

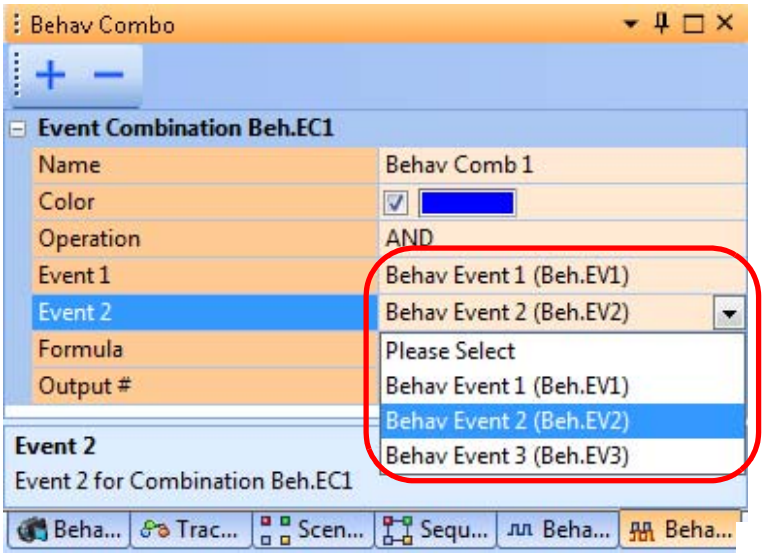
For the combination of two events, the **AND**, **OR** and **XOR** operators are available. The resulting combination event is considered true under the following conditions:

AND—Both of the individual events are true

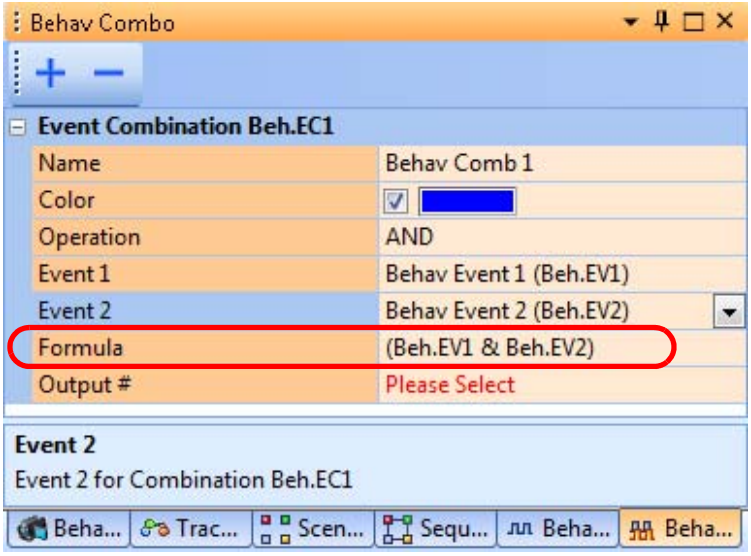
OR—Either one of the individual events is true, or both of the individual events are true

XOR—Either one of the individual events is true, but not both of them

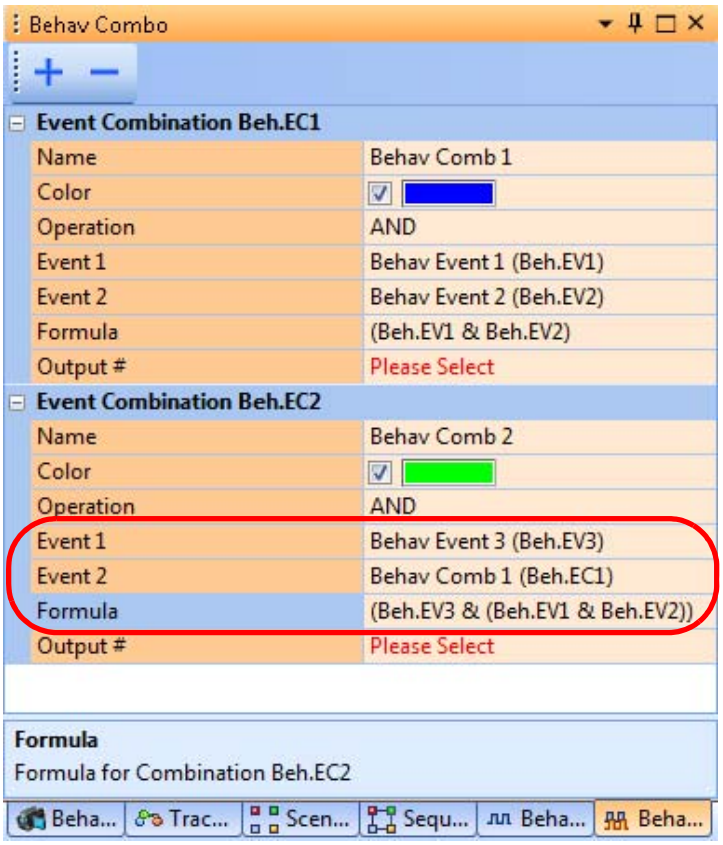
4 Select each event in the combination from the drop down list of available events.



The system generates an entry in the Formula line based on the Operation and Event(s) that the user selected in the above steps.



5 If desired, a Combination Event can contain other Combination Events, as shown in the following example. Events of arbitrary complexity can be created by combining already combined events with combined or non-combined (single) events.



Note: For information on the **Output #** parameter in the Events tab, see Section 4.12, “Specifying Digital Outputs when Events Occur” on page 85. This parameter is optional—use it only if you want to transmit digital outputs from the DIGITAL OUTPUT port on the Trigger Box.

Note: You can also create *global combination events* across multiple video sources. For that procedure, see Section 9.3, “Creating Global Combination Output Events” on page 228.

8.10 Detecting Proximity Events

If you are tracking an animal by means of LEDs or color markers, you can use the dynamic zones capability to detect when a tracked LED or marker moves within a certain distance of another animal.

Example—Social interaction—Proximity of animal to another animal

This example defines a behavioral event for an animal's proximity to another animal. For one of the animals, you can define a dynamic zone of a certain radius around a marker on the animal's head. Then you can cause an event to be considered true when a marker on the head of a second animal touches or crosses the boundary of the dynamic zone around the first animal. In this example, we will set the radius of the zone to 3cm, so if the animals approach within 3cm, the event will be considered true.

In the Tracking toolbar:

1. Select **LED** or **Color Markers** tracking mode. In this example, we will select markers.

In the Tracking tab:

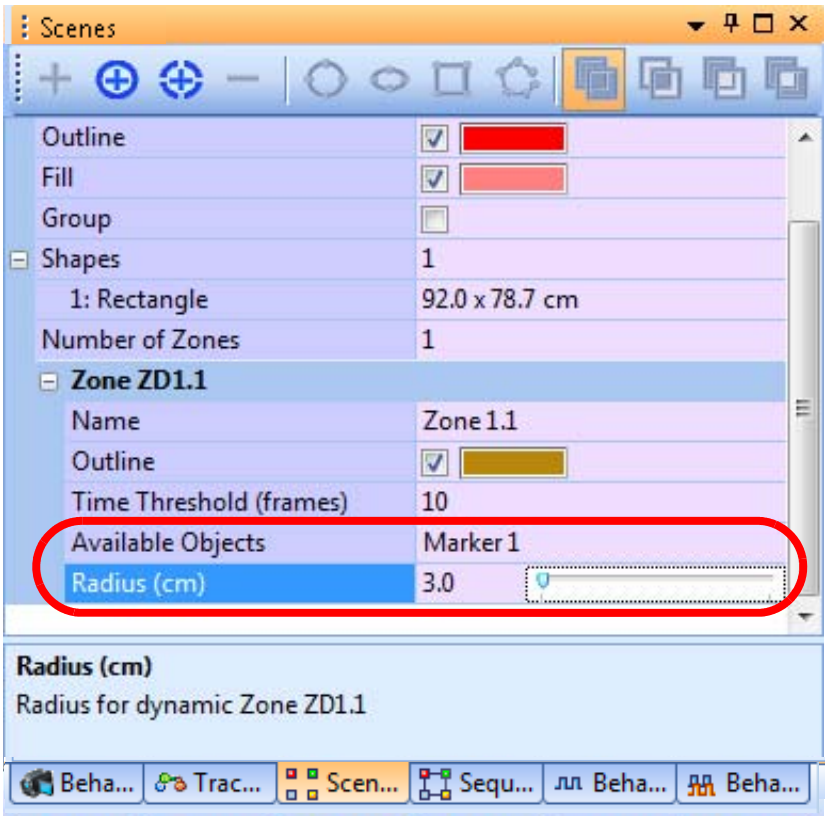
2. Select the checkboxes for Marker 1 (the marker on the head of the first animal) and Marker 2 (the marker on the head of the second animal).

In the Sources tab:

3. Calibrate the arena in cm.
4. Select the **Use Calibration** checkbox.

In the Scenes tab:

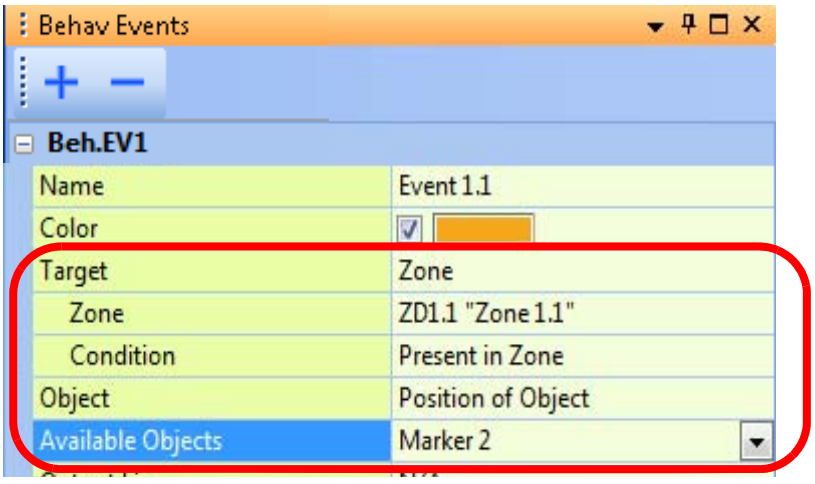
5. Draw the arena.
6. Define a dynamic zone around Marker 1.
7. Set **Available Objects** to Marker 1 and **Radius (cm)** to 3.0cm.



In the Events tab:

8 Set the **Target** to the dynamic zone that was established previously for the 3cm radius around Marker 1 on the head of the first animal.

9 Set the **Condition** as Present in Zone and **Available Objects** as Marker 2 (the marker on the second animal's head).



When Marker 2 comes to within 3cm of Marker 1, regardless of which animal is moving, the event (EV1.1 in this example) becomes true.

10 Event statistics can be viewed in the Event Statistics window of the user interface while the experiment is running.

11 (Optional) If you want to send a signal to an external device, configure the line number in the **Output Line** row. Follow the procedure in Section 4.12, “Specifying Digital Outputs when Events Occur” on page 85. This parameter is optional—use it only if you want to transmit digital outputs from the DIGITAL OUTPUT port on the Trigger Box.

8.11 Where to Go Next

After you have completed the configuration of the behavioral camera, go to Chapter 9, Configuring and Viewing Global Events.

Chapter 9
Configuring and Viewing Global Events

9.1 Getting Started

The procedures in the previous chapters of this book configured individual events and combination events in the photometry and behavioral video streams:

- Section 4.11, “Creating Photometry Events” on page 84
- Section 4.13, “Creating Photometry Combination Events” on page 88
- Section 8.8, “Creating Behavioral Events” on page 200
- Section 8.9, “Creating Behavioral Combination Events” on page 217

This chapter explains how to configure and use global event features, including Input Events, Global Combination events and Event Statistics.

9.2 Configuring and Managing Input Events

The parameters configured in the Input Events tab allow the system to receive an input event from an external source and use it to control the start or stop of a session. The system receives the event through the DIGITAL INPUT port on the Trigger Box. Up to 12 inputs can be received. By default, there are six high true inputs and six low true inputs. However, when you order a system from Plexon, you can request any combination of high true and low true inputs. The **Input Line #** dropdown list will indicate which lines are high true and which are low true. (Use of the Input Events feature is optional.)

The system can detect Input Events in Cameras mode only.

All digital inputs should be kept within the range of 0 to 5V relative to the ground pins during normal operation. The input logic is as follows:

- High True input—The system recognizes voltages greater than 2.0V as asserted and voltages less than 0.8V as de-asserted.
- Low True input—The system recognizes voltages less than 0.8V as asserted and voltages greater than 2.0V as de-asserted.

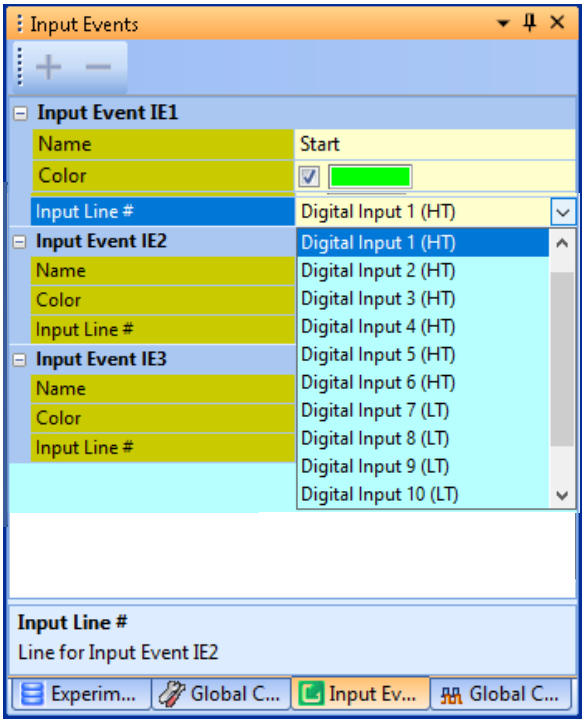
If the input is a pulse, the duration of the pulse should be at least as long as the time between frames for the camera frame rate, which is 30 frames per second.

As an example of using an input event, you can connect a cable from the Plexon PlexBright® 4 Channel Controller to the DIGITAL INPUT port on the Trigger Box and configure the system to start video recording when a stimulation pattern begins playing on the PlexBright controller. (The cable and PlexBright Controller are sold separately.)

For additional details about the input and output ports and signals in the Trigger Box, see Appendix C, Trigger Box and Digital Input/Output.

Configuring the Input Events tab

1. Click on the **Input Events** tab to view the Input Events parameters.
2. Click the **+** sign to begin configuring a new **Input Event**.
3. Double click in the cell on the right side of the **Name** row to enter a name for the input event.
4. In the **Color** row, use the checkbox to show (or not show) a color for the event in the Event Statistics view in the GUI, and click on the color bar to select a color.
5. In the **Input Line #** row, use the dropdown list to select the input line you want to use.



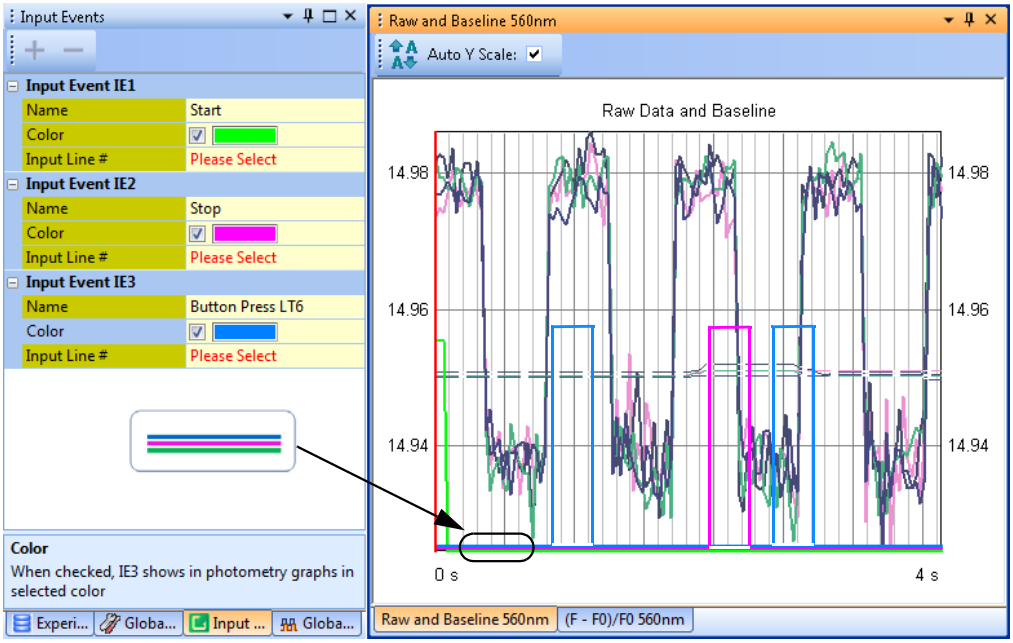
6. Repeat Step 2 through Step 5 for each input line you want to use.

Viewing Input Events in the Photometry Graphs

This section explains how to view the digital input events in the photometry graphs.

Input events defined in the Input Events tab (above) are displayed on the photometry graphs, both the Raw Data and Computed Data graphs. They appear even if the **Input Line #** is not selected in the Input Events tab. When the input event is not detected, the event is displayed as a horizontal line at the bottom of the graph. When the input event is detected, it is displayed as a non-zero horizontal line halfway up the graph. If there are several inputs, you will see one line for each input. The lines are drawn in such a way that they do not overlap, so you will always see the status of each input. Note that the color of each line

matches the color configured in the Input Events tab and will change automatically if the color in the Input Events tab is changed. See the example, below.



If an event is defined but no Input Line # is selected, the input can never be detected and therefore the line will always be displayed at the bottom of the graph. The input event visualizations in the graphs do not show the actual value of the input signal (such as 0 V or +5 V) and they do not indicate whether the input is defined as high true or low true. It shows the logical status of the event – happening or not happening. In other words, the line representing an input event will be at the bottom of the graph if the event is not happening, whether this event is configured in the hardware as high true or low true. Similarly, the line representing an input event will be at the "high" position of the graph if the event is happening, whether this event is configured in the hardware as high true or low true.

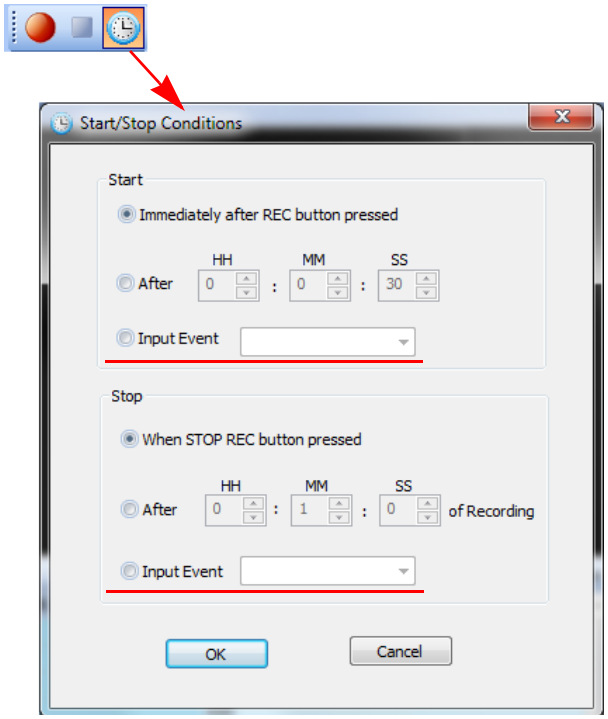
Wiring the Digital Input interface

1. Attach a wire to an input line you want to use on the connector for the DIGITAL INPUT port on the Trigger Box. Attach the other end of the wire to the appropriate connector on the external equipment. (Optionally, you can skip this step now and attach the wire later.)
2. If necessary, attach an appropriate adaptor or converter between the Trigger Box Interface and the external equipment. As required by the user's third party device (for example, Med Associates Inc®, (Lafeyette Instrument®, etc.), an adaptor or cable will be required to interface with the Trigger Box. This can be provided by Plexon at the time of system purchase.

	CAUTION **Limit voltage on the Digital Input/Output interface pins** All digital inputs should be kept within the range of 0 to 5V relative to the ground pins during normal operation. Avoid high voltages. Voltages beyond ±28V are likely to permanently damage the inputs. Avoid accidentally applying voltage to the digital output ports. Voltages beyond ±28V are likely to permanently damage the outputs.

Using an Input Event to start or stop recording

1 Click the **Conditions to start and stop recordings from camera(s)** icon ( ) to open the **Start/Stop Conditions** dialog box.



2 Click the **Input Event** radio button and select the desired **Input Event** from the dropdown list in either the **Start** or **Stop** area.

3 Click **OK**.

Changing the Input Events configuration

You can rewire the lines connected to the DIGITAL INPUT interface, change the values in the Input Events tab, and change the settings in the **Start/Stop Conditions** dialog at any time, even between sessions. The new configuration will apply to all future sessions in that experiment (unless you chose to change the configuration again later).

9.3 Creating Global Combination Output Events

This section explains how to configure global combination output events.

After you create events in the individual video sources (the three photometry sources and the behavioral source), you can create one or more global combination event(s) that combine events from multiple video sources. Here are two examples of global combination events:

- 465nm and 560nm fluorescence intensities both rise above certain levels,
- 465nm fluorescence rises above a certain level and the subject turns its head toward a target object in the experimental arena.

You can also create combination events that contain other (existing) combination events.

TIP

Configuring combination events for single and multiple video sources

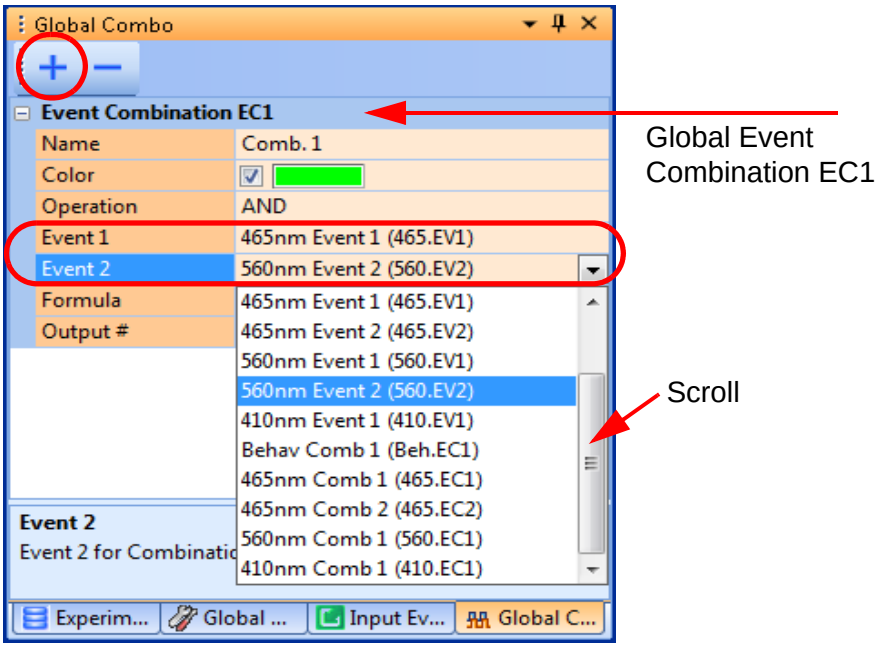
It is usually more convenient to configure combination events in the individual video sources whenever possible (as described in Section 4.13, “Creating Photometry Combination Events” on page 88 and Section 8.9, “Creating Behavioral Combination Events” on page 217), and create a global combination event only when it is necessary to combine events occurring across multiple video sources.

1 Click on the **Global Combo** tab in the global settings window.

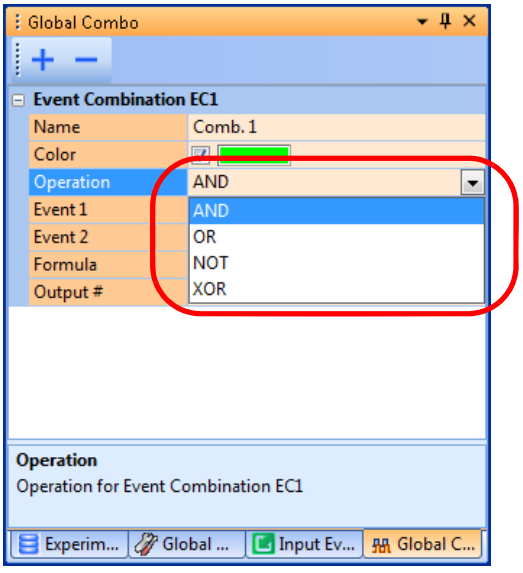
2 Click the **+** icon to create a new event.

3 Configure values for the **Name** and **Color**.

4 Select any existing behavioral or photometry event from the dropdown menus for **Event 1** and **Event 2**.



5 Configure the **Operation** row parameter.



The **Operation** parameter is used to specify events that are combinations of one or more existing events on which logical operations are performed. The existing events can be any single or combination event. The four logical operators are **NOT**, **AND**, **OR** and **XOR**:

NOT—For single events, an event can be defined which is the opposite of it by using the **NOT** operator. The new event is true when the original event is not true and vice versa (the new event is not true when the original event is true). For example, **NOT**[animal is inside feeding zone] means the animal is not inside the feeding zone.

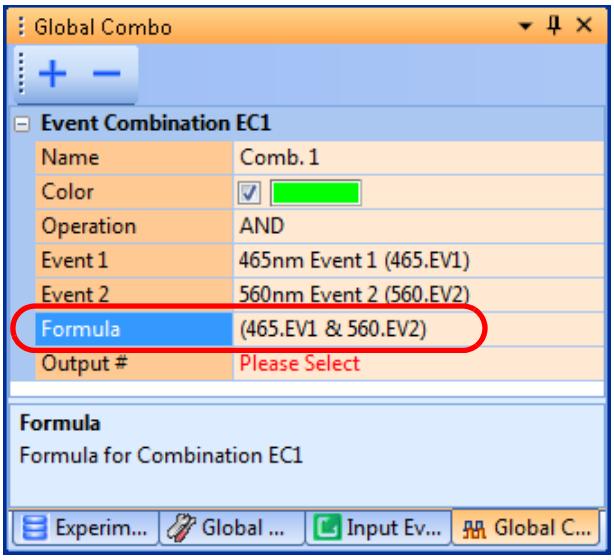
For the combination of two events, the **AND**, **OR** and **XOR** operators are available. The resulting combination event is considered true under the following conditions:

AND—Both of the individual events are true

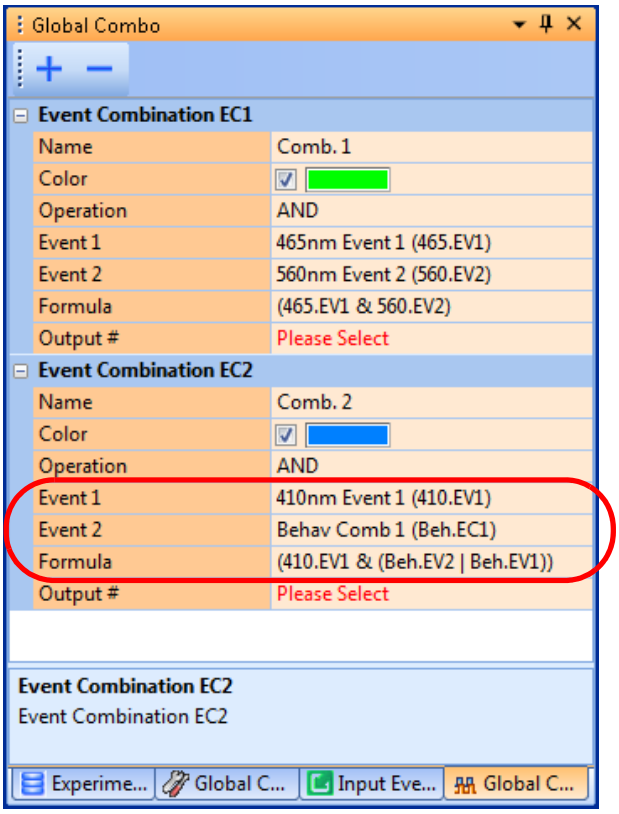
OR—Either one of the individual events is true, or both of the individual events are true

XOR—Either one of the individual events is true, but not both of them

The system generates an entry in the **Formula** line based on the Operation and Event(s) you selected in the above steps.



6 If desired, you can create a global combination event that contains other combination events, as shown in the following example. Events of arbitrary complexity can be created by combining already combined events with combined or non-combined (single) events.

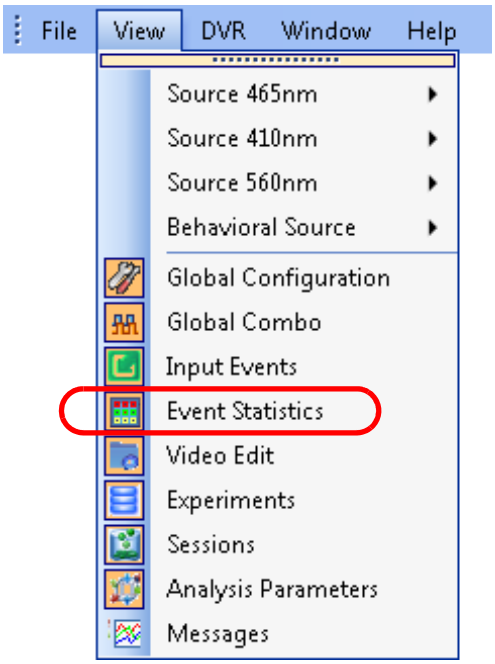


7 Configure the **Output #** parameter, using the method described in Section 4.12, “Specifying Digital Outputs when Events Occur” on page 85.

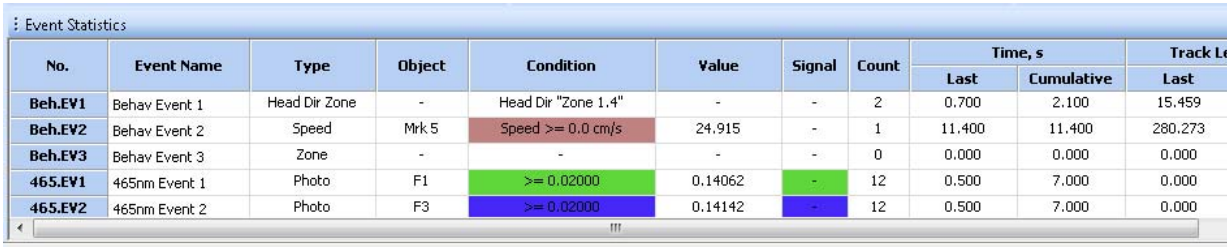
8 Repeat Step 2 through Step 7 to add more global combination events.

9.4 Displaying Event Statistics As They Occur

To open the Event Statistics window, select **Event Statistics** from the **View** dropdown menu.

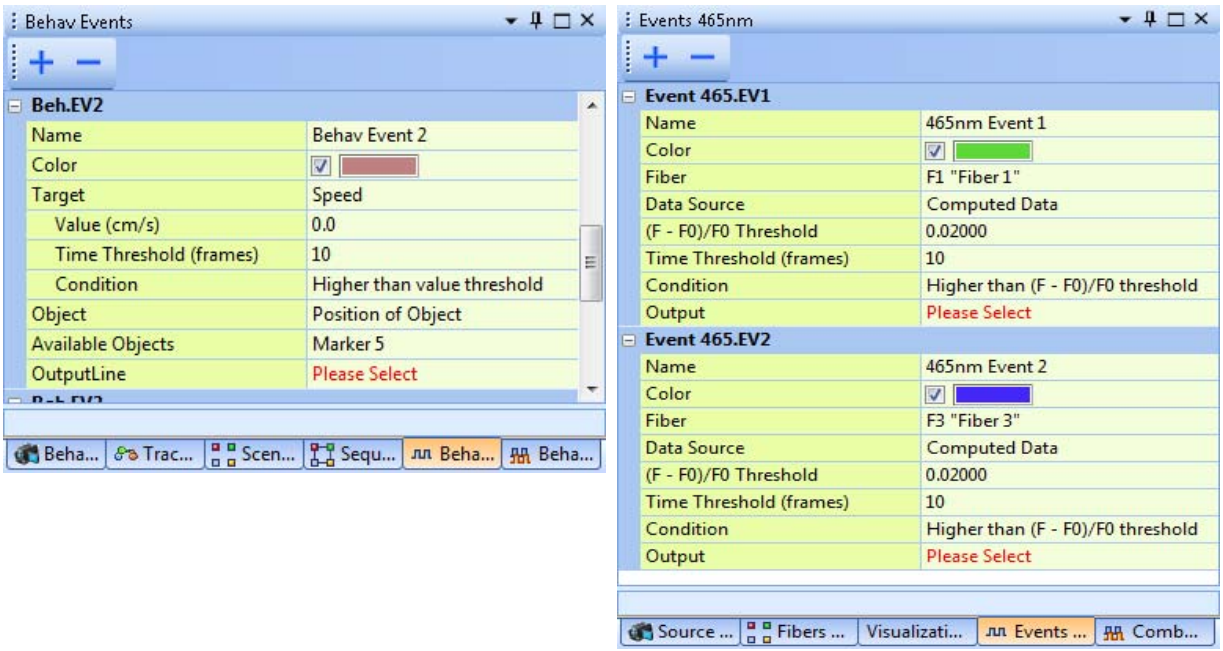


The image below is an example of an Event Statistics display for several behavioral and photometry events. The colored cells in the display indicate that an event was occurring at the moment this image was captured.



No.	Event Name	Type	Object	Condition	Value	Signal	Count	Time, s		Track L
								Last	Cumulative	Last
Beh.EV1	Behav Event 1	Head Dir Zone	-	Head Dir "Zone 1.4"	-	-	2	0.700	2.100	15.459
Beh.EV2	Behav Event 2	Speed	Mrk 5	Speed >= 0.0 cm/s	24.915	-	1	11.400	11.400	280.273
Beh.EV3	Behav Event 3	Zone	-	-	-	-	0	0.000	0.000	0.000
465.EV1	465nm Event 1	Photo	F1	>= 0.02000	0.14062	-	12	0.500	7.000	0.000
465.EV2	465nm Event 2	Photo	F3	>= 0.02000	0.14142	-	12	0.500	7.000	0.000

Following is the configuration that generated the Event Statistics display shown above.



.

TIP

Viewing the Event Statistics

The **Event Statistics** window is populated only during an experiment, not if you are viewing data from a previously saved experiment.
You must rerun the video of the previously saved experiment to populate these fields.

9.5 Where to Go Next

When you are ready to start video recording, see Chapter 10, Recording and Monitoring.

Chapter 10
Recording and Monitoring

10.1 Before You Start

Verify that you have completed the procedures in the previous chapters as applicable to your experiment.

Note: (Optional) If you are using any digital input or output lines for signaling between the system and external devices, ensure you have attached the input/output wires you want to use on the appropriate Trigger Box interfaces. For details of these connections, see AppendixC, Trigger Box and Digital Input/Output.

10.2 Starting the System

Perform these steps to start the system.

1. Ensure that the system is connected as described in Chapter 2, Installing and Starting the System.
2. If not already done, double-click the Plexon® Multi-Wavelength Photometry icon  to open the application.

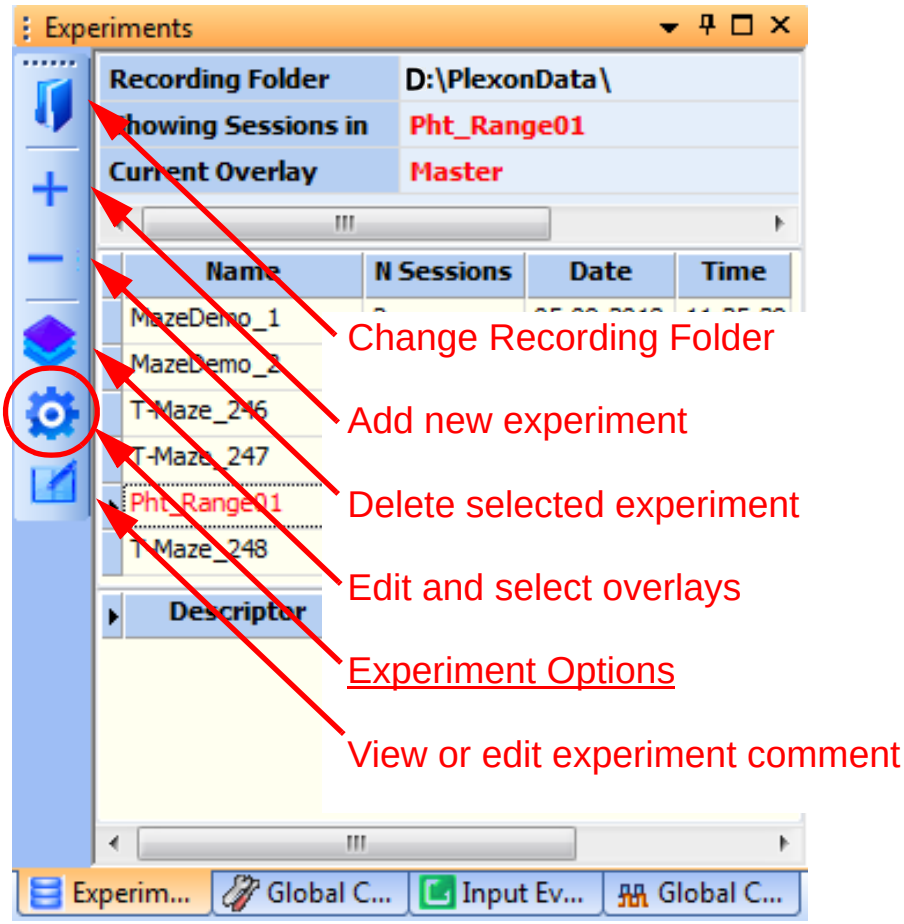
TIP

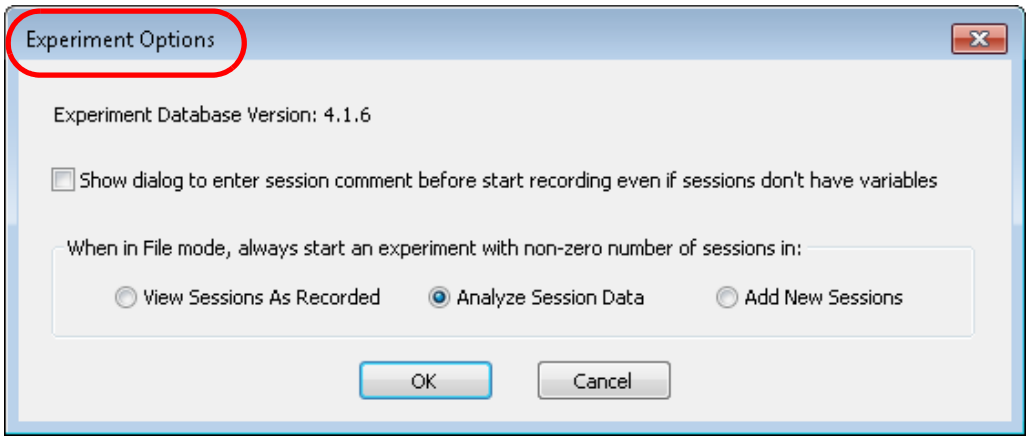
Reset to Default Layout

It is often helpful to reset the screen display to the default layout (unless you have created a customized layout that you prefer). The reset ensures that the system is displaying all of the tabs and options you are likely to use in configuring your experiment. In the main window, select **Window > Layout > Reset to Default Layout**.

10.3 Setting Experiment Options

If you click on the **Experiment Options** icon (see the image below), it opens a dialog that allows you to set one of the recording behaviors.





You can select the checkbox for **Show dialog to enter session comment before start recording even if sessions don't have variables** if you want that option. (If a session *does* have variable(s) the system will always show that dialog before recording, which allows you to enter a value for the session variables).

The display of the sessions variables prior to recording is described in the procedure in Section 10.5, “Configuring, Starting and Stopping the Recording” on page 240.

The **Experiment Database Version** number in the above dialog is system generated. It does not require any user action, but it can be useful information if it becomes necessary to do any system troubleshooting.

The **When in File mode...** default is **Analyze Session Data**. The options are discussed in Section 11.3, “Understanding Files Submode Settings” on page 254.

10.4 Ensuring Consistent Parameter Settings in an Experiment

Once there are existing sessions (recordings) within an experiment, certain parameter settings are disabled for that experiment (the settings cannot be changed). This is done so that all the sessions within an experiment are recorded with consistent parameters. The disabled parameter settings are listed in Section 3.12, “Ensuring Consistent Parameter Settings in an Experiment” on page 51.

10.5 Configuring, Starting and Stopping the Recording

1. Verify the system is in **Cameras** mode.
2. In the **Experiments** tab, open the **Recording Folder** browser by clicking the **Change Recording Folder** icon then navigating to the folder that contains the individual subfolders for your experiments.

   Note: You must select a folder that contains one or more experiment subfolders. If you select a single experiment subfolder, you will not be able to perform any analysis on it. (You can select the experiment subfolder but you will not be able to access the experiment in the Experiments tab.)
3. Click **OK**.

Preparing to start recording a session (manual/immediate)

The **Start recording** icon on the toolbar remains grayed out and inactive until the preconditions for starting a recording have been met. It turns red and active after the preconditions have been met. The icon will become active when [1] you create a new experiment, or [2] you select a previously saved experiment. When enabled, the toolbar should look like this:

4 If not already done, create a new experiment or select an existing experiment in accordance with the procedures in Chapter 3, Preparing Your Experiment Database.

5 Click the Experiments tab to display a list of all your experiments in the folder.

6 In the Experiments tab, click in the row for the experiment to which you want to add sessions.

After you click on the row for your experiment, notice that the list of previously saved sessions (if any) for this experiment appears in the Sessions window.

7 (Optional) In the Global Config tab you have the option to change the settings for **Layers Transparency** (default value 0.5), and additional parameters if Tracking is enabled in the behavioral video. The default values are suitable for many experiments.

8 Click on the **Source** tab under the photometry device windows and the behavioral video window, as applicable to your experiment, to view these parameters. The settings for these parameters were entered in the procedures in Chapter 4, Photometry Features and Procedures and Chapter 5, Setting Up the Behavioral Camera. If any of these settings need to be changed, make those changes now.

9 In the **Scenes** tab for the behavioral camera, adjust the size and position of the arena and any zones if necessary. For example, it might be necessary to increase the size of an arena or zone, or to move an arena or zone slightly to properly track the subject.

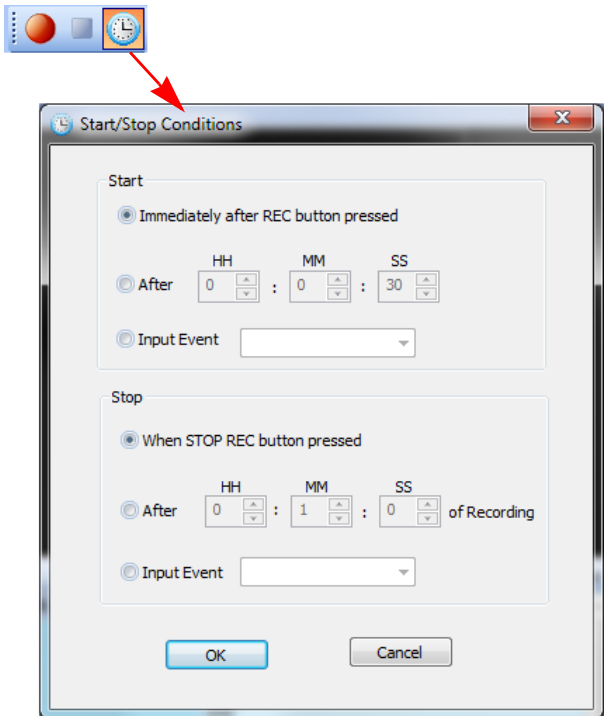
10 In the **Tracking** tab for the behavioral camera, ensure that the subject is being tracked properly. Note that the status bar flashes red if the camera is not ready to track the subject.

Note: Changes in the calibration, dimensions and positions of arenas and zones apply to all future recordings within an experiment. However, if you add or delete an object (shape) in an arena or zone, that addition or deletion affects all sessions (past and future) for the current experiment.

Note: Once sessions have been recorded for an experiment, you cannot select additional colors or uncheck any that have already been selected. This ensures that all sessions in the experiment have the same tracking objects, and ensures that the analysis is consistent across all sessions.

Setting start and stop conditions

11 (Optional) If desired, set delays for starting and stopping the video recording. Select the **Conditions to start and stop recordings from camera(s)** icon to display the **Start/Stop Conditions** dialog box.



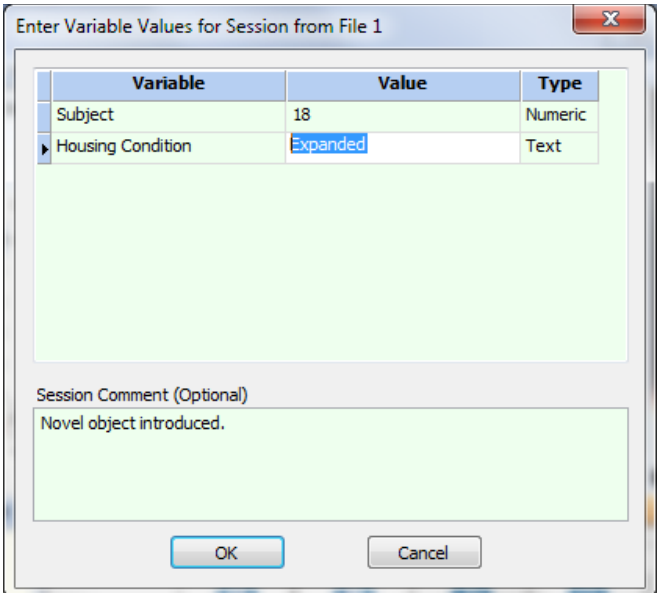
For more details about using this option, see Section 10.6, “Setting Start and Stop Conditions” on page 246.

Starting and stopping the recording of a session (manual/immediate)

12 To start recording video manually, click the **Start recording** icon  on the toolbar.

13 If the session has user-defined variables, the system opens the **Enter Variable Values for Session** dialog box and prompts you to enter the values.

In that case, double click in the **Value** column and enter the value(s) of the variables for this session.



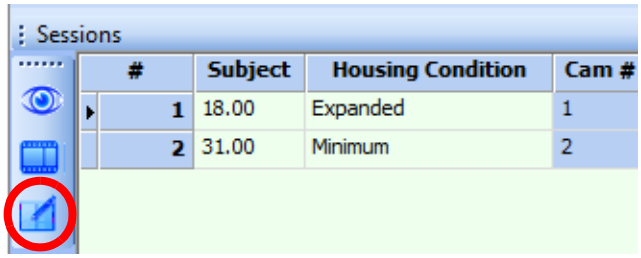
Note: You can also set the system to open the above dialog even if there are no variables for the session. You might want to do this if you plan to enter a Session Comment for some of your sessions. See Section 10.3, “Setting Experiment Options” on page 239.

14 You can also add or edit a **Session Comment** for this session. (See the image above.) You can enter up to 200 characters in this field.

15 Click **OK**.

Note: The system presents a dialog box for each video stream (camera) in the session.

Note: At any time, you can view or edit the **Session Comment** by clicking the **View or edit experiment comment** icon.



After you press the **Start recording** icon, the **Stop recording** icon becomes active on the toolbar, and a green cassette icon appears on the status bar at the bottom of the user interface to indicate that the recording is in process.

Note: The system automatically checks that there is at least 10 GB of free disk space on the PC before it starts recording a file. The file size can grow to fill the space available, except that the system automatically stops recording the current file when the available disk space has been reduced to 10 GB.

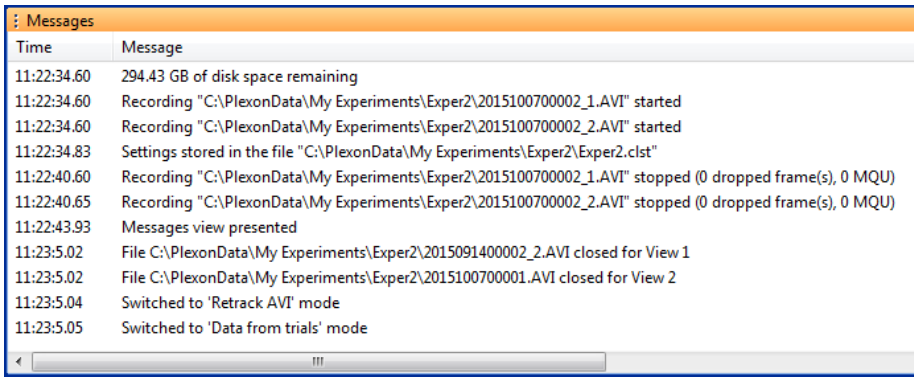
Note: The I/O icon, as seen in the image above, is not related to the recording function. (It is described in Section 10.7, “Monitoring the Video During Recording” on page 250.)

16 To stop the recording manually, press the **Stop recording** icon on the toolbar.

The system stops recording and saves the video file(s) to the location that was specified as **Recording Folder** in the **Experiments** tab. The system also clears the recording information from the status bar and re-arms itself for the next recording.

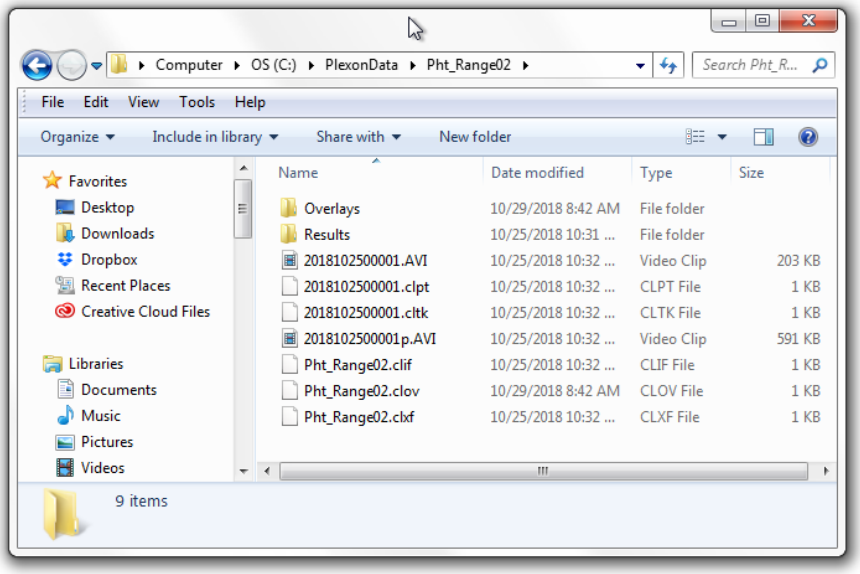
Viewing messages and files after recording a session

To display the Messages window, from the main menu select **View** > **Messages**.



17 To view the video (AVI) files, navigate to the folder where the files were saved, as specified in the **Experiments** tab, **Recording Folder** parameter) and double click on the desired file to view it. You can view the video files within

the system GUI or with Windows® Media Player® or many other available video players.



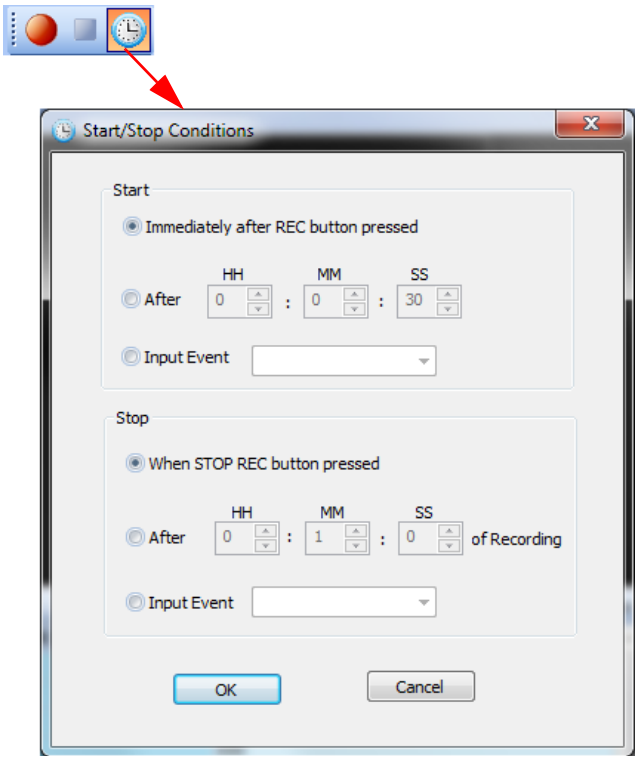
18 To transfer files to an analysis computer (if different than the recording computer), follow standard Windows procedures. A flash drive with a valid Plexon “PHT + BEHV” user license is required to open the files on the analysis computer.

10.6 Setting Start and Stop Conditions

This section explains how the **Start/Stop Conditions** dialog box works and how to use it.

10.6.1 Understanding the Start/Stop Conditions

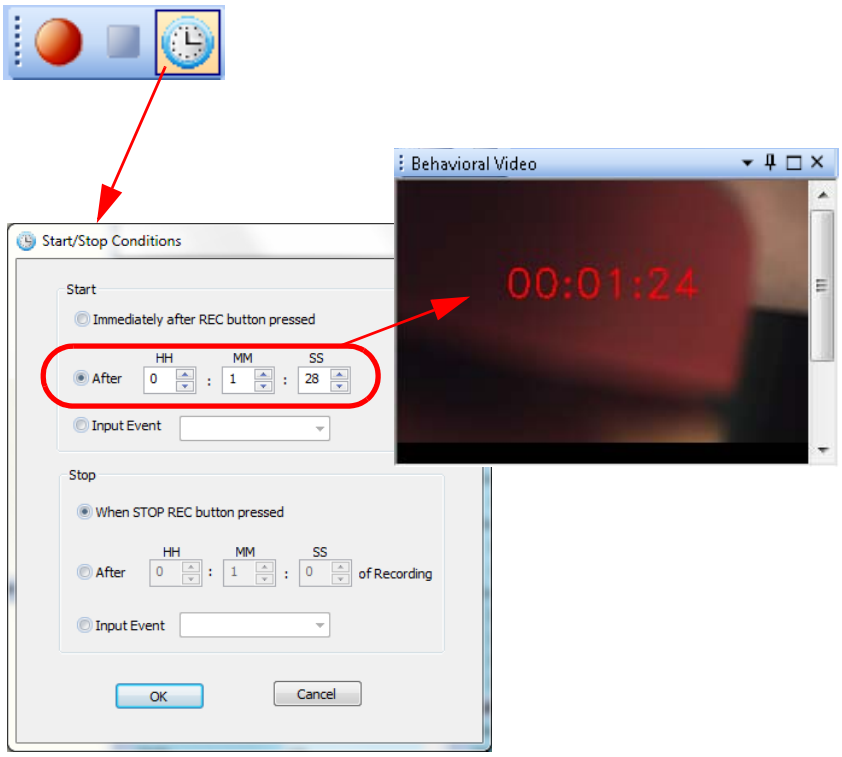
Clicking the clock icon  opens the dialog box.



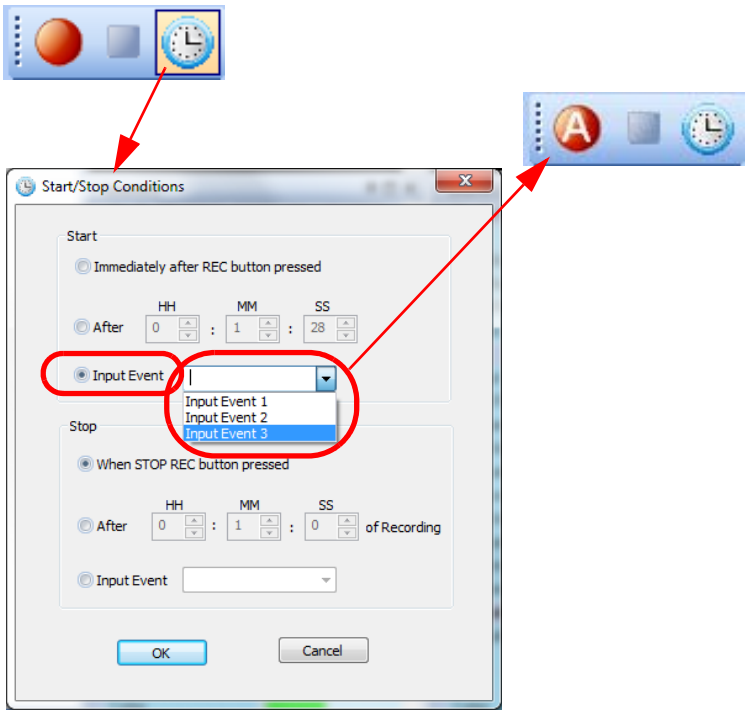
There are three options for starting a recording and three options for stopping the recording. The default settings are to immediately start and stop recordings when you press the **Start** and **Stop** buttons.

In the **Start** area of the dialog box,

- If you set a time for **After**, the recording starts after you press the **Start** button and the time you specified elapses. The system displays the time counting down to the actual start of the recording.



- If you select an **Input Event** from the dropdown list, the recording starts after the input event is received (becomes TRUE). The input event must be one that was configured in the Input Event tab. Input Events are received through the DIGITAL INPUT interface on the Trigger Box (see Section 9.2, "Configuring and Managing Input Events" on page 226). After you click **OK**, the Start button displays the letter "A" to indicate it is ready to be armed.



10.6.2 Procedure

This section explains how to use the **Start/Stop Conditions** settings.

Displaying the Start/Stop Conditions dialog box

1. Select the **Conditions to start and stop recordings from camera(s)** icon to display the **Start/Stop Conditions** dialog box.
2. If the default settings are displayed, the system will start and stop the recording immediately after the REC (**Start**) and STOP REC (**Stop**) buttons are pressed.

Adjusting the Start time

3. If you want the recording to start after a delay period when you press the **Start** button, click on the **After** radio button and adjust the settings for hours, minutes and seconds in that row. Then click **OK**.

After you press the **Start** button, the video will display the time counting down to 00:00:00, at which time the recording will start.

4 If you want the recording to start after the system receives an input event through the Digital Input interface, perform the following steps:

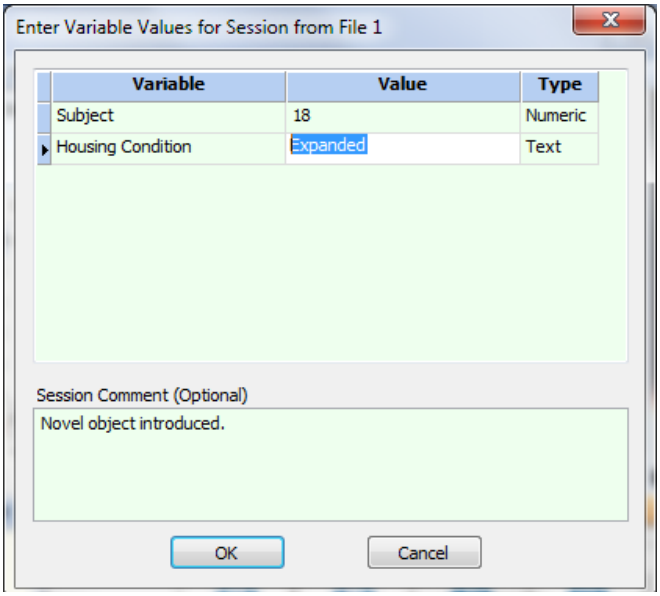
a Click on the **Input Event** radio button and select an **Input Event** from the dropdown list. After you click **OK**, the **Start** button displays the letter "**A**" to indicate it is ready to be armed.

b Click on the **Start** button. The following events occur:

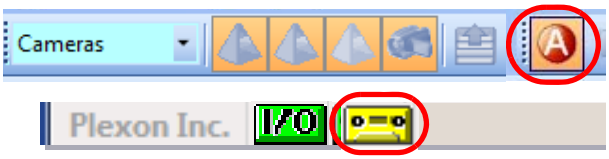
– The background of this icon turns orange to indicate that the system is armed and will start recording when the specified Input Event is received.

– The rest of the main toolbar is disabled.

c If the session has user-defined variables, the system prompts you to enter the values. In that case, double click in the **Value** column and enter the value(s) of the variable(s) for this session, then click **OK**.
Each time the system completes a session and is ready to start a new session (awaiting the next external input), the system will prompt you to enter value(s) of the variable(s) for the next session.



The status bar at the bottom of the screen displays a flashing yellow cassette icon indicating that the system is armed and ready to record.



Note: The **I/O** icon, as seen in the image above, is not related to the recording function. (It is described in Section 10.7, "Monitoring the Video During Recording" on page 250.)

5 If you want to return the system to manual start mode, click the **Start** icon to disarm the system. then open the **Start/Stop Conditions** dialog box and select **Immediately after REC button pressed**. Then click **OK**.

Adjusting the Stop time

6 If you want the recording to stop after a specified amount of time has elapsed (since the start of the recording), click on the **After** radio button and adjust the settings for hours, minutes and seconds in that row. Then click **OK**.
After the recording starts, the system allows recording to continue until the specified time elapses, at which point it stops the recording. (The system displays the elapsed time in the usual manner in the status bar at the bottom of the screen.)

7 If you want the recording to stop after the system receives an input event through the Digital Input interface, click on the **Input Event** radio button and select an **Input Event** from the dropdown list. Then click **OK**.

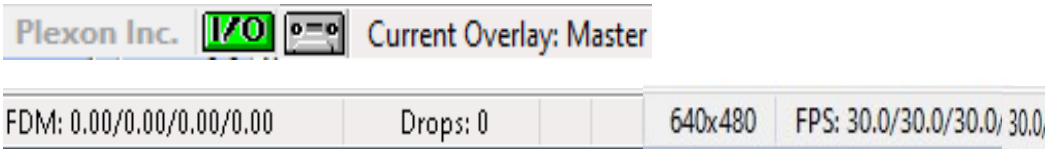
.

TIP
You can stop a recording manually at any time
You can stop an in-progress recording manually by pressing the **Stop** icon at any time, even if the recording is set to stop after a specified time or an Input Event.

10.7 Monitoring the Video During Recording

Before recording has started

When recording has not started, the status bar in the user interface displays the parameters shown in the example below. (The image is split into two sections to fit the page.)



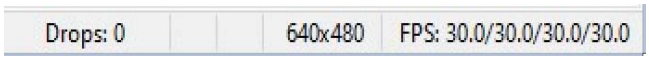
The contents of the status bar include

- The I/O icon. This icon indicates the presence of digital input and output ports in the Trigger Box. In **Cameras** mode, this icon is always green, because the system requires the Trigger Box to be connected and turned on for **Cameras** mode to function. In **Files** mode, if the Trigger Box is turned off or disconnected, this icon turns red. (Files mode can function whether the Trigger Box is present or not.)
- A cassette icon that is displayed as grey when recording is not occurring.
- The name of the currently active Overlay, which contains the settings for the arena and zones for each video stream.

- The Frame Difference Metric (FDM), which is a measure of the relative noise level in each video stream source (camera or file). If there is no change occurring in the video stream, the FDM is 0 or close to 0. (Electronic noise in the system can cause the FDM to be nonzero.)
- A count of the number of dropped frames (0 prior to recording). After recording starts, any dropped frames could indicate a problem, typically that the PC is being overloaded with applications other than the Plexon software, for example Internet browsing or heavy calculation programs.
- The camera frame resolution for the current experiment, or the experiment run most recently, in pixels (horizontal x vertical).
- The video recording rates in frames per second (FPS).

When recording starts

The status bar in the user interface displays information that allows you to monitor the video recording process. This image shows the status bar during a typical recording. (In the example below, the image is split into three sections to fit the page.)



The contents of the status bar include

- The I/O icon (discussed above).
- A cassette icon that flashes yellow if recording is armed and is steady green when recording is in process.
- A display of the size of the recorded file in time and file size, and the remaining recording time and storage space available on the disk where the **Recording Folder** resides.
- A count of the number of dropped frames during the current recording.
- Numbers identifying the resolution of the current recording in pixels (horizontal x vertical) and frames per second (FPS) for each of the four video streams.

10.8 Managing CPU Demand and Avoiding Dropped Frames

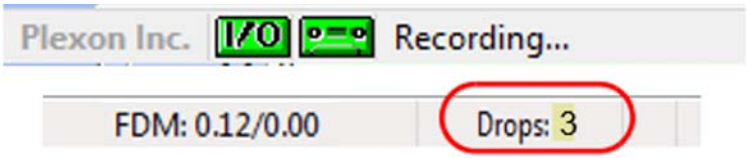
The standard configurations of the system hardware and software are intended to cover the vast majority of needs for the neuroscience research community. Nevertheless, certain combinations of settings may cause performance issues. Such settings might include:

- Large numbers of colors being tracked
- Difficulty tracking several colors (mostly in cases where the number of tracked colors exceeds five) due to their blocking most of the time, or if the tracking colors were not set up correctly
- Large numbers of complex events

Other applications running on the computer may interfere with operation of the Plexon system. These include:

- Windows Update
- Backup software
- User installed software
- Browsers
- Slower disk activity (can occur as a disk comes close to 100% full)

The result of any of the causes listed above is usually reflected in dropped video frames while recording. These are shown in the status bar at the bottom of the GUI window.



Any non-zero drop count is a potential cause for concern and the sessions being recorded when the drops occurred should be carefully inspected for anomalous results. If you find these, you should attempt to eliminate the cause of the drops by examining and correcting the possible causes shown in the lists above. If you continue to observe dropped frames during recording and cannot immediately correct the problem, contact Plexon for assistance or upgrades as needed. It might be possible for Plexon to assist you in upgrading components of your system, including the computer, so it will better handle the high CPU loads.

You can reach Plexon support at +1 214-369-4957 or support@plexon.com.

10.9 Where to Go Next

Go to Chapter 11, Analyzing Data and Adding Sessions.

Chapter 11
Analyzing Data and Adding Sessions

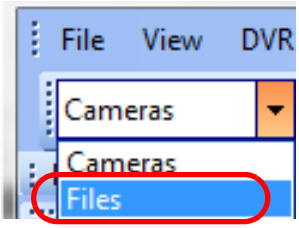
11.1 Introduction

This chapter explains how to manage your Experiment folders and session video files, how to perform analyses and view the results, how to input and output photometry and behavioral events data, and how to add more sessions to an existing experiment.

11.2 Placing the System in Files (Offline/Analysis) Mode

The system has two modes of operation: **Cameras** (online/recording) mode and **Files** (offline/analysis) mode.

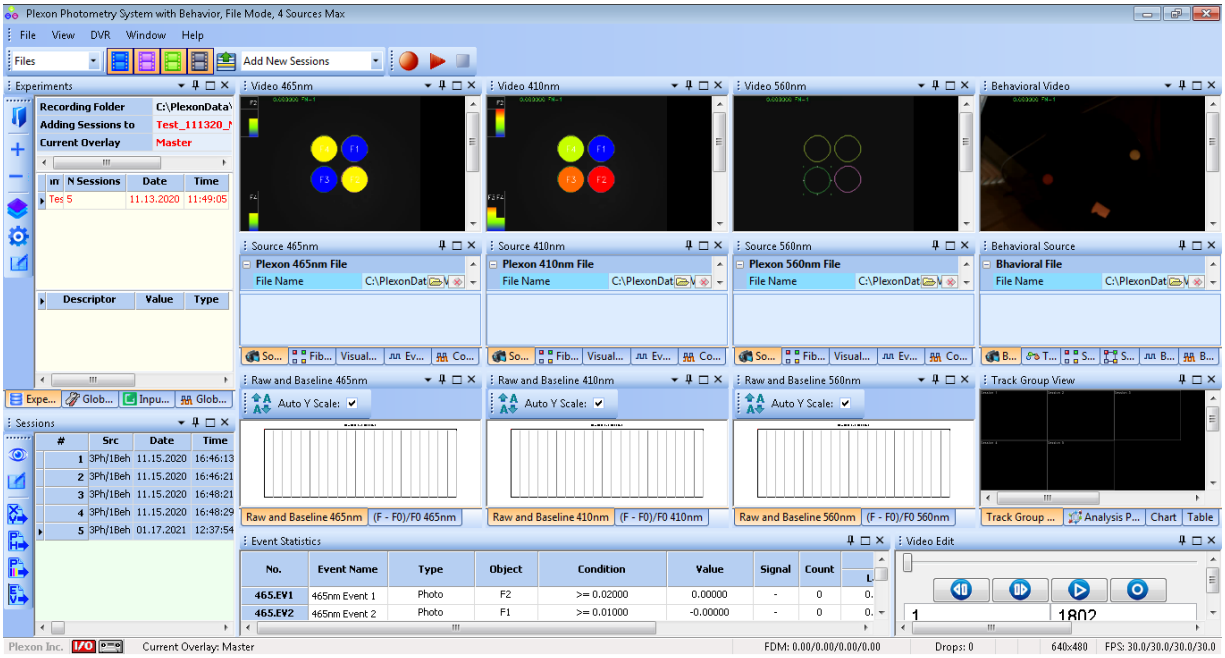
1 If your system is not already in **Files** mode, switch from **Cameras** mode to **Files** mode using the video source dropdown list on the main toolbar, as shown in this image.



11.2.1 User Interface Layout

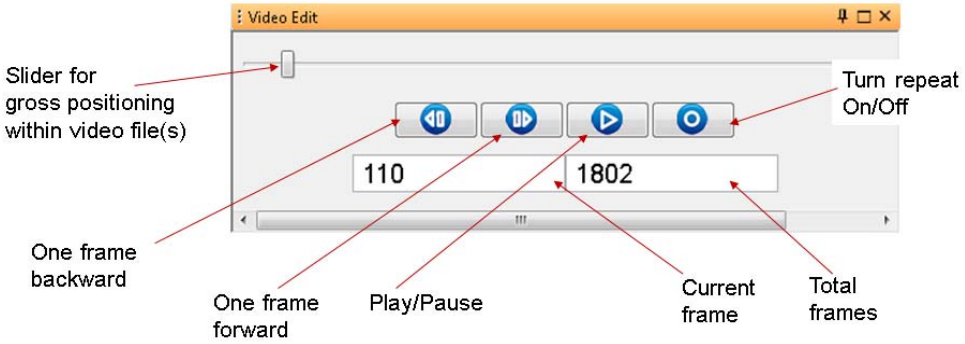
The image below shows the default user interface layout with an experiment selected and the files for all four devices loaded.

Note: You can easily restore the default GUI layout at any time: In the **Window** dropdown menu, select **Layout**, then select **Reset to Default Layout**. All the device windows (photometry devices and behavioral camera) will be displayed in the default GUI arrangement.



11.2.2 Video Edit Window

You can use the icons in the Video Edit window to replay the video file frame by frame. If the Video Edit window is not displayed, you can display it by selecting **Video Edit** from the **View** dropdown menu in the main toolbar.



Note: The Video Edit functions are disabled while a recording is in progress.

11.3 Understanding Files Submode Settings

There are three submodes you can select when the system is in **Files** mode. Each of these submodes has a specific purpose, as described below.

- **View Sessions As Recorded—**For each individual session, you can view the original settings of the session source and global settings, that is, the settings that were in place when the session was recorded. You can export photometry data, tracking coordinates from the behavioral source (if present), and timestamps of digital input and output events (per-frame values and intervals) as described in Section 11.10, “Exporting Photometry Results per Frame” on page 285 through Section 11.12, “Exporting Recorded Events per Session” on page 287. No changes to the original settings are allowed in this mode.

Note: The software automatically loads AVI files for the selected session in “View Sessions As Recorded” and “Analyze Session Data” submodes.

- **Analyze Session Data—**For each individual session that has been recorded, you can modify visualization settings, number of behavioral zones, arena and zone shapes, and compute behavioral statistics (e.g., time in zones, etc.). You cannot export data in this Files submode; however, you can export analysis results as described in Section 11.8, “Setting the Analysis Parameters and Analyzing the Data” on page 271 and Section 11.9, “Exporting Analysis Results” on page 281.

Note: Once you modify the session settings, the new settings are saved in the current master for use in further analysis of this and other sessions. Settings from sessions as recorded are not changed.

- **Add New Sessions**—In this mode, you can add new sessions to the experiment. This mode is similar to **Cameras** mode, except that the video streams are coming from existing AVI files instead of live video from cameras. In this mode, prior to recording the first session in an experiment, you can modify all configurable parameters for the photometry and behavioral video streams, add/remove zones and fibers, and move or resize the zone and fiber boundaries. Note that the video files must be loaded to the photometry sources and the behavioral source to be able to move/resize fibers and behavioral arena/zones. You cannot export data in this Files submode; however, you can export analysis results as described in Section 11.8, “Setting the Analysis Parameters and Analyzing the Data” on page 271 and Section 11.9, “Exporting Analysis Results” on page 281.

Note: If you create a new experiment in Files mode, the software automatically switches to **Add New Sessions** submode.

Note: For “Add New Sessions” submode, video files will have to be loaded to each video source manually.

See the additional details below.

IMPORTANT—Notes on adding new sessions to an existing experiment

Note the following restrictions when you load an existing experiment that has recorded session(s) and then switch to **Add New Sessions** submode.

1. You must load video files to all four sources to be able to see fibers for the photometry sources and arena and zones for the behavioral source.
2. You must load video files to all four sources to start recording.
3. You will be able to move and resize fibers, but you will not be able to delete fibers or add new fibers. Video files must be loaded to the photometry sources to be able to move/resize fibers.
4. You will not be able to change the behavioral tracking mode; you will not be able to change from tracking to non-tracking or non-tracking to tracking.
5. You will not be able to change the number of tracked objects or number of animals in the arena (LED or color marker tracking).
6. You will be able move, resize, delete, and add arena and zones for the behavioral source. A video file must be loaded to the behavioral source to be able to move/resize the arena and zones.

Summary of Files submode functions for photometry

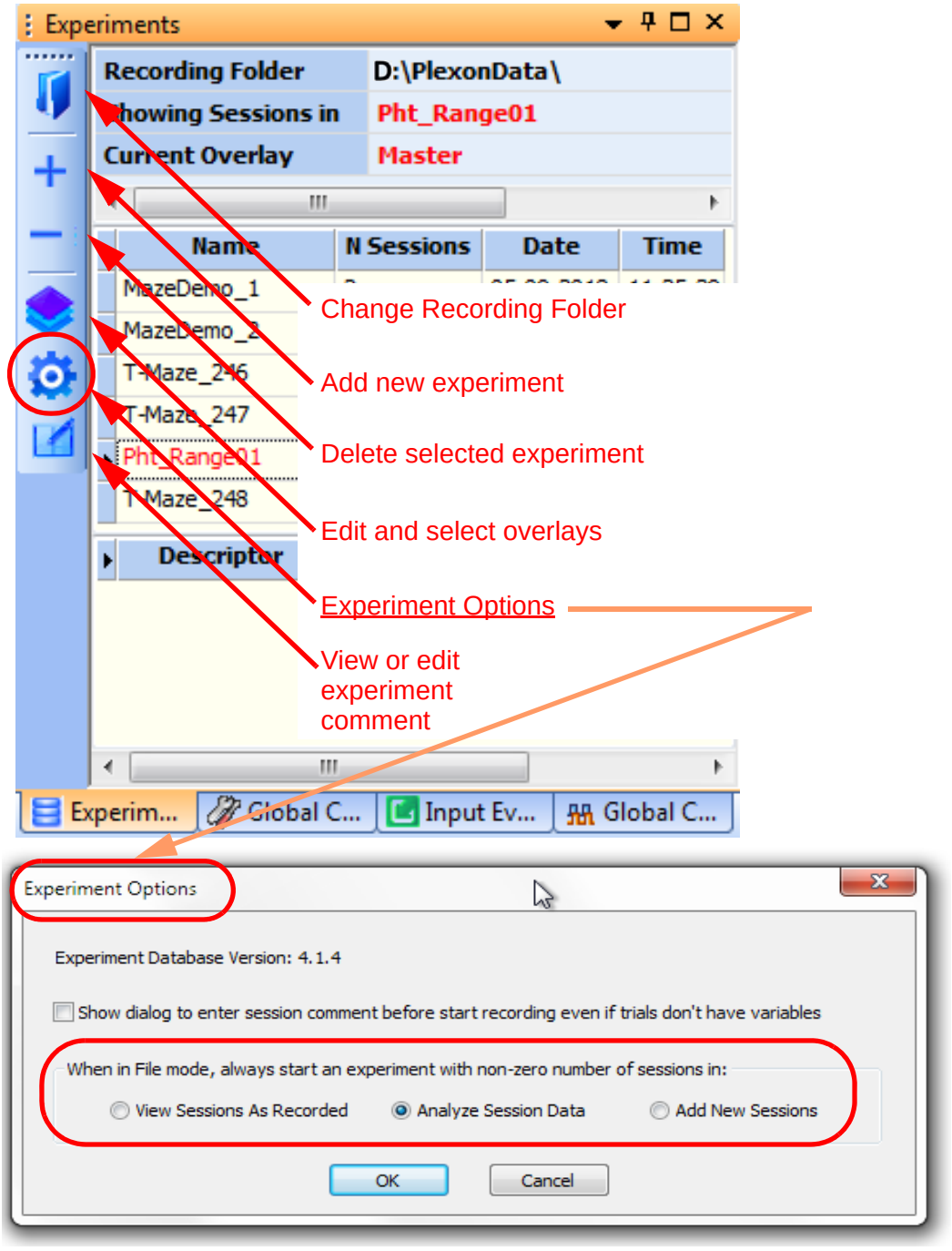
Parameters		Files submodes: View Sessions as recorded	Add New Sessions	Analyze Sessions Data
Source Tab	DVR parameters	No	Yes	Yes
	LED parameters	No (shows if there was LED stimulation for this sessions)	No (Doesn't show in this mode, since LED stimulation cannot be added offline)	No (shows if there was LED stimulation for this sessions)
Fibers Tab	Fiber Name	No	Yes	Yes
	Fiber Outline and Graphs	Yes	Yes	Yes
	Time Variables	No	Yes	No (will be available in the next version when we allow to re-analyze PHT data)
	Add/delete fibers	No	Yes (only for experiments without sessions)	No
Video View	Move/resize fibers	No	Yes (the change will be applied to all newly recorded sessions)	No
Visualization Tab	All parameters	Yes	Yes	Yes
Events, Combo Events and Global Combo	Add/delete/modify events	No	Yes	Yes

Summary of Files submode functions for behavior

Parameters		Files submodes: View Sessions as recorded	Add New Sessions	Analyze Sessions Data
Source Tab	DVR parameters	No	Yes	Yes
	Calibration	No	Yes	Yes
Scenes Tab	Arena/Zones Name	No	Yes	Yes
	Arena/Zones Outline and Fill	Yes	Yes	Yes
	Time Variables	No	Yes	Yes
	Add/delete arena/zones	No	Yes (For the experiments with sessions the change will be applied to all sessions - already recorded and all added after this change)	Yes (For the experiments with sessions the change will be applied to all recorded sessions)
Video View	Move/resize arena/zone shapes	No	Yes (the change will be added to the sessions added after this modification)	Yes (the change will be added only to the currently selected session)
Tracking Tab	All visualization parameters	Yes	Yes	Yes
	Selecting markers/LEDs to track or Motion Measure for the Whole Body	No	Yes (only for an experiment before sessions are recorded)	No
Events, Combo Events and Global Combo	Add/delete/modify events	No	Yes	Yes

Setting the default submode

If an existing experiment already has one or more sessions recorded, when you open the experiment in **Files** mode, the system sets the submode as **Analyze Session Data** by default. However, you can change this behavior if you prefer. If you click on the **Experiment Options** icon, it opens a dialog (see the image below).



You can select the checkbox for the preferred submode as highlighted in the image above. Your selection will take effect the next time you select an experiment.

11.4 Creating or Selecting an Experiment

To create a new experiment or select a previously saved experiment, see these procedures, as applicable.

- Section 3.1, “Data Storage and Organization” on page 30
- Section 3.2, “AVI Video Format, Data Rate, Timestamps and Compression” on page 31
- Section 3.3, “Planning the Database” on page 32
- Section 3.4, “Setting Parameters for an Experiment with Multiple Sessions” on page 33
- Section 3.5, “Setting the Recording Folder Location” on page 35
- Section 3.6, “Creating a New Experiment” on page 36
- Section 3.7, “Selecting a Previously Saved Experiment” on page 44
- Section 3.8, “Editing the Experiment Name, Descriptor and Variable Values” on page 45

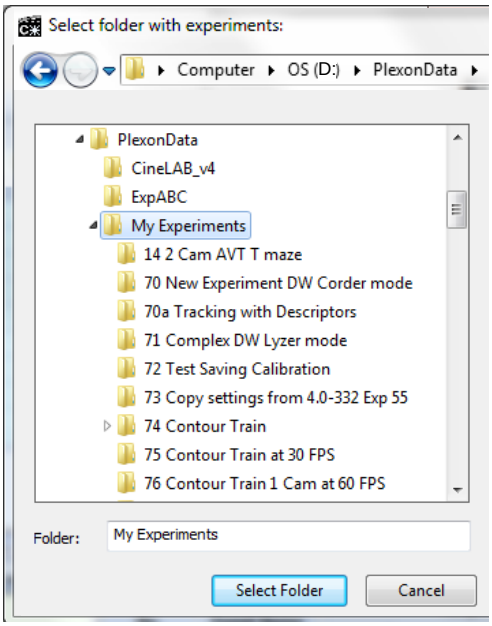
11.5 Viewing Videos of Completed Sessions

This section explains how to use the system in **Files** (offline) mode to analyze the video data in existing files (sessions that have been completed). This procedure uses existing AVI files, which were recorded previously by means of the procedure in Section 10.5, “Configuring, Starting and Stopping the Recording” on page 240.

The offline functions can be run on the host PC where the video files were originally recorded, or on a separate PC with appropriate specifications (see Section 1.8, “Computer to Run Photometry Software” on page 11). To perform offline functions on the separate PC, the required Experiment folders and their contents must be copied to that PC into a recording folder (with a path and name configured in the **Recording Folder** line of the **Experiments** tab). The Plexon® Multi-Wavelength Photometry System license must be plugged into that computer.

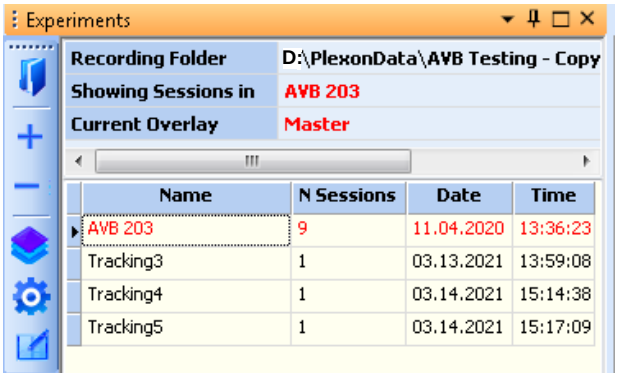
11.5.1 Selecting Files Mode and Navigating to the Experiments Folder

1. Place the system in Files mode if it is not already in that mode.
2. In the **Experiments** tab, open the **Recording Folder** browser by clicking the **Change Recording Folder** icon then navigating to the folder that contains the individual subfolders for your experiments. In the following example, the folder D:\PlexonData\My Experiments is being selected.



Note: You must select a folder that contains one or more experiment subfolders. If you select a single experiment subfolder, you will not be able to perform any analysis on it. (You can select the experiment subfolder but you will not be able to access the experiment in the Experiments tab.)

3. Click **OK**.
4. Click the Experiments tab to display a list of all your experiments in the folder.
5. In the Experiments tab, click in the row for the experiment you want to analyze.



Note: After you click on the row for your experiment, notice that the list of sessions for this experiment appears in the **Sessions** window.

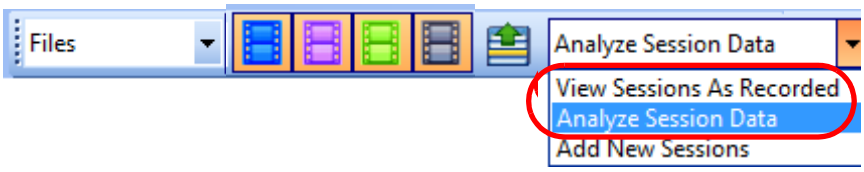
6 (Optional) In the Global Config tab you have the option to change the settings for **Layers Transparency** (default value 0.5), and additional parameters if Tracking is enabled in the behavioral video. The default values are suitable for many experiments.

Note: After a session has been recorded in an experiment, you cannot change the number of fibers or the number or tracked objects in the experiment. This ensures consistency for all sessions in the current experiment.

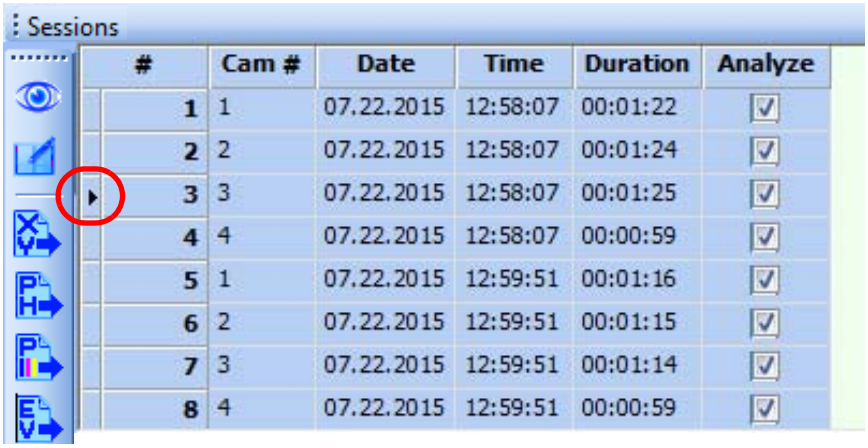
11.5.2 Selecting Sessions to Be Viewed and/or Analyzed

1 Select one of the following submodes depending on your current needs:

- **View Sessions As Recorded** submode—This is a viewing mode. You can export data but you cannot change fibers, arena or zone shapes, or any parameter settings.
- **Analyze Session Data** submode—This is a viewing and reanalysis mode. You can change settings of the visualization parameters in the photometry videos and the behavioral video, and you can change the behavioral arena and zone shapes. You can then analyze behavioral data and export behavioral statistics, as discussed later in this chapter.

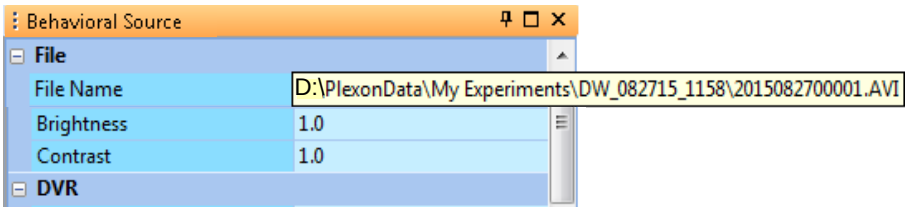


2 In the **Sessions** window, click in the row containing a session for which you want to view the video. In the example below, it is Session 3.

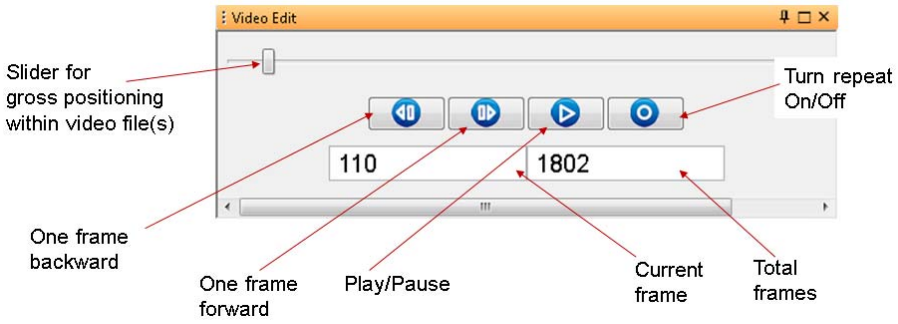


#	Cam #	Date	Time	Duration	Analyze
1	1	07.22.2015	12:58:07	00:01:22	☑
2	2	07.22.2015	12:58:07	00:01:24	☑
3	3	07.22.2015	12:58:07	00:01:25	☑
4	4	07.22.2015	12:58:07	00:00:59	☑
5	1	07.22.2015	12:59:51	00:01:16	☑
6	2	07.22.2015	12:59:51	00:01:15	☑
7	3	07.22.2015	12:59:51	00:01:14	☑
8	4	07.22.2015	12:59:51	00:00:59	☑

The system displays the Video Edit window and the selected videos. You can view the full path and the name of the AVI file associated with the session by moving your mouse over the row to the right of the **File Name**.



3 You can use the icons in the Video Edit window to replay the video file. If the Video Edit window is not displayed, you can display it by selecting **Video Edit** from the **View** dropdown menu in the main toolbar.



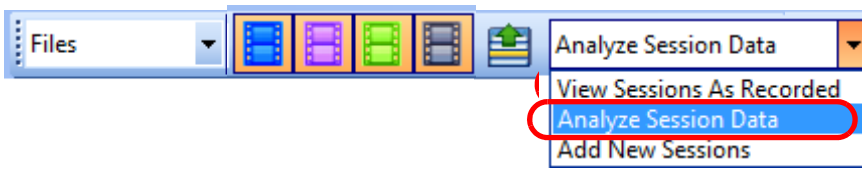
Note: The Video Edit functions are disabled during a recording.

4 To view additional session files, repeat Step 2 and Step 3.

11.6 Modifying Arenas and Zones in the Behavioral Video Stream

After you have recorded several sessions, you might need to move or modify an arena or zone. For example, you might accidentally bump some objects in the arena as you pick up and move an animal and place a new subject in the arena. Zones may have been drawn around those objects, so you might need to move the zones in relation to the new position of the objects in the arena. For information and examples, see Section 11.13, “Using the Overlay Feature during Analysis” on page 292, and Appendix D, Modifying Arenas and Zones.

You need to select **Files/Analyze Session Data** submode (if not already done) to modify the arenas, zones and settings of the previously recorded sessions.



	**CAUTION** **Do not <u>delete</u> an arena or zone shape until you evaluate the impact** If you want to delete an arena, zone or any shape belonging to an arena or zone, evaluate the impact first. Deleting a shape from the current session will delete that same shape from all previous (and future) sessions in the experiment.

	**CAUTION** **Do not <u>modify</u> shapes or settings until you evaluate the impact** In **Files/Analyze Session Data** mode, changes to arena and zone shapes and parameter settings are automatically saved. The original shapes and settings cannot be recovered once you change them.

11.7 Adding Sessions to an Existing Experiment

This section explains how to add sessions to an existing experiment. The added sessions can be AVI files that were not originally created in the existing experiment folder, or AVI files in this experiment folder that you want to re-record with different sessions variables values. The added files become new session numbers within the experiment folder.

11.7.1 Adding a Session in Cameras Mode

You can add a session to a new or previously saved experiment by recording video in **Cameras** mode. For the procedure, see Section 10.5, “Configuring, Starting and Stopping the Recording” on page 240.

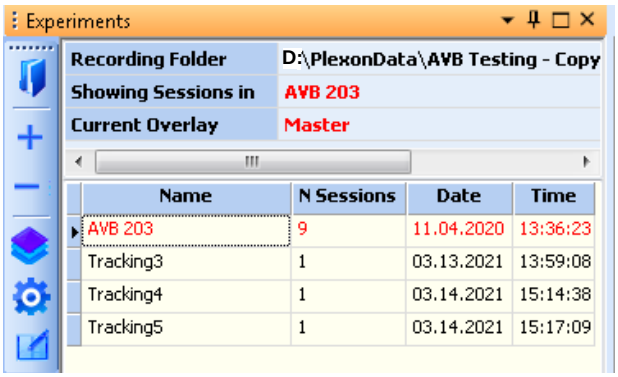
11.7.2 Adding a Session in Files Mode

Adding a session in **Files** mode means re-recording an existing AVI file with different settings.

Selecting the file and managing the Global Config parameters

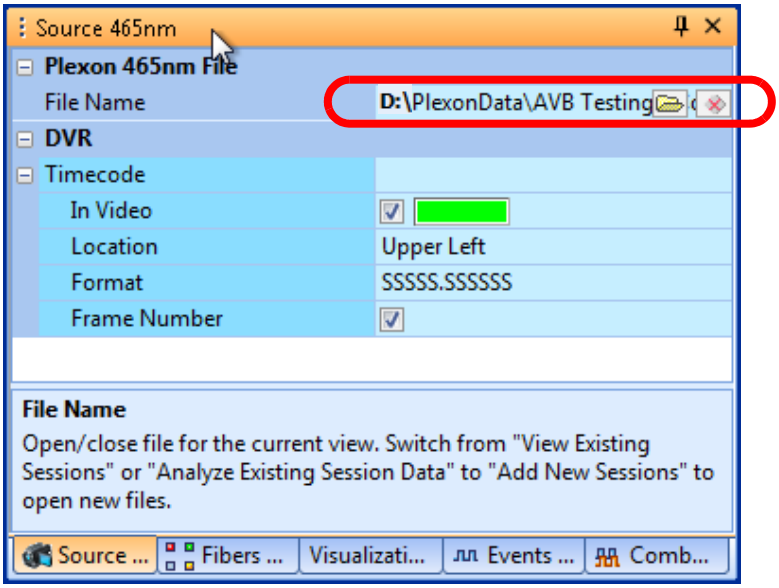
1. Place the system in **Files** (offline/analysis) mode if it is not already in that mode.
2. In the **Experiments** tab, open the **Recording Folder** browser by clicking the **Change Recording Folder** icon then navigating to the folder that contains the individual subfolders for your experiments.

   Note: You must select a folder that contains one or more experiment subfolders. If you select a single experiment subfolder, you will not be able to perform any analysis on it. (You can select the experiment subfolder but you will not be able to access the experiment in the Experiments tab.)
3. Click **OK**.
4. Click the Experiments tab to display a list of all your experiments in the folder.
5. (Optional) In the Global Config tab you have the option to change the settings for **Layers Transparency** (default value 0.5), and additional parameters if Tracking is enabled in the behavioral video. The default values are suitable for many experiments.
6. In the Experiments tab, click in the row for the experiment to which you want to add sessions.



   Note: After you click on the row for your experiment, notice that the list of sessions for this experiment appears in the Sessions window.
7. Select the **Add New Sessions** option from the dropdown menu in the main toolbar, as shown in the image below.

8 In the Source tab, in the **File Name** row, click on the folder icon to navigate to the folder containing the desired AVI file and select the file.



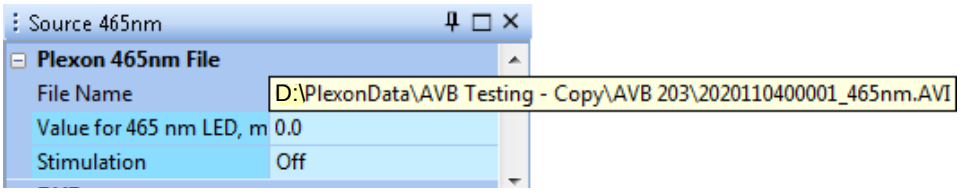
Note: If you are using the behavioral feature and you select a file that was created with a tracking option that differs from the tracking option of the experiment (as set previously in the Behavioral Video tab), the system does not open the file, but displays an informational dialog box.

Similarly, you will not be able to open a file with a different number of fibers than the current experiment. In this case, you will need to create a new experiment in the **Files** mode from the video files.

TIP

View the full file name and path in Files mode

In **Files** mode, you can view the full path and file name by positioning your mouse over the cell on the right side of the File Name row, as shown in the following image.



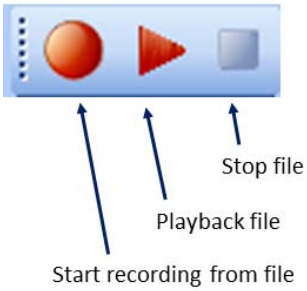
In the above example, the video file name is 2020110400001_465nm.AVI. The format is yyyymmdd#####_N.AVI, that is, the year, month, day, sequential number (a five-digit number generated automatically by the system), and the video stream for which the file was created (N = 465nm, 410nm, 560nm or Beh).

CAUTION

Do not manually change the names of AVI files

Do not attempt to change the file name of an AVI file in the Windows environment, because that will cause the system to be unable to locate it automatically in the future.

9 Repeat Step 8 for each of the files you want to include in the session. You will need to load an existing file for all four of the video windows (the three photometry wavelengths and the behavioral camera). After all four video files are loaded, the system enables the recording (and Play) toolbar icons.



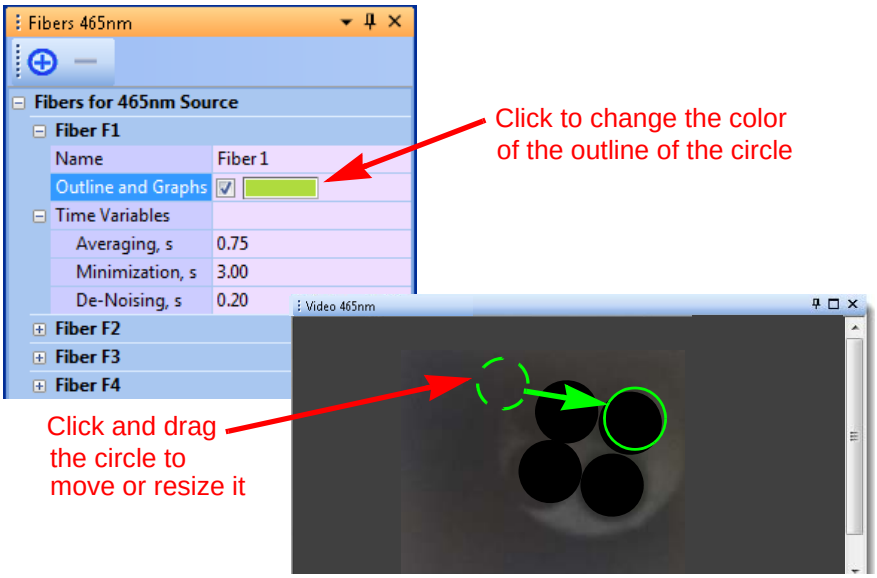
10 Modify any of the settings you want to change in the four video configuration tabs, or modify shapes in any of the four video windows. For example, you can move or resize a fiber in a photometry window, change the settings for events, or move or resize a zone or arena shape in the behavioral window.

If necessary, modify the position and diameter of a fiber outline in a photometry video window so it matches the circular region of the fiber. You can move and resize the fiber outline as shown in the image below. You can also change the color of the outline and the associated data graphs that will be displayed later for this fiber. However, after a session is recorded in the

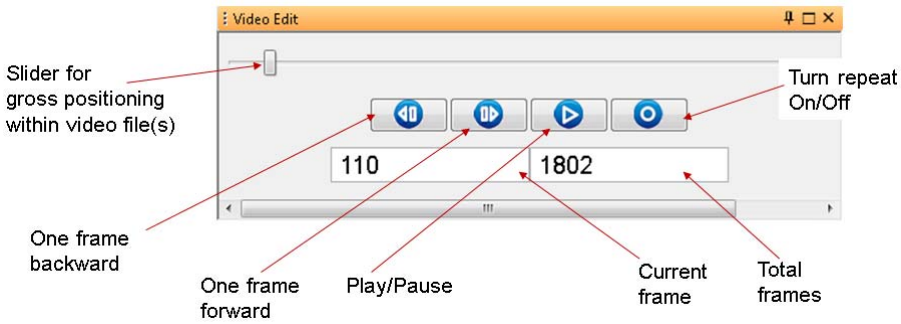
current experiment, you cannot change the number of fibers configured for each wavelength.

If necessary, modify the size, location or number of zone or arena shapes in the behavioral video. (Arena and zone shapes are described in Chapter 7, Configuring the Tracking Parameters and Chapter 8, Behavioral Features and Procedures.) However, you cannot change the tracking mode or the number of tracked objects. Those parameters are already set in the AVI file that was loaded in the behavioral Source tab.

The example below shows how to move and resize a fiber circle.



11 (Optional) You can use the icons in the Video Edit window to replay the video file frame by frame. Note that the Video Edit controls are active when you are viewing a previously saved video file, but inactive when a new video is being recorded.



Preparing to start recording a session

The **Start recording from file** icon on the toolbar remains grayed out and inactive until the preconditions for starting a recording have been met (in the previous steps). It turns red and active after the preconditions have been met.

12 Click on the **Source** tab under the Video window to view the parameters in the Source tab. The settings for these parameters were entered in the procedures in Chapter 4, Photometry Features and Procedures and Chapter 5, Setting Up the Behavioral Camera. If any of these settings need to be changed, make those changes now.

13 IMPORTANT—
If you plan to make any changes in the behavioral **Tracking**, **Scenes** or other tabs, first be sure to read and understand the information in Section 11.14, "Caveats—Understanding Tracking Data in Computations" on page 296.

Starting the recording of a session

14 To create (add) the new session, click the red **Start recording from file** icon.

The system prompts you to insert values for the user defined variables. Double click in each cell and enter the values you want to use for this session. Then click **OK**.

The system starts the recording.

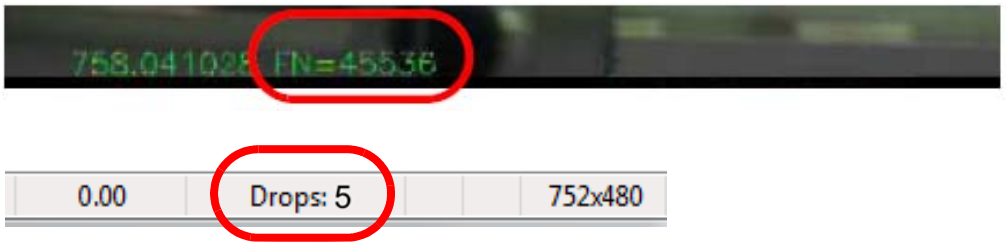
15 You can stop the recording by clicking the **Stop file** icon  . Or you can allow the video to continue until it is finished.

Note: The Video Edit functions are disabled during a recording.

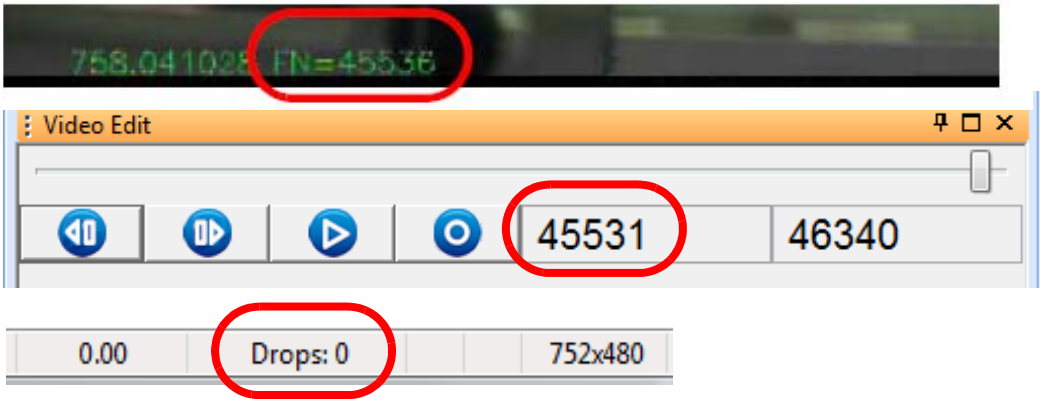
Monitoring dropped frames when adding a session (re-recording a file)

One or more video frames can be dropped (fail to be recorded to an AVI file) during a recording session if the computer is experiencing partial congestion, which can be caused by running high-CPU applications at the same time as the Plexon software. It is important to understand how the system displays information about dropped frames when your system is in **Files** mode vs. **Cameras** mode.

During original recording in Cameras mode—In the example below, notice that the frame number displayed in the Video window is 45536 and the status bar at the bottom of the GUI shows that five frames have been dropped. This means that only 45531 frames have been successfully recorded.



During playback or re-recording (adding sessions to an existing experiment) in Files mode—In the example below, notice that the frame number displayed in 7the Video window is 45536, but the frame count in the Video Edit bar is 45531 (the number of frames that have been successfully recorded). However, since *no additional frames* can be dropped during playback or re-recording session, the status bar at the bottom of the GUI is disabled—it will always display **Drops: 0** in **Files** mode.



Note: In the event that processing ever experiences problems, see "Issues with Dropped Frames and PC Overload" on page E-15. If problems persist, please contact Plexon Support for help in identifying and resolving the issue.

11.8 Setting the Analysis Parameters and Analyzing the Data

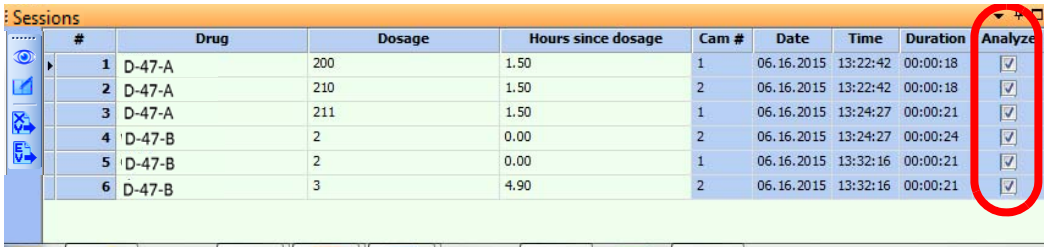
This section explains how to view behavioral statistics for existing sessions in an experiment. It applies only to the behavioral data, not the photometry data. You can use these procedures in any of the **Files** submodes.

11.8.1 Setting the Tracking Visualizations, Scenes and Events

Set the parameters as described below according to the analytical needs of your experiment. These settings are described in other chapters in this document.

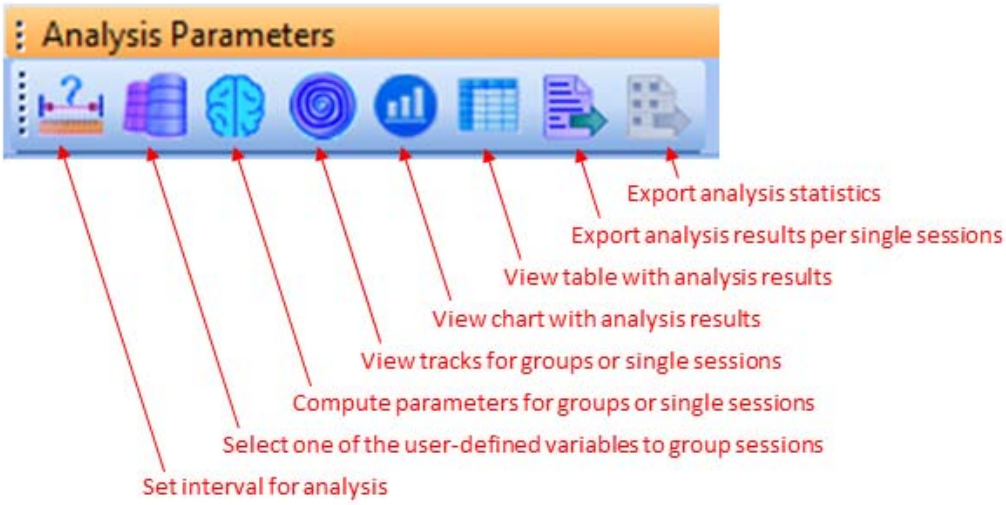
1. Set the parameters in the **Marker Visualizations**, **Whole Body Visualizations** or **LED Visualizations** section of the Tracking tab
2. Set the parameters in the Scenes tab
3. Set the parameters in the Sequences tab
4. Set the parameters in the Events tab
5. Set the parameters in the Behav Combo tab
6. Configure digital output lines if desired
7. Configure global combination events if desired
8. In the Sessions tab, select one or more of the sessions that you want to include in the visualization and analysis. You can select and deselect individual check boxes in the **Analyze** column, or you can right click anywhere in the Sessions tab and click the option **Mark All Sessions As Included to Analysis**.

   Note: If no tracking data was recorded for the sessions in a particular experiment, the **Analyze** column displays “N/A” instead of the checkboxes.



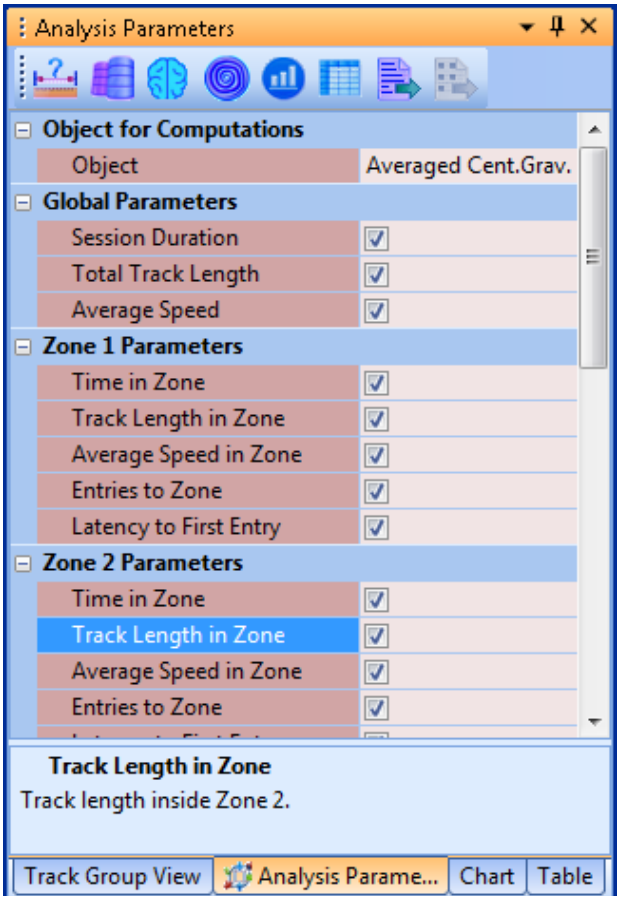
11.8.2 Navigating the Analysis Parameters Toolbar and Features

The Analysis Parameters tab and the Analysis toolbar are shown below. Some of the icons in the toolbar will be grayed out (unavailable) until the **Compute parameters for groups or single sessions** icon is clicked.



A shown in the following image, a list of parameters is displayed in this tab. You can click in the check boxes to select which parameters you want displayed in the **Chart** tab and exported to the comma separated values (CSV) file.

- **Object for Computation** parameters (available for the LED and Color Markers tracking modes but not for Object Contour mode)
- **Global Parameters** (**Session Duration**, **Total Track Length** and **Average Speed**)
- **Zone 1/2/3/... Parameters**. Zone-specific parameters become available after you create a zone in the Scenes tab.



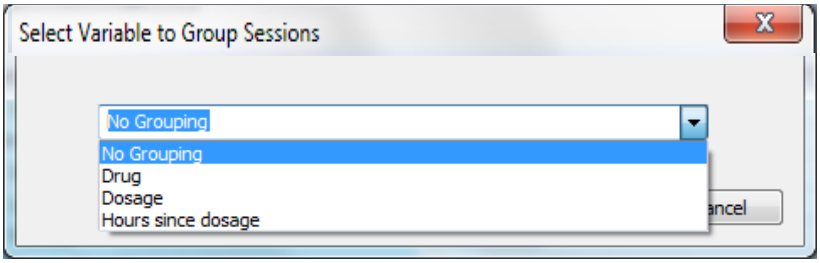
If you create a zone, the **Analysis Parameters** tab will automatically display the zone-specific parameters list, as shown in the above image, and the system will be able to provide zone-specific data. However, if you want to generate a digital output signal when the animal enters a zone, you must set up an event for the zone entry (in the Events tab). You can use that zone entry event to send a signal to an external device, for example, to have that external device turn on an LED or activate a pellet dispenser. To configure a zone-specific event, see Section 8.5, “Adding Zones (Static and Dynamic)” on page 188 and Section 8.8.1, “Events Based on a Zone” on page 201.

11.8.3 Perform an Analysis and Group Event Results by Session Number

This section explains how to set the options for the analysis by session number.

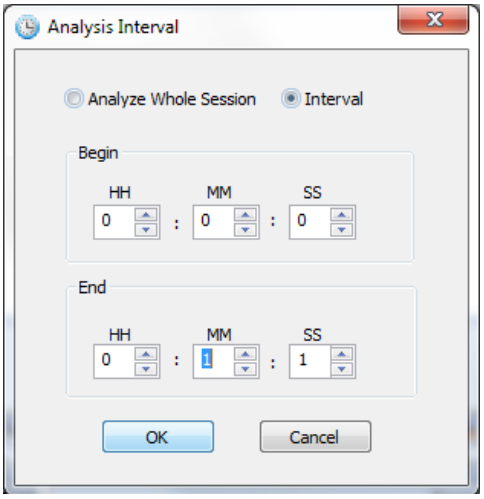
1 In the Analysis toolbar, click the **Select one of the user-defined variables to group sessions** icon.

2 The **Select Variable to Group Sessions** dialog box opens.



3 Ensure that the choice in the dropdown list is **No Grouping**, which is the default value. Selecting this option (**No Grouping**) instructs the system to sort results according to Session number.

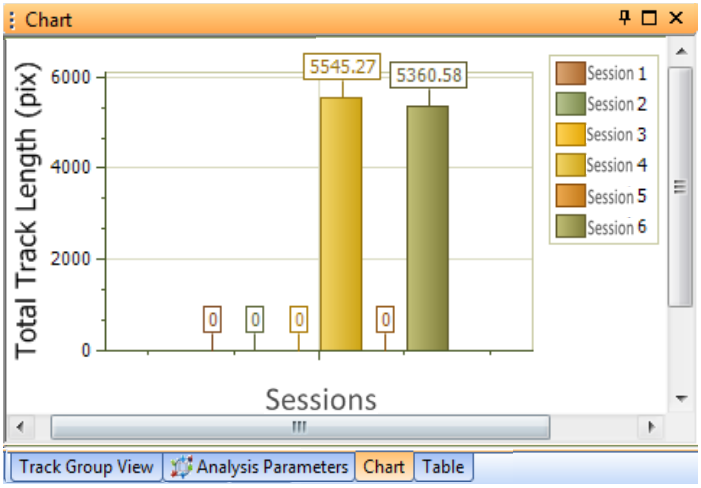
4 (Optional) Adjust the time interval by clicking the **Set Interval for Analysis** icon and adjusting **Begin** and **End** times to apply to all sessions in the experiment. The default value is **Analyze Whole Session**.



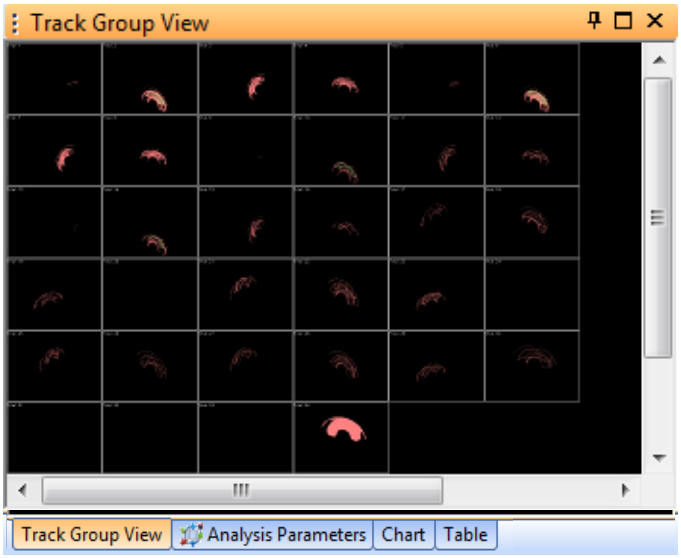
After you set the Analysis Interval, the **View chart with analysis results**, **View table with analysis results** and **Export analysis results per single sessions** icons become active. (The **Export analysis statistics** icon remains inactive because no meaningful statistics can be generated for individual sessions for this type of data.)

5 Click the **Compute parameters for groups or single sessions** icon to instruct the system to perform the calculations that will be used for behavioral analysis.

6 View the **Chart** tab. Notice in this example that the **Total Track Length** (the total distance the animal moved during the specified time interval) is shown for each session. The length is displayed in pixels if you have not calibrated the arena dimensions, or in inches or centimeters if you have calibrated the arena dimensions.

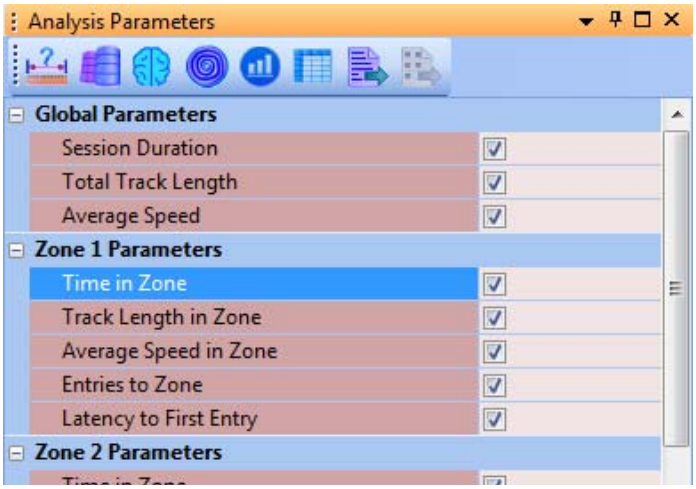


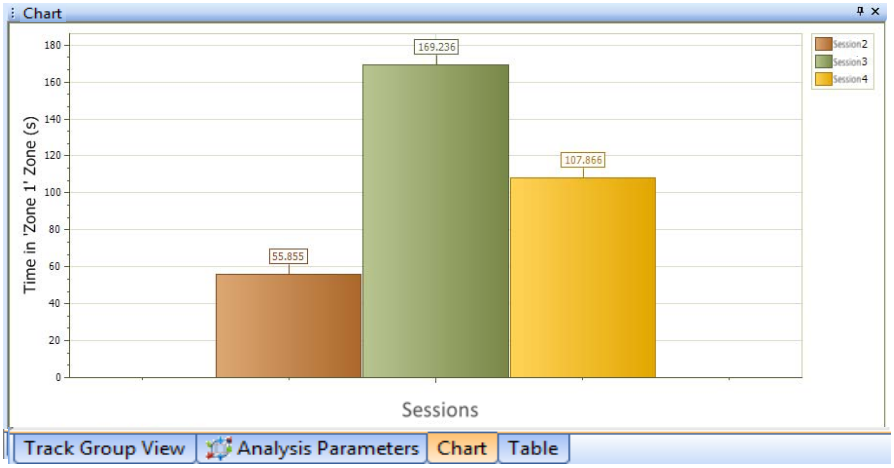
7 Click on the **Track Group View** tab to see a record of the animal's movements during the session.



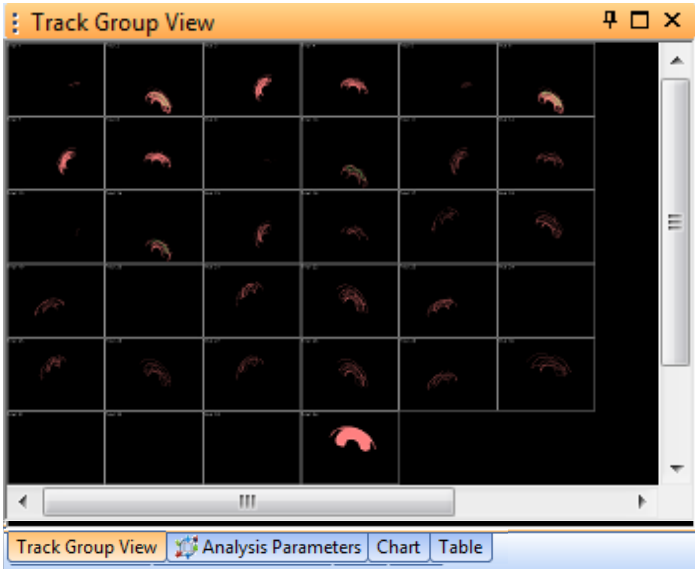
8 You can visualize (display) various sets of data in the **Chart** tab by highlighting an item in the Analysis Parameter list. In the example below,

Time in Zone is selected for Zone 1 and the times are displayed for each session.

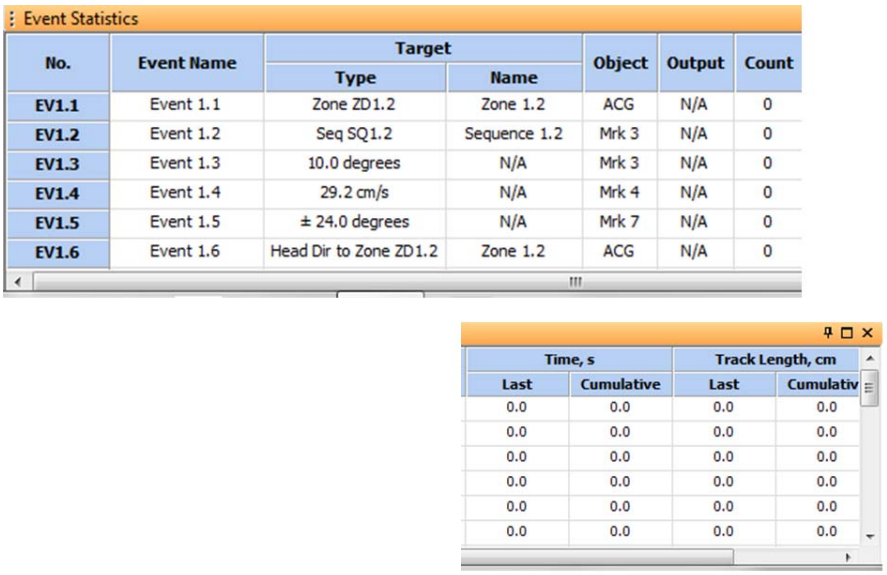




9 Select the Track Group View to view the actual path of the animal for each session.



10 If the animal's movements cause behavioral events to be detected during the session, you can view the event data in the Event Statistics window. The Event Statistics window is populated only during an experiment, not if you are viewing data from a previously saved experiment. You must rerun the video of the previously saved experiment to populate these fields.



Event Statistics

No.	Event Name	Target		Object	Output	Count
		Type	Name			
EV1.1	Event 1.1	Zone ZD1.2	Zone 1.2	ACG	N/A	0
EV1.2	Event 1.2	Seq SQ1.2	Sequence 1.2	Mrk 3	N/A	0
EV1.3	Event 1.3	10.0 degrees	N/A	Mrk 3	N/A	0
EV1.4	Event 1.4	29.2 cm/s	N/A	Mrk 4	N/A	0
EV1.5	Event 1.5	± 24.0 degrees	N/A	Mrk 7	N/A	0
EV1.6	Event 1.6	Head Dir to Zone ZD1.2	Zone 1.2	ACG	N/A	0

Time, s		Track Length, cm	
Last	Cumulative	Last	Cumulativ
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0

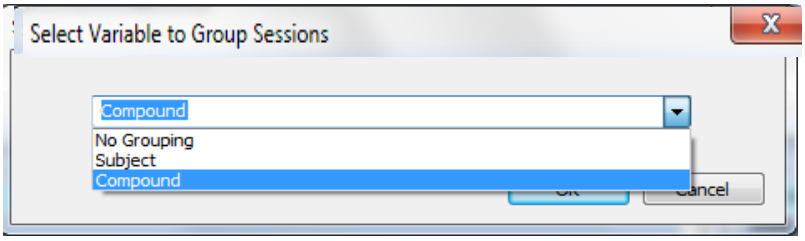
11.8.4 Perform an Analysis and Group Event Results by Variables

This section explains how to set the options for data analysis based on the variables associated with the sessions in an experiment.

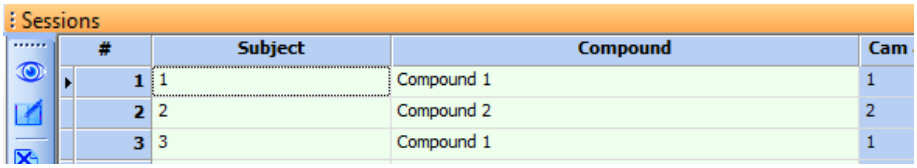
1 In the **Analysis** toolbar, click the **Select one of the user-defined variables to group sessions** icon.

2 The **Select Variable to Group Sessions** dialog box opens.

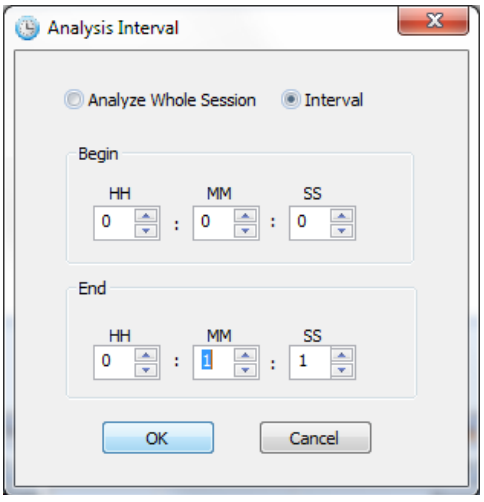
3 In the dropdown list, select one of the variables for the analysis.



Note that the variable options in the dropdown list are the same as the headings of the variable columns in the Sessions window.



4 (Optional) Adjust the time interval by clicking the **Set Interval for Analysis** icon and adjusting **Begin** and **End** times to apply to all sessions in the experiment. The default value is **Analyze Whole Session**.



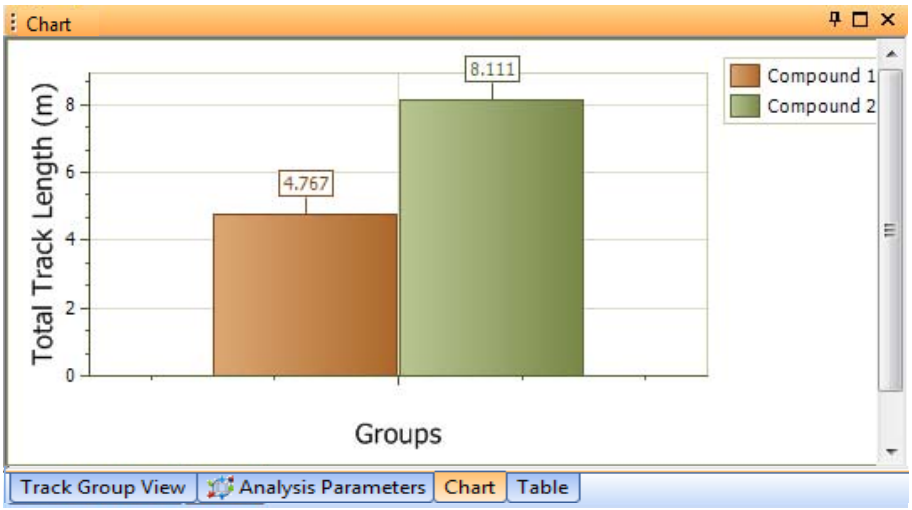
After you set the Analysis Interval, the **View chart with analysis results**, **View table with analysis results**, **Export analysis results per single session**, and **Export analysis statistics** icons become active.

5 Click the **Compute parameters for groups or single sessions** icon  to instruct the system to perform the calculations that will be used for behavioral analysis.

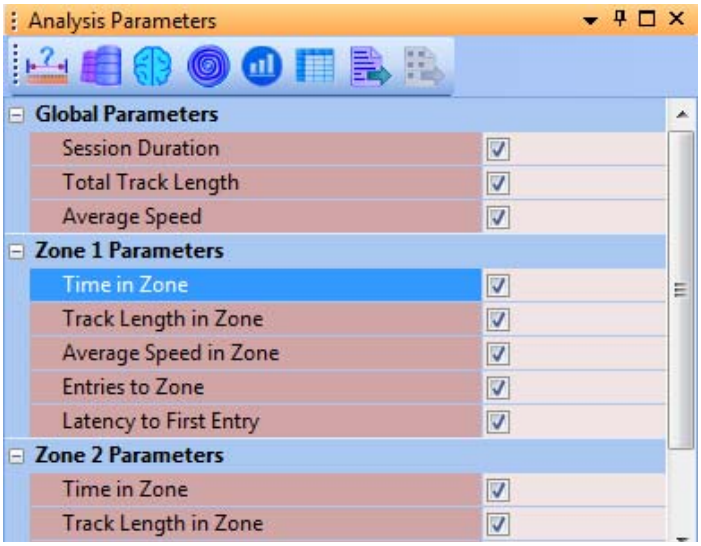
6 View the results.

Notice that in this example, the **Total Track Length (m)** is shown for the variable group that was selected in Step 3, which was **Compound**. Each of these compounds was used in several sessions. The average total track length for all sessions involving Compound 1 was 4.767m and the average total track length for all sessions involving Compound 2 was 8.111m.

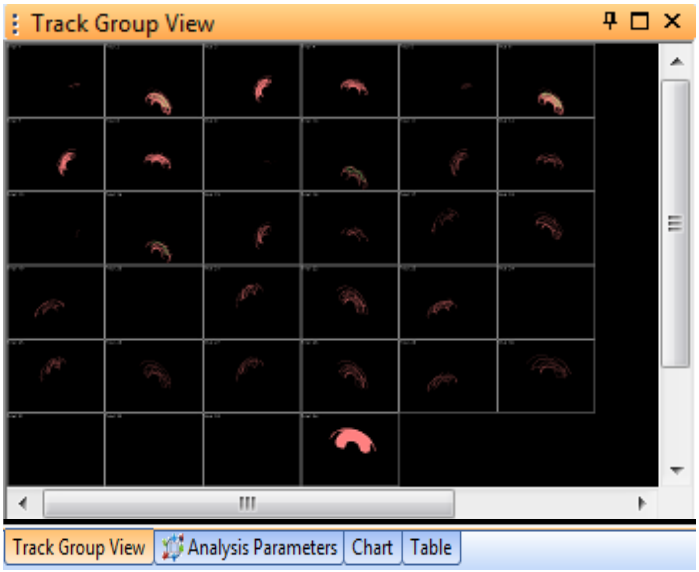
In general, the chart shows the average value for all sessions in each Group.



7 You can visualize (display) various sets of data by highlighting an item in the Analysis Parameter list.

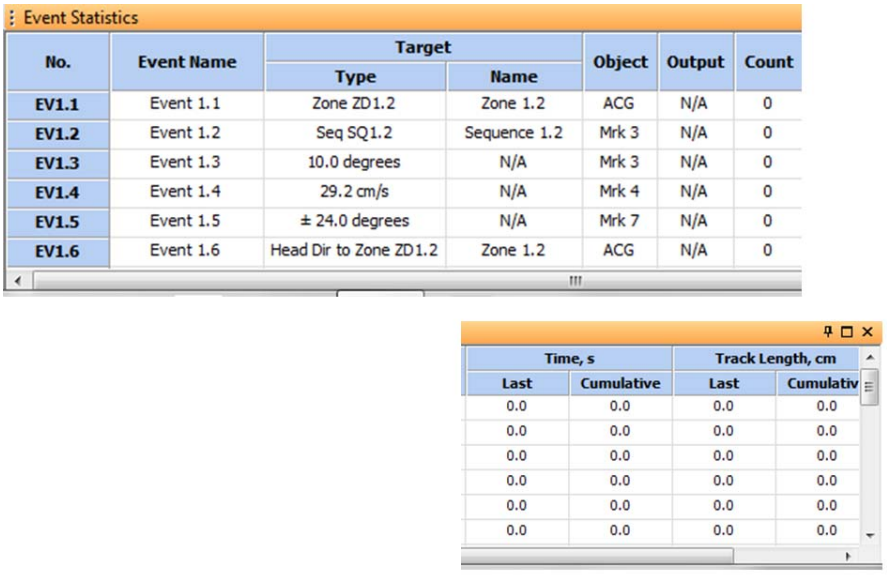


8 Select the Track Group View to view the actual path of the animal for each session.



9 If the animal's movements cause behavioral events to be detected during the session, you can view the event data in the Event Statistics window. The Event Statistics window is populated only during an experiment, not if you are

viewing data from a previously saved experiment. You must rerun the video of the previously saved experiment to populate these fields.



Event Statistics

No.	Event Name	Target		Object	Output	Count
		Type	Name			
EV1.1	Event 1.1	Zone ZD1.2	Zone 1.2	ACG	N/A	0
EV1.2	Event 1.2	Seq SQ1.2	Sequence 1.2	Mrk 3	N/A	0
EV1.3	Event 1.3	10.0 degrees	N/A	Mrk 3	N/A	0
EV1.4	Event 1.4	29.2 cm/s	N/A	Mrk 4	N/A	0
EV1.5	Event 1.5	± 24.0 degrees	N/A	Mrk 7	N/A	0
EV1.6	Event 1.6	Head Dir to Zone ZD1.2	Zone 1.2	ACG	N/A	0

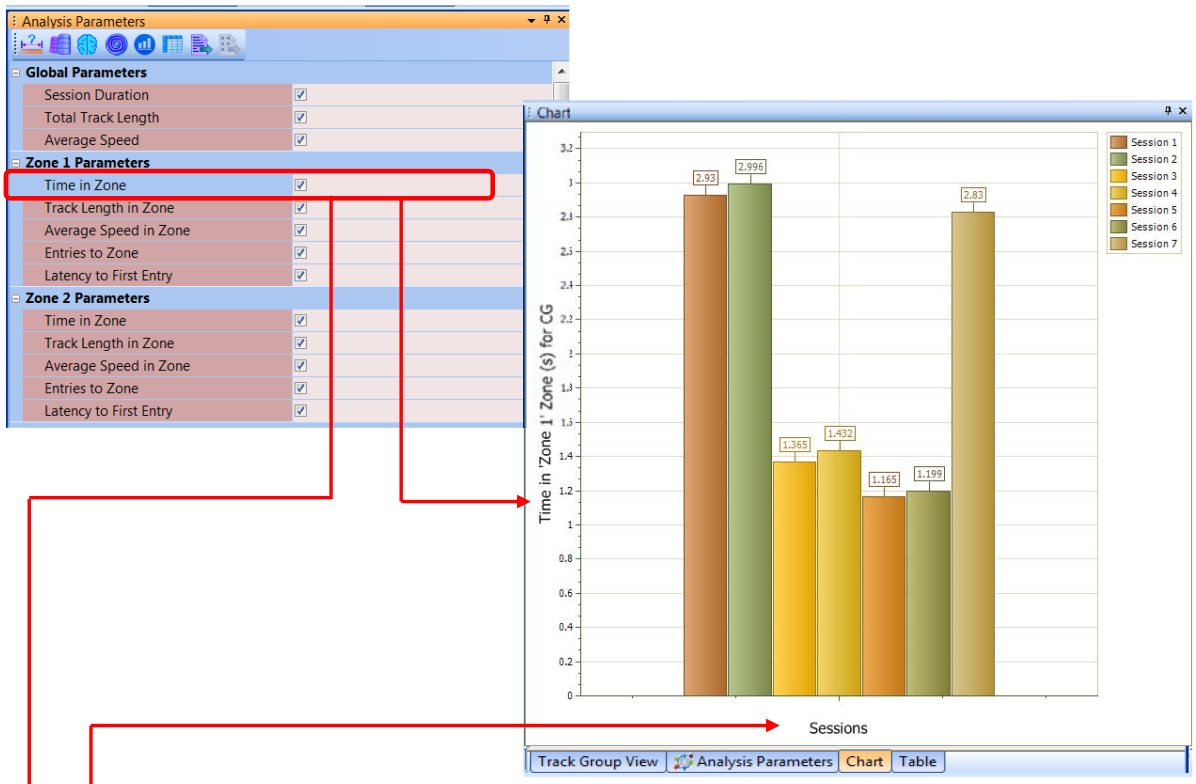
Time, s		Track Length, cm	
Last	Cumulative	Last	Cumulativ
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0

11.9 Exporting Analysis Results

Export analysis results per single session

Use the **Export analysis results per single sessions** icon  to export results in comma-separated values (CSV) format for further analysis in other software applications.

As shown in the example below, the exported data includes global parameters (session duration, total track length and average speed) and zone-related parameters (time in zone(s) and zone sequence(s), track length, average speed, entries to zone, and latency to first entry). The data are the same as what is shown in the **Chart/Table** tabs and **Sessions** tabs. See the example below.



#	ID	Condition	Cam #	Date	Time	Duration	Analyze
1	1.00	learning	1	05.05.2016	15:04:53	00:00:59	☑
2	2.00	testing	1	05.05.2016	15:06:37	00:02:36	☑
3	3.00	learning	1	05.05.2016	15:15:45	00:01:38	☑
4	4.00	testing	1	05.05.2016	15:26:53	00:01:07	☑
5	5.00	learning	1	05.05.2016	15:30:34	00:01:10	☑
6	6.00	testing	1	05.06.2016	12:26:53	00:00:56	☑
7	7.00	testing	1	05.09.2016	10:13:52	00:04:44	☑

Exported data

Trials	ID	Condition	Session Duration (s)	Total Track Length (pix) for CG	Average Speed (pix/s) for CG	Time in 'Zone 1' Zone (s) for CG	Track Length for 'Zone 1' Zone (pix) for CG	Average Speed for 'Zone 1' Zone (pix) for CG	Number of Entries to 'Zone 1' Zone for CG	Latency to First Entry to 'Zone 1' Zone (s) for CG
Session 1	1	learning	59.529	2145.998	36.049	2.93	596.461	203.581	2	0.1
Session 2	2	testing	156.614	5163.135	32.967	2.996	600.889	200.535	2	30.863
Session 3	3	learning	98.516	2211.052	22.444	1.365	299.107	219.119	1	84.866
Session 4	4	testing	67.62	2930.435	43.337	1.432	296.95	207.421	1	30.863
Session 5	5	learning	70.982	2488.866	35.063	1.165	285.296	244.83	2	10.654
Session 6	6	testing	56.932	2373.671	41.693	1.199	287.984	240.272	2	10.621
Session 7	7	testing	284.628	8941.24	31.414	2.83	710.566	251.086	5	30.863

The global data types are defined as follows:

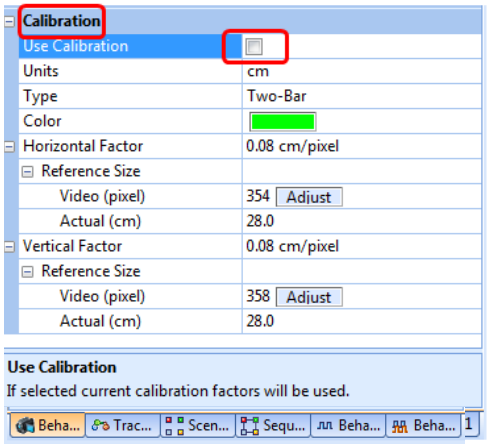
- **Session Duration** (seconds)—Total recording time for the session.
- **Total Track Length** (pixels, cm or inches)—Cumulative track length the animal travelled during the session. If the arena is calibrated, the total track length is displayed in the user-specified units (cm or inches); if it is uncalibrated, the total track length is displayed in pixels.
- **Average Speed** (pixels, cm or inches per second)—The average speed during the session. See Section 8.8.4, “Events Based on Speed” on page 208 for the method the system uses to calculate average speed.

TIP

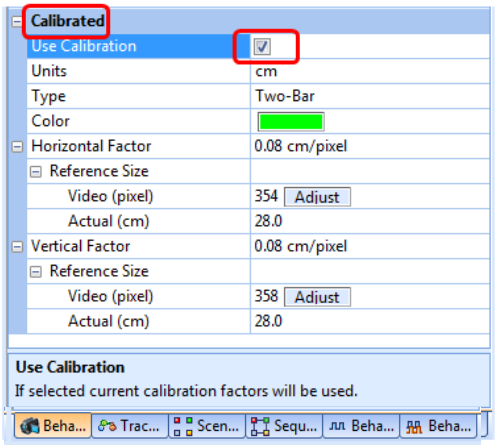
Select the “Use Calibration” checkbox for calibrated data

Remember that the positional coordinates in the exported file will be in pixels if the **Use Calibration** checkbox was not checked during recording, or if it was deselected prior to clicking the **Compute** ( ) button. See the examples below. The calibration procedures are provided in Chapter 6, Calibrating the Arena Dimensions.

Extracted coordinates will be in pixels if:
Use Calibration is unchecked
when recording is started
-or--
before clicking the **Compute** button



Extracted coordinates will be in cm or in. if:
Use Calibration is checked
when recording is started
--or--
before clicking the **Compute** button



The zone-specific data types are defined as follows:

- **Time in Zone** (seconds)—Cumulative time that the event was true during the session.
- **Track Length in Zone** (pixels, cm or inches)—Cumulative track length the animal travelled during the time(s) the event was true in the session. If the

arena is calibrated, the track length is displayed in the user-specified units (cm or inches); if it is uncalibrated, the track length is displayed in pixels.

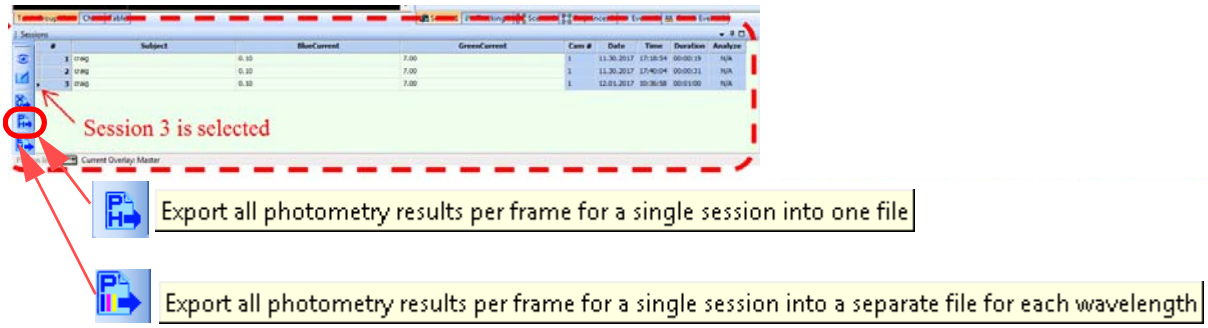
- **Average Speed in Zone** (pixels, cm or inches per second)—The average speed of the animal during the time(s) the event was true in the session. See Section 8.8.4, “Events Based on Speed” on page 208 for the method the system uses to calculate average speed.
- **Entries to Zone**—The number of times the event was true in the session.
- **Latency to First Entry** (seconds)—The time that elapsed between the start of the recording and the first time the event was true during the session.

Export analysis statistics

Use the **Export analysis statistics** icon  to export results in comma-separated values (CSV) format for use in other software applications.

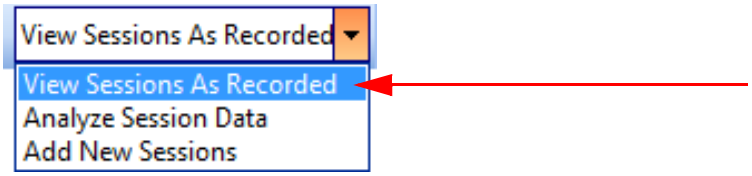
11.10 Exporting Photometry Results per Frame

To view the procedures for exporting photometry results, see Section 4.15, “Exporting Photometry Results” on page 92.

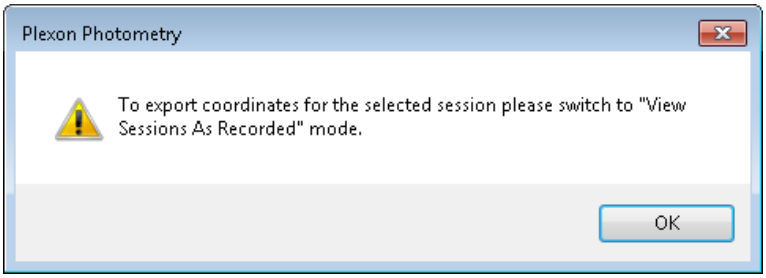


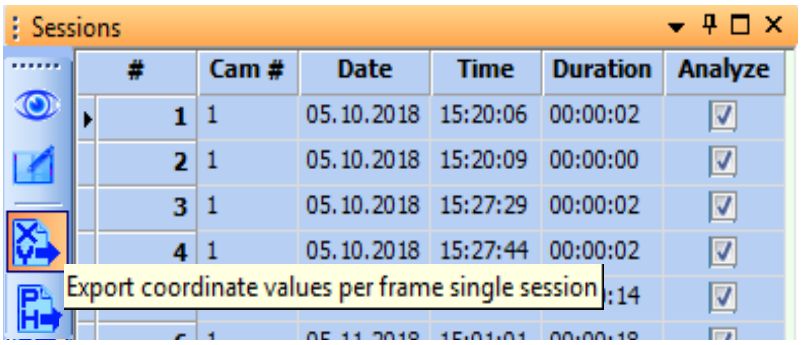
11.11 Exporting Coordinate Values and Motion Measure per Frame

This section describes the coordinate values in the behavioral video stream that you can export to a comma separated values (CSV) file. You must be in **Files** mode and **View Sessions as Recorded** submode to export.



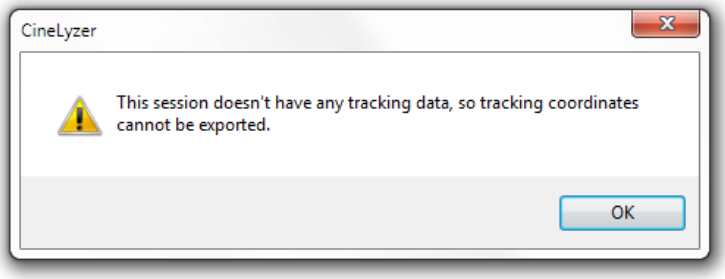
Note: If you attempt to export data in either of the other submodes (Analyze Session Data or Add New Sessions), the system will notify you to switch to View Sessions As Recorded submode.





When you click this button, the system prompts you to name and save the CSV file. A typical file will look similar to the example below. (Spaces have been added to this image for clarity, but in an actual file, there are no spaces.)

Note: If the selected session does not have any tracking data to export, the system displays a message to that effect.



Data Format

The exported file (CSV format) will contain the frame number, time (seconds from start of the recording), X coordinate, Y coordinate and Motion Measure. Motion Measure will be present if **Object Contour** tracking mode has been used. (Motion Measure is explained below.)

Frame,	**time,**	**X,**	**Y,**	**MotionMeasure**
71,	2.33057,	262.382,	216.407,	1
72,	2.36387,	260.231,	218.248,	20
73,	2.39716,	266.992,	259.734,	354
74,	2.43046,	264.150,	228.365,	337
75,	2.46375,	262.435,	216.526,	34
76,	2.49704,	264.183,	221.678,	40
77,	2.53034,	261.822,	241.334,	163
78,	2.56363,	259.304,	219.754,	189
.....				
.....				

Units for the X and Y coordinate values—If the **Use Calibration** box has been checked prior to recording the session, the values will be exported in the calibration units (cm or inches). Otherwise the values will be exported in pixels.

Motion Measure is an **Object Contour** mode option that you can use to detect animal “freezing” in fear-conditioning experiments. The Motion Measure option is separate and distinct from the target-tracking capability. The system analyzes the images of the target animal in consecutive frames. The pixels that lie on the animal in the current video frame but not on the animal in the previous video frame (and vice versa) are counted. This number is divided by the total number of animal pixels in the current frame. The maximum result is nominally 2.0, and the range 0.0 to 2.0 is scaled to the range 0 to 1023.

A value of **1023** indicates that the animal moved so quickly that no pixels were in common with the previous frame.

A value of **1** indicates that the frames were literally identical.

A value of **0** results when any one of the following conditions are present:

- The frame is the first frame of the file,
- The previous frame and the current frame are identical,
- The tracking window switched from small in the previous frame to large in the current frame,
- The tracking window switched from large in the previous frame to small in the current frame
- The object in the previous frame and the object in the current frame are of 0 size.

The Motion Measure frame-to-frame pixel overlap technique provides information on the movement of the animal's body over time. For example, a rotating animal's centroid might not change, but the Motion Measure feature would indicate that the animal is not actually "frozen" because consecutive video frames are not identical.

Animal outside arena or not visible in the arena

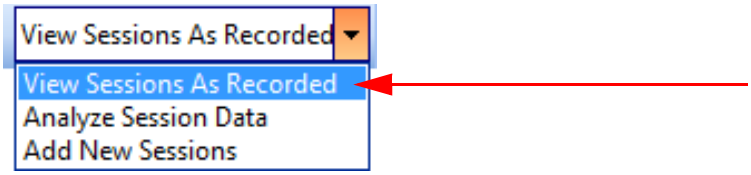
If the animal travels outside the arena, or if it is obscured from the view of the camera (for example, if it travels under an object inside the arena), the system inserts a value of "-10000" in the exported data for that frame. Frames that contain a "-10000" should be excluded from your analysis, or in some cases you may want to consider manually interpolating data in those frames.

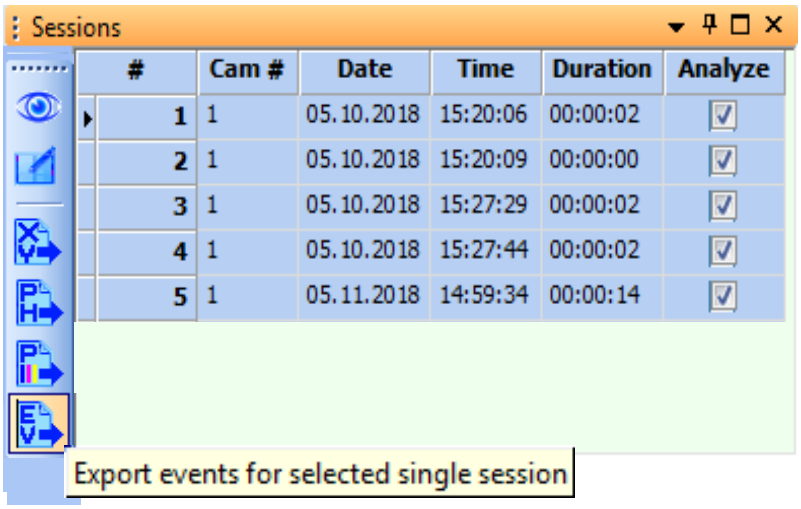
11.12 Exporting Recorded Events per Session

This section describes the event data you can export to a comma separated values (CSV) file. You must be in **Files** mode and **View Sessions as Recorded** to export.

Use the following procedure to export event data.

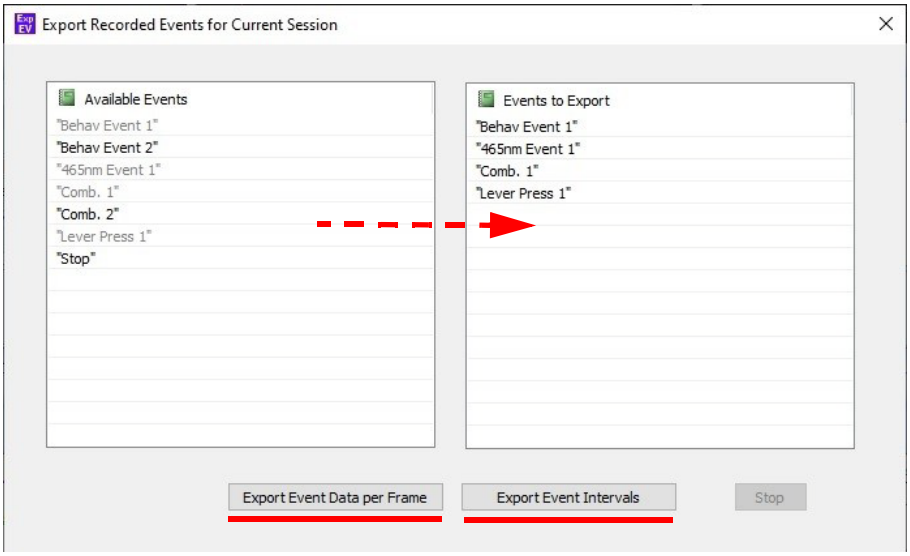
1 In **Files/View Sessions As Recorded** mode, click the **Export events for selected single session** button.





The system opens the export dialog.

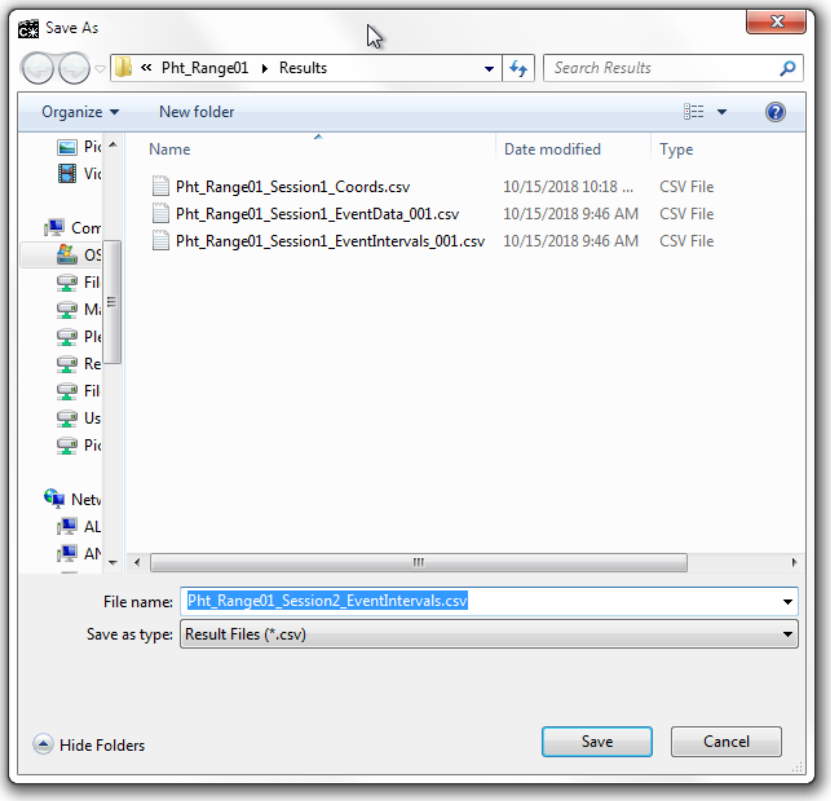
2 In the export dialog, drag the data types you want from the **Available Events** column and drop them in the **Events to Export** column.



Note: If the selected session does not have any event data to export, the **Available Events** list in the export dialog will be empty.

3 To export event data on a per-frame basis, click the **Export Event Data per Frame** button at the bottom of this dialog. To export a file with event intervals data, click the **Export Event Intervals** button.

4 The system displays a **Save As** dialog and prompts you to name and save the CSV file. In most cases, the system provides a suitable file name and location. However, you can modify either or both of these in the displayed dialog.



The exported files will look similar to the examples below. (Spaces have been added to these images for clarity, but in an actual file, there are no spaces.)

Data Format—Export Event Data per Frame

The exported file (which is in CSV format) will contain the frame number, Timestamp (seconds from start of the recording), and indicators for the events being true (1) or false (0). For each behavioral event, the system will also export the track length the animal covered during each interval when this event was happening. The track length is exported either in pixels or, if the behavioral arena has been calibrated before recording, in centimeters or inches. CSV files can be easily opened in Excel. The example below shows an exported event data file opened in Excel:

#	Timestamp	Behav Event 1	Track Length (cm) for Behav Event 1	465nm Event 1	Comb. 1	Lever Press 1
97	3.2	0	0	0	0	0
98	3.233333	0	0	0	0	0
99	3.266667	0	0	1	0	0
100	3.3	0	0	1	0	0
101	3.333333	0	0	1	0	0
102	3.366667	0	0	1	0	0
103	3.4	0	0	1	0	0
104	3.433333	0	0	1	0	0
105	3.466667	0	0	1	0	0
106	3.5	0	0	0	0	0
107	3.533333	0	0	0	0	0
108	3.566667	0	0	0	0	0
109	3.6	0	0	0	0	0
110	3.633333	0	0	0	0	0
111	3.666667	0	0	0	0	1
112	3.7	0	0	0	0	1
113	3.733333	0	0	0	0	1
114	3.766667	0	0	0	0	1
115	3.8	0	0	0	0	1
116	3.833333	0	0	0	0	1
117	3.866667	0	0	0	0	1
118	3.9	0	0	0	0	1
119	3.933333	0	0	0	0	1
120	3.966667	0	0	0	0	1
121	4	0	0	0	0	1
122	4.033333	0	0	0	0	0
123	4.066667	0	0	0	0	0
124	4.1	0	0	0	0	0
125	4.133333	0	0	0	0	0
126	4.166667	0	0	0	0	0
127	4.2	0	0	0	0	0
128	4.233333	0	0	0	0	0
129	4.266667	0	0	1	0	0
130	4.3	0	0	1	0	1
131	4.333333	0	0	1	0	1
132	4.366667	0	0	1	0	1
133	4.4	0	0	1	0	1
134	4.433333	0	0	1	0	1
135	4.466667	0	0	1	0	1
136	4.5	0	0	0	0	1
137	4.533333	0	0	0	0	1
138	4.566667	0	0	0	0	0

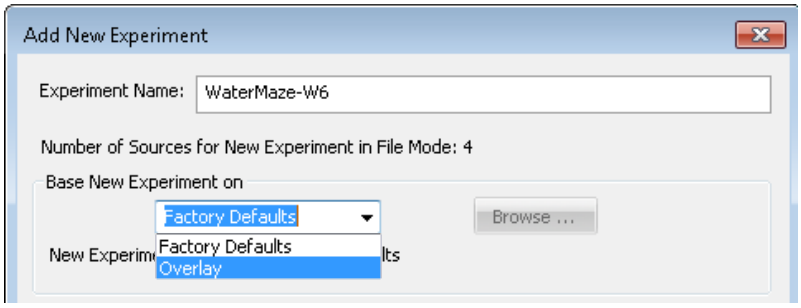
Data Format—Export Event Intervals

This CSV file will contain start and end times for each interval of every event in the right window of the Export dialog above. The example below shows an example of such a file loaded to Excel:

	Event	Start Time (s)	End Time (s)
2	Behav Event 1	30.233333	31.466667
3	Behav Event 1	42.266667	49.466667
4	Behav Event 1	57.266667	59.466667
5	465nm Event 1	16.266667	18.466667
6	465nm Event 1	23.266667	51.466667
7	Lever Press 1	15.033333	15.366667
8	Lever Press 1	34.033333	35.233333

11.13 Using the Overlay Feature during Analysis

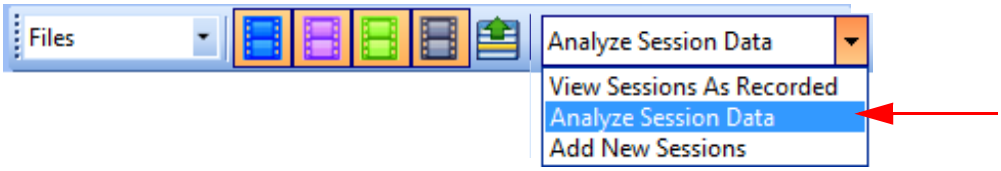
As described in Section 3.6, “Creating a New Experiment” on page 36, when you create a new experiment, the system gives you the option of basing its geometry and video parameter values on [1] **Factory Defaults** or [2] an **Overlay** (a set of geometry and video parameter values that was previously saved as part of another experiment).



If you select an existing overlay when you create a new experiment, and then start recording, the parameter values in that overlay will be saved as the Master overlay for that experiment. For example, if you load an Experiment 6 Master overlay to Experiment 7, those parameter values are saved as the Master overlay for Experiment 7. In this case, both Experiment 6 and Experiment 7 would have identical Master overlays.

Modifying the overlay when analyzing sessions

The flexibility of overlays is helpful when you are analyzing data in previously recorded sessions. That analysis is done in **Files** mode with **Analyze Session Data** submode selected.



A typical reason you would want to modify the arena and zone settings on a per-session basis would be if you had the camera slightly off target for a particular session. This happens sometimes if you accidentally bump the camera while changing subjects and cleaning the arena, or sometimes the camera position may shift during a session unexpectedly. You still want to save and analyze that particular session in your current experiment, but first you need to realign the arena and zone settings with the images you are viewing on the video stream. Using a separate overlay, you can alter the arena and zone positions for that individual session and perform your analysis. The other previously recorded sessions are not affected. You can recalibrate the modified arena if necessary. You can also use this feature during your analysis to focus more precisely on certain regions of the test apparatus or the subject’s behavior in a certain location.

Note: If you make changes to the Master overlay, for example, modifying an arena or zone, or changing parameter values, those changes to the Master overlay are automatically saved and carried forward to future sessions in that experiment.

TIP

Preserving the Master overlay in each experiment

You can modify arenas, zones and other parameters for each experiment. These modifications are allowed only in **Files/Analyze Session Data** mode, and are automatically saved in the Master overlay for the experiment. If you have a current Master overlay that you would like to preserve for future analyses, you can preserve it as follows:

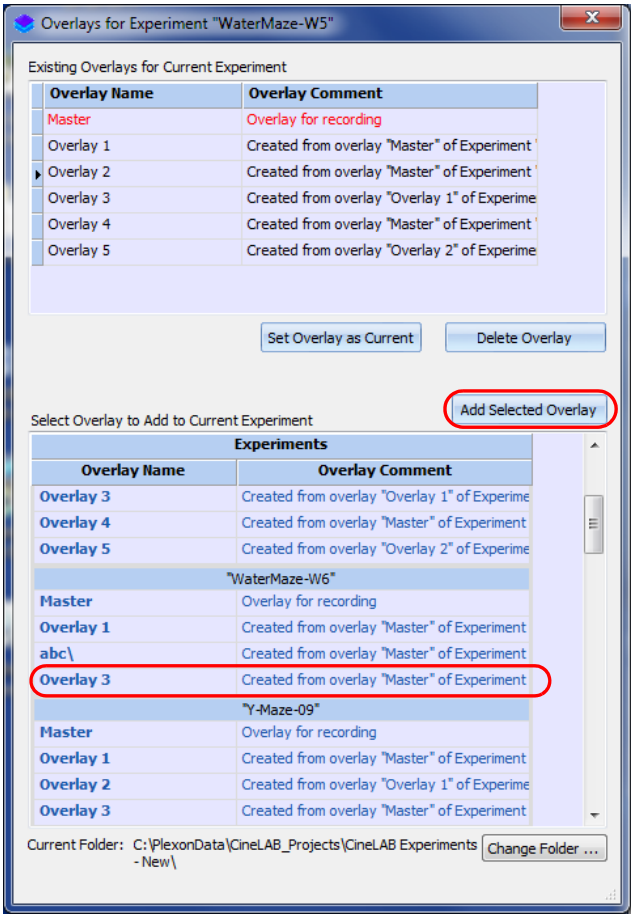
- Create a new experiment that has the same number of video streams and tracking mode as the original experiment, and give it an appropriate name, such as SavedOverlay_1
- Record a session in the new experiment (the data is not important in this case)
- In the future, you can reload that new Master overlay whenever needed
- Return to your original experiment and continue recording sessions, modifying the Master overlay if needed

Procedure

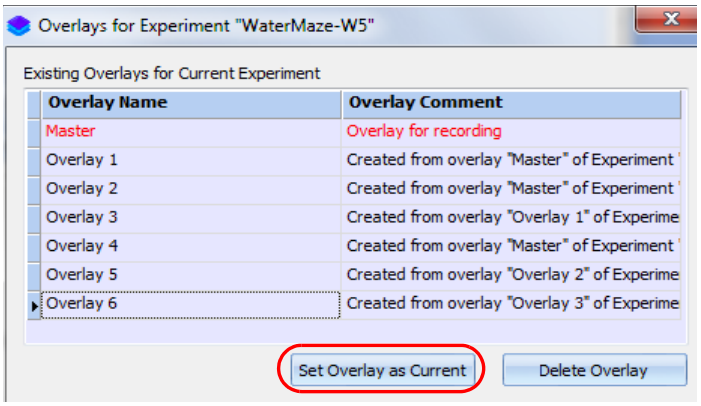
1 Ensure that the system is in **Files/Analyze Session Data** mode.

2 To apply a different overlay to an existing session, click on the **Edit and select overlays** icon .

3 Select the desired overlay from the list in the dialog box. For example, in the list below, select Overlay 3 of WaterMaze-W6 to be added to the available overlays for WaterMaze-W5. Then click the **Add Selected Overlay** button.



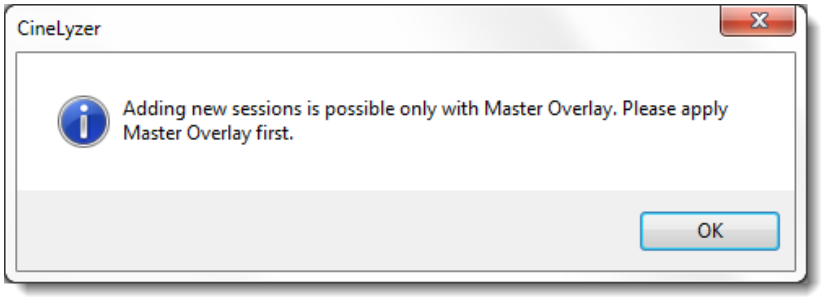
4 Select the appropriate overlay (in this example, Overlay 6, which you previously added to the list of available overlays), then click the **Set Overlay as Current** button.



5 If desired, you can modify the name or comments by double clicking in the field and entering new text.

Adding New Sessions

The applied overlay is used for analysis of *existing* sessions. Later, when you want to add more sessions to that experiment, the system will prompt you to reapply the **Master Overlay** before continuing. The system allows you to add new sessions only when the **Master Overlay** is selected.



11.14 Caveats—Understanding Tracking Data in Computations

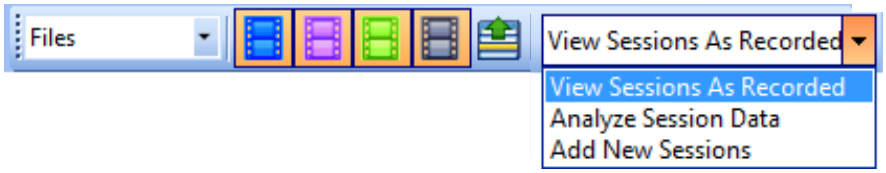
In any experiment, once a session has been recorded with tracking data enabled (that is, a session for which tracking data has been recorded), the tracking data for that session cannot be changed. However, you can still analyze the existing data and use the existing recorded video in several ways.

In Cameras Mode

If you move or resize an existing arena or zone shape, there is no effect on *previously recorded* sessions. All of the original tracking data from the previously recorded sessions are used in the system's computations. Of course, for *future* sessions, tracking data is generated based on the resized or moved shape.

If you add a new shape or delete an existing shape, the system applies the change to *all* sessions, past and future. For computations, the system uses only those tracking data that correspond to the region inside the new arena. (Adding or deleting shapes in an experiment after sessions have been recorded is NOT recommended.)

In Files Mode with "View Sessions As Recorded"



In this mode, you cannot modify an existing arena or zone shape.

In Files Mode with "Analyze Session Data"

If you move or resize an existing arena or zone shape, there is no effect on *previously recorded* sessions. All of the original tracking data from the previously recorded sessions are used in the system's computations. For computations involving sessions you are *currently analyzing*, the system uses only those tracking data that correspond to the region inside the new arena; note, however, that no new tracking data is generated.

If you add a new shape or delete an existing shape, the system applies the change to *all* sessions, past and future. For computations, the system uses only those tracking data that correspond to the region inside the new arena. (Adding or deleting shapes in an experiment after sessions have been recorded is NOT recommended.)

In Files Mode with "Add New Sessions"

In this case, you are generating a new recording and new tracking data using an AVI video file that was created previously. The effects are the same as for **Cameras Mode** described above.

For additional details and examples, see Section 7.16, "Modifying Arenas and Calibration During an Experiment" on page 179.

Appendix A
Optimizing Behavioral Camera Positioning

A.1 Overview

This appendix provides details of the behavioral camera setup and usage. It is intended to assist the scientist in preparing the camera for his or her experiment.

In any experiment where video is required, there is a physical area of interest in which it would be meaningful to record video. This area of interest can be a simple enclosure, a maze, a geometrical track, or any other area needed for the experiment. For video recording of behaving animals, this area of interest is known as the arena. The arena shape and size are critical in determining the distance from the camera to the arena. Colors used and target visibility are also extremely important.

Here are some general tips for obtaining good results:

- Mount the camera securely and ensure that it can view the experimental arena fully.
- Ensure that the experimental arena is well lit (and uniformly lit).
- Consider displaying the timestamp and frame number in the video frame, as described in Section 5.3, “Configuring Behavioral Source Parameters” on page 105.
- Become familiar with the many parameters that are configurable in the user interface. These parameters can help you more accurately track your subject as it moves around the experimental arena.

The sections that follow this introduction provide information about camera selection, arena layout, and camera installation.

Plexon® hopes that this procedure solves these issues for most installations. Plexon welcomes feedback as to how to improve the experience. Please contact Plexon Support at support@plexon.com or +1 214-369-4957 with comments.

A.2 Camera Positioning

The camera should be mounted as far as possible from the arena, while still close enough to allow the zoom function to fill the sensor with the arena image. Be sure to allow space for camera mounting.

Investigators may find that they need to compromise arena size and/or camera distance to accommodate the physical limitations of the camera. The limiting factors are that the lens must be able to focus the arena image and that the arena image should fill the screen. If the camera’s distance to the arena is fixed, the arena size must be kept within certain limits for best results. Alternatively, if the arena size is fixed, the distance between the camera and arena must be kept within certain limits for best results.

TIP

Consider calibration requirements

Plan the mounting of the camera with calibration in mind. Calibration of linear dimensions (in inches or centimeters) works most accurately when the camera is orthogonal to the arena.

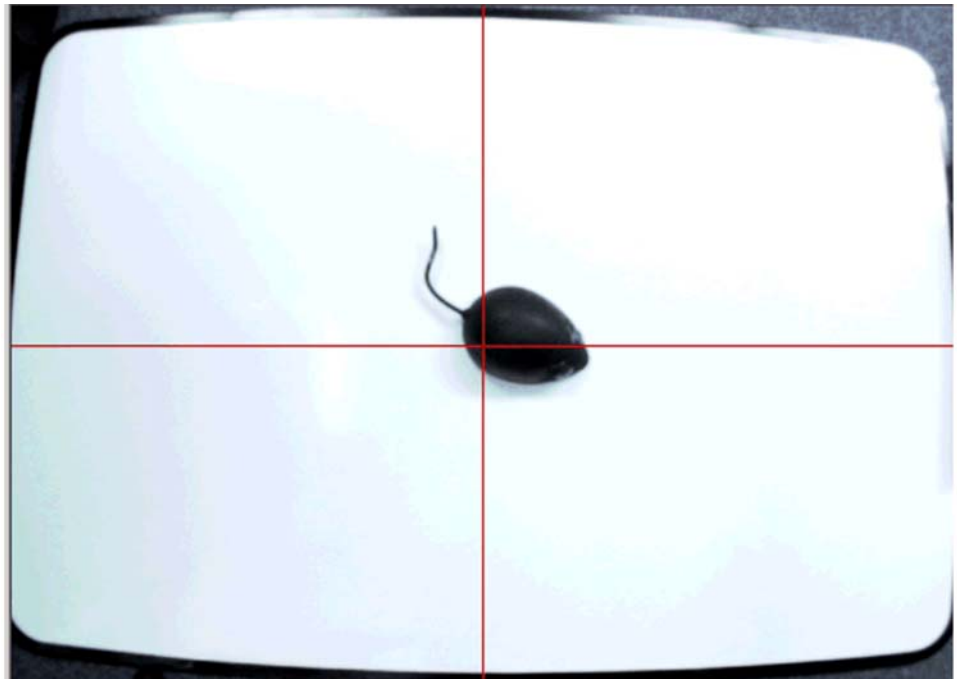
The calibration procedure is explained in Chapter 6, Calibrating the Arena Dimensions.

A.3 Experiment Design

A.3.1 Field of View

The field of view (what the sensor of the camera sees) depends on the distance between the camera and the surface and the angles of view of the lens that is used with the camera. The camera supplied by Plexon with the standard system generate screen images that are 640x480 pixels.

Orient the camera to the experimental area to take maximum advantage of the field of view. Best results will be obtained when the longest dimension of the arena is parallel to the longer sensor dimension of the camera.



The best way to avoid optical distortions is to mount the camera as high (or far) as possible from the experimental area and zoom as much as possible so that the entire experimental area is inside the image.

A.3.2 Colors

In contour tracking, to achieve the best tracking accuracy, choose colors with maximum contrast. For example, if the target animal is white, choose black or another dark color for the arena floor. Likewise, if the target is dark-colored, choose a bright white or other light color for the floor. For multi-colored animals like Long-Evans rats, “salmon pink” has been shown to have good contrast to the animal’s fur colors, so using a salmon pink background will make it easier for the system to track the whole body of the animal. It may be necessary to experiment to determine the best background color in individual situations.

In all tracking modes, use materials with solid colors as floors, if at all possible. Avoid floor materials with patterns or textures.

A.3.3 Visibility

Unless otherwise required, the design of the arena should ensure that the target is completely visible to the camera in all areas. If the target is partially obscured by overhangs or other obstacles during its travel around the arena, the centroid calculations that determine target position and orientation can be affected.

Appendix B
Navigating the Plexon User Interface

B.1 Plexon User Interface

The Plexon® User Interface embodies a standard look and feel. To illustrate the underlying concepts behind the look and feel, this appendix uses screenshots from the Plexon CineLyzer® System.

Note: Your product or version could be different from the examples shown here; but the navigation techniques will be the same.

The discussion includes the following sections:

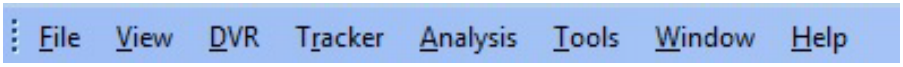
- Screen Elements
- Standard Menu Items and Dialogs
- Customization

TIP
Reset to Default Layout
It is often helpful to reset the CineLyzer screen display to the default layout (unless you have created a customized layout that you prefer). The reset ensures that the system is displaying all of the tabs and options you are likely to use in configuring your experiment. In the main window, select **Window** > **Layout** > **Reset to Default Layout**.

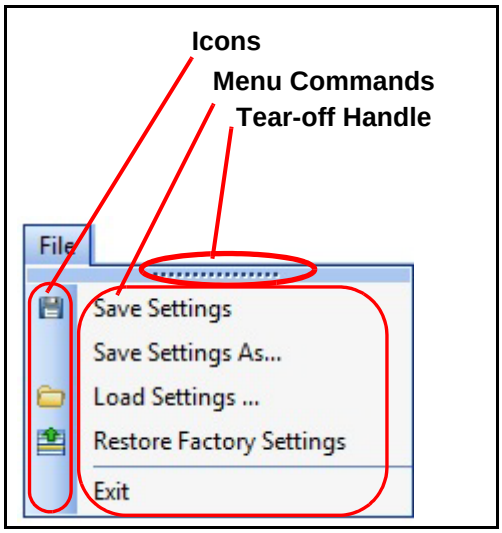
B.2 Screen Elements

B.2.1 Menus

The menu bar of the application contains the names of all of the menus for the application. Each menu name has a letter underlined which indicates the hot-key combination for that menu. To activate a particular menu, click the left mouse button on the name or press and hold the **ALT** key while pressing the underlined letter. The image below shows the main menu of the CineLyzer System.

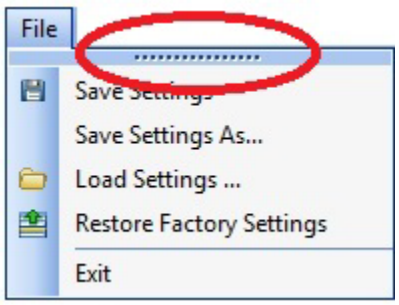


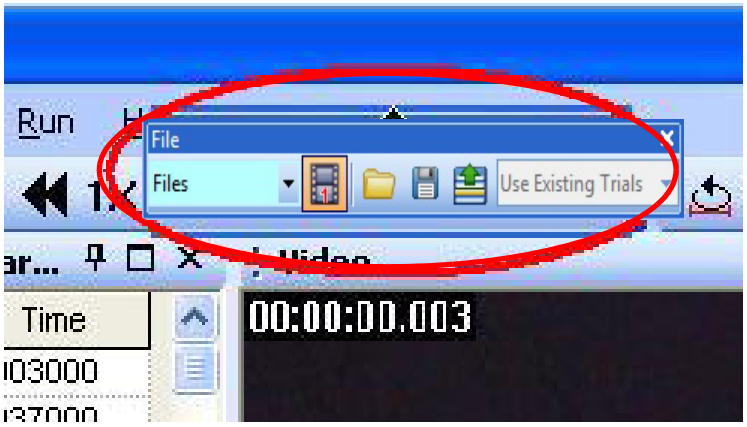
Each menu of the Plexon User Interface contains menu commands and may also contain Icons, Hot Keys and a Tear-off Menu Handle as shown by the illustration below.

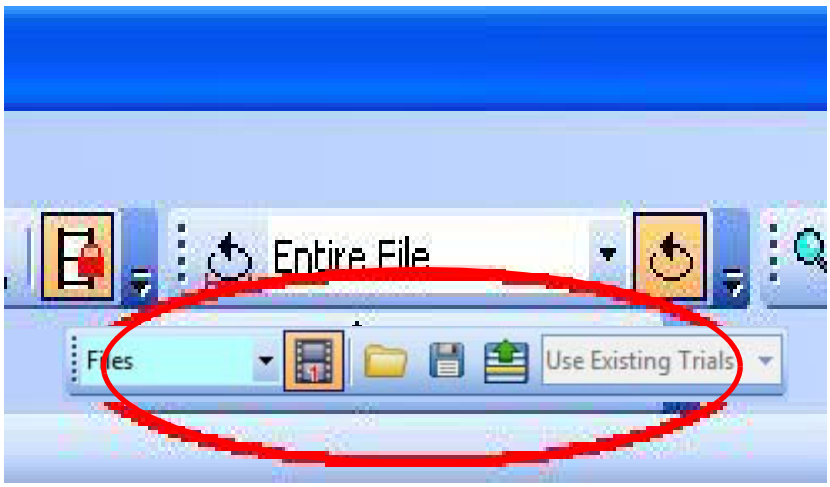


While all Plexon applications are shipped with their available menu commands organized into a set of menus, the contents of the menus can be customized and new menus can be created.

- **Icons** - Icons may be located immediately to the left of the menu commands. These icons will be displayed on the toolbar associated with the menu. In the example menu, there are icons associated with all but the last three of the menu commands. The presence of an icon next to a menu command means that the icon is also a label on a toolbar button and will execute the same command when clicked as the menu command on the menu.
- **Tear-off Menu Handle** - The Tear-off Menu Handle is an area (the one containing the dots) at the top of the menu, present on many menus. The tear-off feature allows the quick creation of a toolbar that contains all of the commands in the menu that have command icons. You can hover the mouse over the tool to highlight it, drag the mouse to the toolbar area and place the menu as a toolbar there. The illustrations below show highlighting the Tear-off Menu Handle to begin dragging the toolbar, dragging the toolbar across the screen, and finally docking the toolbar in place.







B.2.2 Windows

The image below shows a typical window title bar. It contains (from left to right) a **Title**, a **Auto Hide** button, a **Maximize** button, and a **Close** button.

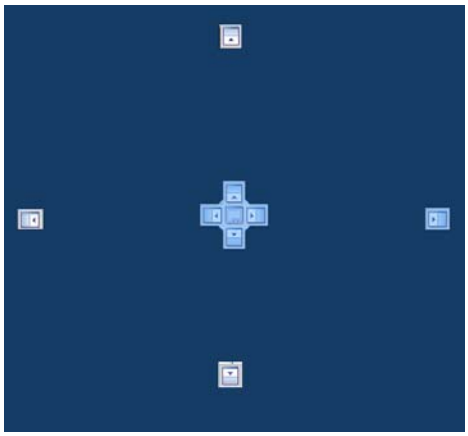
- **Auto Hide Button**  - The **Auto Hide** button "pins" a window to the screen to keep it visible or "rolls up" a visible window into a tab. When the window is pinned, the **Auto Hide** button points in a vertical direction. If the window is rolled up, the **Auto Hide** button points in a horizontal direction.

- **Maximize Button**  - The **Maximize** button may not appear on all windows. It is the standard Windows® maximize button. Clicking the **Maximize** button on a window will maximize the original window and hide other windows occupying the same horizontal or vertical space. Clicking the

Maximize button again will restore the previous layout. When clicked the image on the button toggles between one window and overlapping windows.

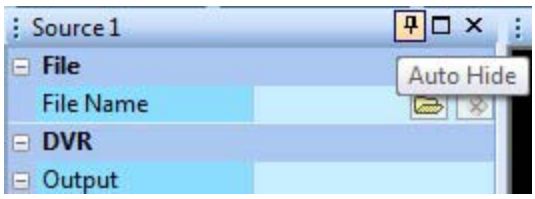
- **Close Button**  - The **Close** button closes the window.

Plexon software applications often display several windows simultaneously. These windows may be resized by using standard resizing methods and may also be repositioned by dragging and dropping and by using **Docking Stickers**. The image below shows the repositioning of a window (denoted by the blue transparent rectangle) and various **Docking Stickers**. These **Docking Stickers** allow you to dock the window being moved in one of several ways described below.



You can position a window by floating it, docking it at the desired docking sticker at a window edge or in a tab.

- **Floating a Window** - You can drag a window by the Caption Bar near the center of the screen and release it causing the window to float. Holding down the CTRL key while dragging will always float the window. Double-clicking on the Caption Bar will also float a window. Note that the size and position of the floating window is remembered.
  - **Rolled-up Windows** - Floating windows may be enabled for roll up by pressing the **Auto Hide** button. The window will roll up when the focus is changed to a different window. The first image below shows a floating window before rolling it up. The second image below shows the rolled-up window after the focus has changed.

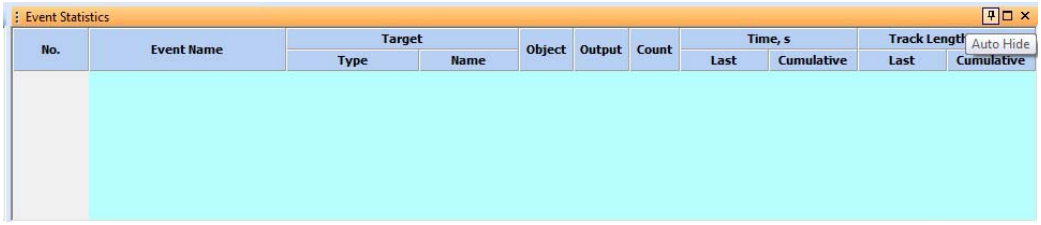


- **Docking a Window at the Application Frame** - When you begin to drag a window, a transparent blue rectangle appears to indicate the position of the window and the four **Docking Stickers** appear individually at each edge of the application frame to allow you to dock the window to the respective edge. To dock just move the mouse to the desired **Docking Sticker** and release the mouse button. The docked window will extend along the entire length of the edge to which it is docked. The image below shows all four screen **Docking Stickers**.

- **Docking a Window at a Window Edge** - When you move the window inside another window, the window **Docking Stickers** appear inside the window grouped together near the center of the window. Releasing the mouse button while it is over one of these stickers (except the center one) will dock the moving window to the respective edge of the window associated with the window **Docking Stickers**. The image below shows the window **Docking Stickers**. Note that the shading of these **Docking Stickers** is different that the shading of the **Docking Stickers** in the previous image.

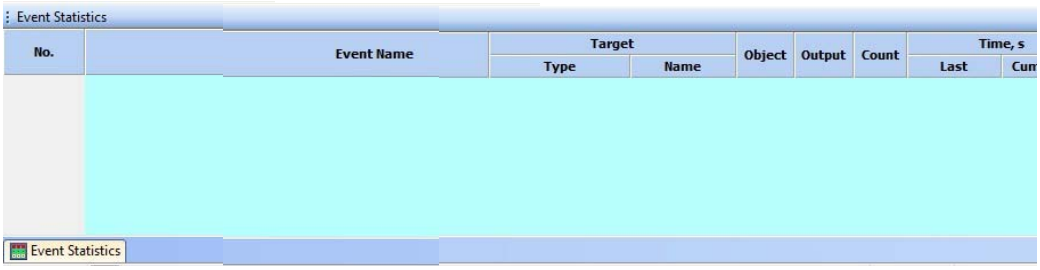
- **Placing Windows into Tabs** - Releasing the mouse button over the center **Docking Sticker** will allow the moving window to occupy the same space as the window beneath and will create tabs along the bottom for switching the view between the two windows. The image below shows the Marker Occurrences window without sidebar tabs on the left. On the right, a Channels window has been placed into the same space by using the center docking sticker. Note that there are two sidebar tabs at the bottom of the window.

— **Hidden Windows** - Docked windows whose access is not needed often can be hidden or “rolled up” by pressing the **Auto Hide** button. When hidden, the window is represented by a sidebar tab. Sidebar tabs may be located at the left, right, or bottom of the screen and indicate hidden windows. To show one of these windows, just hover the mouse over one of the tabs and the window will appear. For example, if you click the **Auto Hide** button on the Event Statistics window:



The sidebar tab for this window will show up at the bottom left corner of the screen:

Here is the Event Statistics window opened by the sidebar shown on the picture above:



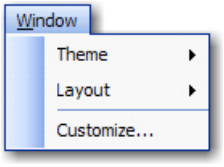
If you click the **Auto Hide** button again, the window will remain open after you move the mouse away from the sidebar.

B.3 Standard Menu Items and Dialogs

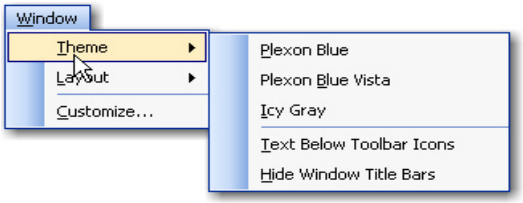
In Plexon software applications, some menu items may have the same functionality across several applications. These items are standard menu items and consist of the **Window** menu, the **Run** menu, and the **Help** menu.

B.3.1 Window Menu

The **Window** menu contains three items: **Theme**, **Layout**, and **Customize**.

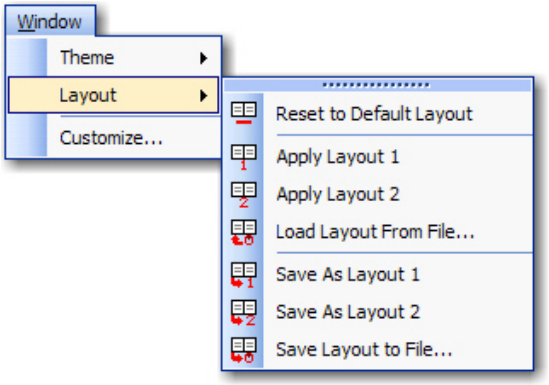


- **Theme** - Clicking on **Theme** displays a menu as shown below. A theme is a color scheme that is part of the look-and-feel of the user interface.



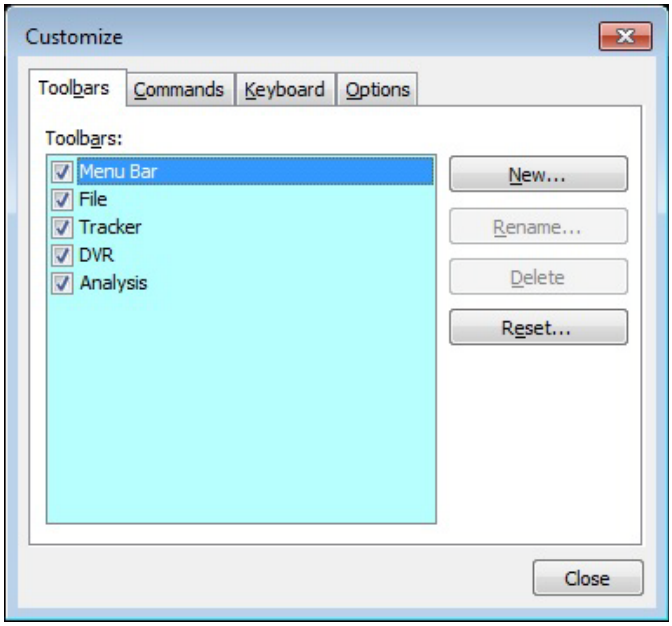
  - **Theme Group** - The top group of items are theme toggle items that may be selected to apply to the user interface look-and-feel. Only one of the themes may be selected at a time.
  - **Text Below Toolbar Icons** - This item is a toggle item to show or not show text below the toolbar icons.
  - **Hide Window Title Bars** - This item is a toggle item to hide or show window title bars.

- **Layout** - Clicking on **Layout** displays a menu as shown below. A layout is the size and placement of the windows on the screen. It also remembers the number and placement of toolbars.



  - **Reset to Default Layout** - Clicking on this item resets the layout to the factory default.
  - **Load Layout Group** - Clicking on **Apply Layout 1** or **Apply Layout 2** applies one of the standard layouts to the user interface. Clicking on **Load Layout From File** will allow you to select a layout file to apply to the user interface.
  - **Save Layout Group** - Clicking on **Save As Layout 1** or **Save As Layout 2** saves the current screen layout as one of the two standard layouts. You can also click **Save Layout to File** to save the current screen layout to a file that can be loaded by the **Load Layout From File** item.

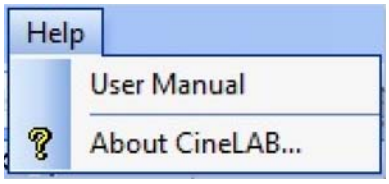
- **Customize** - Clicking **Customize** displays the **Customize** dialog box as shown below



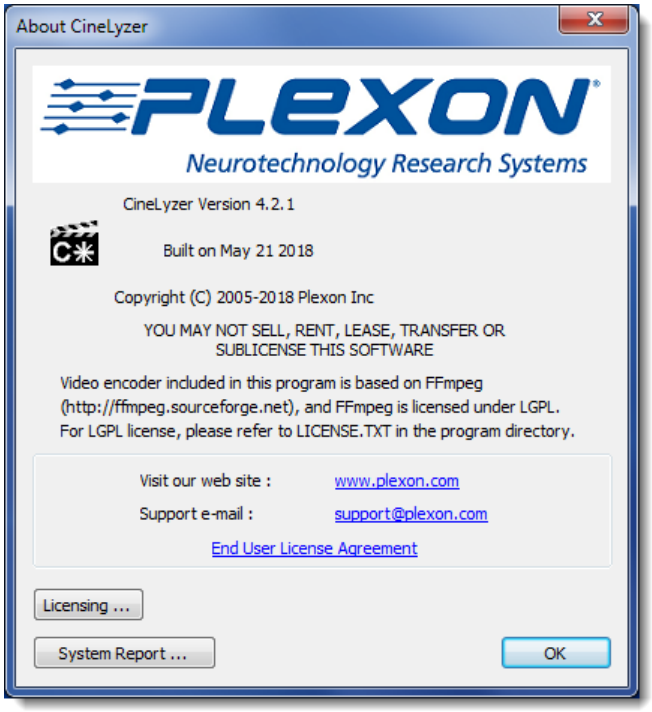
For details on using the **Customize** dialog box, see “Customization” on page B-15.

B.3.2 Help Menu

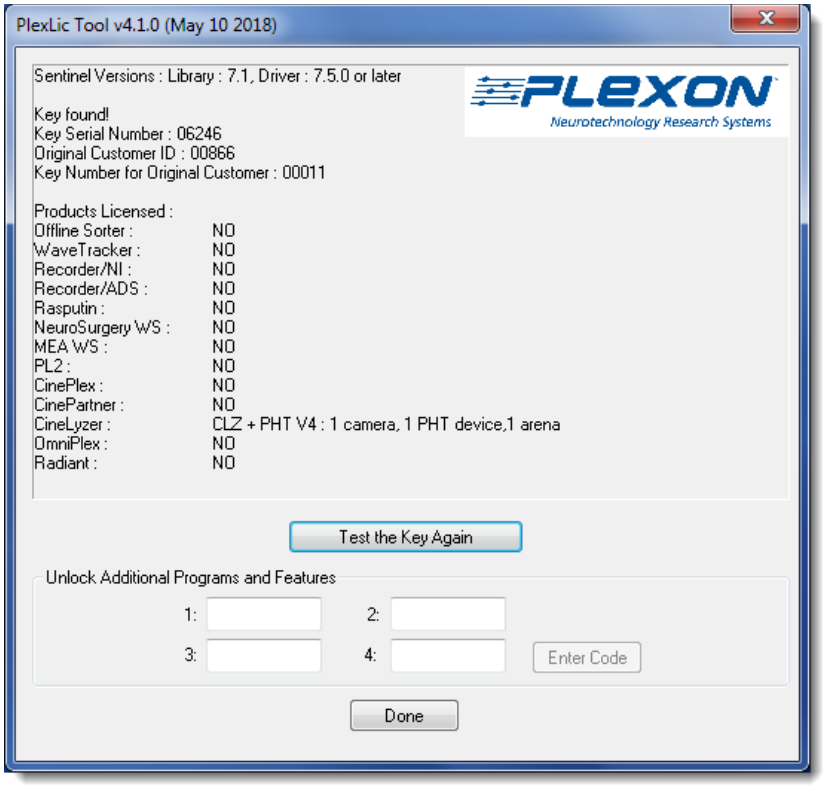
The **Help** menu contains four items: **Help**, **Quick Reference**, **Web Update**, and **About XXXXX** where **XXXXX** is the name of the application.



- **User Manual** - Clicking **User Manual** displays the User Guide for the application.
- **About** - Clicking the **About** item displays the **About** dialog box. The text of the **About** item varies according to the application. The **About** dialog contains the version number and build data of the application, links to the Plexon website and support e-mail, and buttons for **Licensing**, **System Report**, and **Manage File Extensions**.



— **Licensing** - Clicking the **Licensing** button displays the **Plexon License Management** dialog box. The **Plexon License Management** window includes the complete licensing information for Plexon products. The window includes the following three areas: information, key testing, and code entry.



– The information area includes information on license keys and a list of all the Plexon products and their licensed status on this computer.

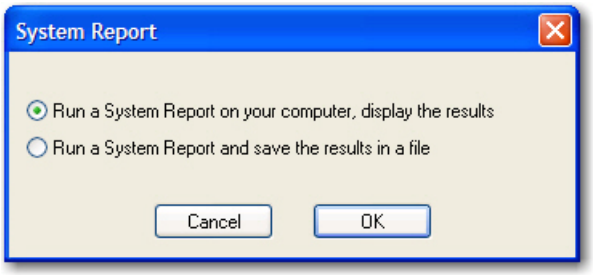
– If you have moved or added a key, the **Test the Key Again** button provides a convenient tool to test license keys to ensure they function correctly.

– If you have more than one key installed, the **Next Key>>** and **<<Prev Key** buttons appear. You may use these buttons to cycle through and test all keys.

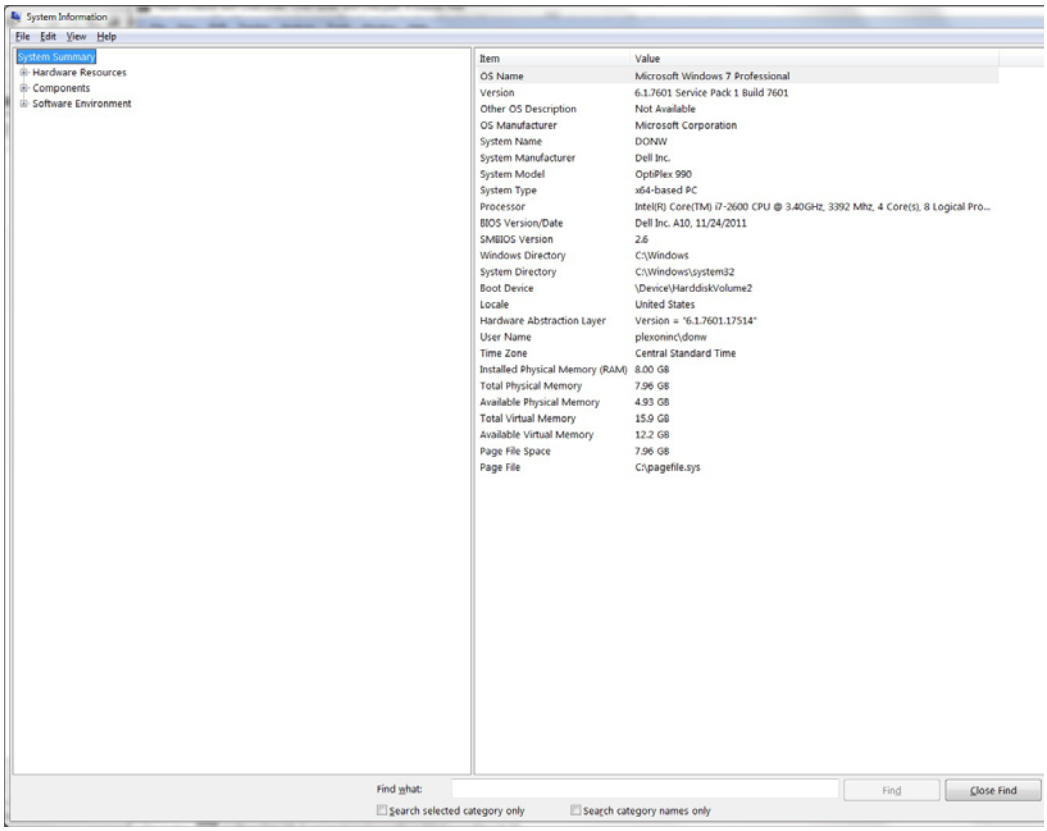
– The code entry area is used to enter the unlock codes for optional programs and features. If you have licensed optional items, instructions for entering codes and testing keys are included with Plexon installation programs.

— **System Report** - The purpose of the **System Report** button is to help Plexon Support diagnose problems by listing system information. Clicking the **System Report** button will first display a dialog box to

allow you to display the system report on the monitor or save the report to a file that can be sent via E-mail to Plexon Support.



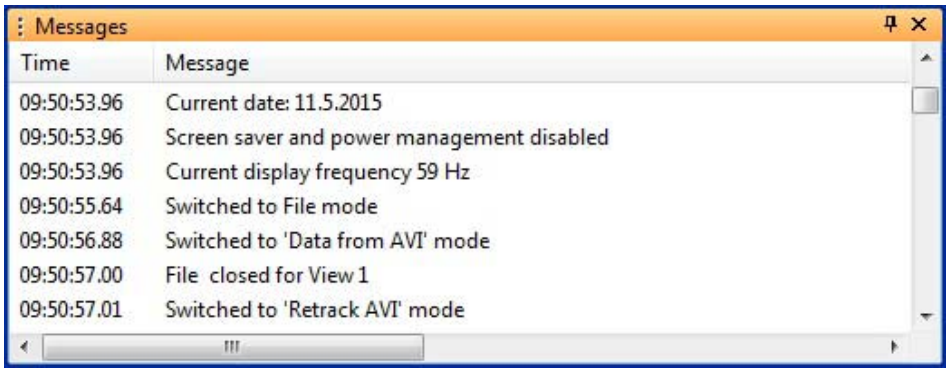
– After selecting a choice, clicking **OK** will launch the standard Microsoft® System Information tool. The image below shows the System Information report displayed on the monitor.



B.3.3 Messages Window

The **Messages** window displays a log of timestamps and associated application events that Plexon Support can use for troubleshooting purposes.

Note: The Messages window is not available for all applications.



Right-clicking the mouse in the Messages window will display a right-click menu with the following items:

- **Erase** - This item clears the window of all messages
- **Pause** - This item stops the logging of messages
- **Show Debug Messages** - This item is a toggle to show or hide debug messages
- **Select and Copy All** - This item allows you to copy all of the messages to another application - such as a word processor
- **Save Log to File** - This item allows you to save the messages to a log file.
- **Mail Log to Plexon** - This item allows you to send the message log to Plexon for troubleshooting purposes

B.3.4 Right-click Menus

Most windows have right-click menus that control their behavior and options. To open a right-click menu, place the cursor inside a window and click and release the right mouse button. The right-click menu appears where the mouse is clicked. To select a menu item, move the cursor over it and click the left mouse button.

B.3.5 Current Selections

In grid-based windows, the currently selected item always appears with a >> or > in the left column of the appropriate grid-based window.

B.3.6 Undo

Plexon applications provide multiple *undo* levels. To undo an operation, on the **Edit** menu, click **Undo** or click the **Undo** button on the toolbar. You can undo

operations that change the contents of the project file but may not undo operations that change the user interface options or colors.

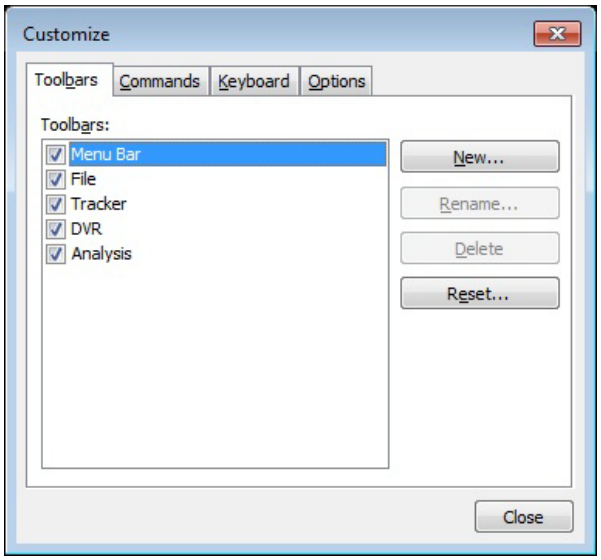
B.4 Customization

Although the menus and toolbars offer a rich set of commands and functions that should meet the needs of most Plexon customers, the **Customize** dialog box also allows you to customize several areas of the interface should the need ever arise. This section describes the uses of the **Customize** dialog box.

To open the **Customize** dialog box, from the **Window** menu, select **Customize**. The **Customize** dialog box contains several tabs. See the image below. Although the content of the tabs will vary according to the application, the functional operation of each tab is respectively the same across all Plexon software applications.

B.4.1 Toolbars Customization

An image of the **Toolbars** tab follows:

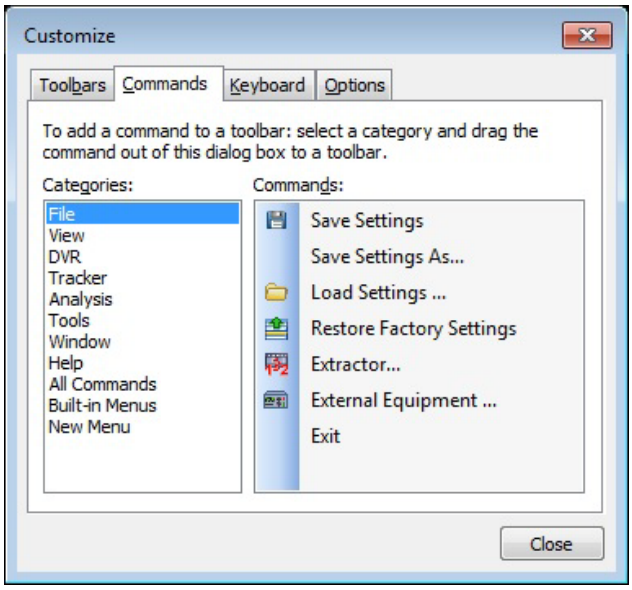


- **Toolbars** - This box contains a list of the toolbars for the application. Click a toolbar checkbox to have it appear in the main application window.
- **New** - This button opens the **New Toolbar** dialog box. You can use this feature to create a custom toolbar for commands frequently used. Enter a toolbar name in the **Toolbar name** box.
- **Rename** - If you have selected a toolbar that was previously defined, click **Rename** to change the name of the toolbar. The **Rename Toolbar** dialog box displays.

- **Delete** - If you have defined a new toolbar and selected it, click **Delete** to remove that toolbar. There are no default values for newly defined toolbars. Standard toolbars may not be deleted. A confirmation dialog box displays.
- **Reset** - If you have selected a standard toolbar, click **Reset** to restore the toolbar to its default contents. If new buttons have been dragged to a toolbar, click **Reset** to restore the default version of the toolbar. A confirmation dialog box displays.

B.4.2 Commands Customization

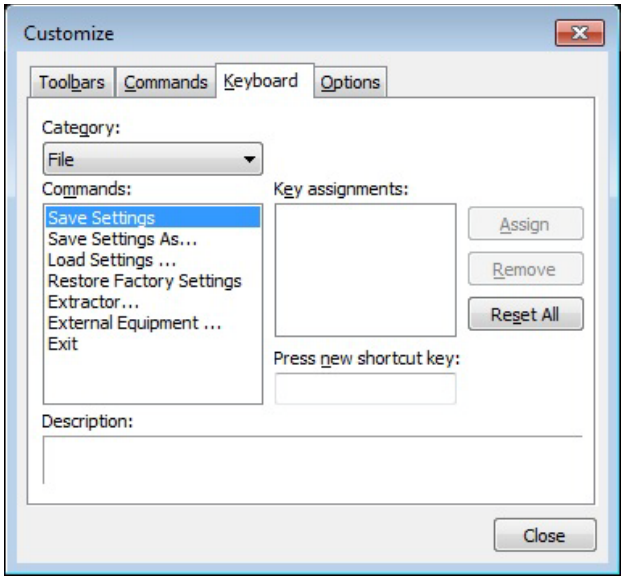
The **Commands** tab is used to customize which commands are available in toolbars. An image of the **Commands** tab follows:



- **Categories** - This is a list of all the toolbar categories. Select a toolbar category to see the buttons in the **Commands** area.
- **Commands** - This area shows all the buttons and the associated menu commands that belong to the selected category. You can select the desired command and drag it to the toolbar.

B.4.3 Keyboard Customization

The **Keyboard** tab allows you to bind keystrokes to commands. An image of the **Keyboard** tab follows:



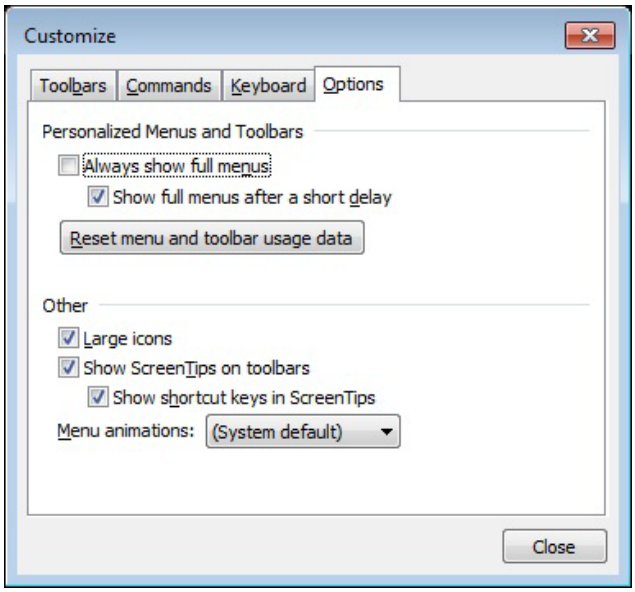
- **Category** - This is a list of all the main menu headings. Select a menu heading to category to see the associated commands in the **Commands** area.
- **Commands** - This is a list of all the commands associated with the selected main menu heading in the **Category** area.
- **Key assignments** - This displays the current key assignment for the command selected in the **Commands** area.
- **Press new shortcut key** - This allows you to enter a shortcut key combination for the command selected in the **Commands** area.
- **Description** - This area displays a description of the currently selected command in the **Commands** area.
- **Assign** - This button assigns the shortcut in the **Press New Shortcut Key** area to the selected command in the **Commands** area. If the shortcut key is already assigned to another command, a confirmation dialog box displays to allow or cancel the reassignment.
- **Remove** - This button removes the selected shortcut key in the **Key Assignments** area from the selected command in the **Commands** area.
- **Reset All** - This button removes all custom key assignments. A confirmation dialog box displays to allow or cancel the operation.

Procedure for Customizing Keystroke Shortcuts

1. From the **Window** menu, select **Customize,** and then click the **Keyboard** tab of the **Customize** dialog box.
2. Choose a category from the **Category** dropdown and from the **Commands** list, select the desired command to bind to a keystroke shortcut.
3. If there is already a key assignment listed in the **Key assignments** area, remove it by clicking the **Remove** button if so desired. You can also just reassign a new key combination to the selected command. (See Step 5)
4. Click the mouse in the **Press new shortcut key** area.
5. Click the **CTRL** or **ALT** key and hold it down while clicking another key. The dual key combination will be displayed in the **Press new shortcut key** area. This combination will be the key assignment for the selected command.
6. Click the **Assign** button to assign the key combination to the selected command. If there already is a key assignment for the command, a confirmation box will display to confirm or cancel the reassignment.

B.4.4 Options Customization

An image of the **Options** tab follows:



- **Personalized Menus and Toolbars** - This area contains two check boxes and a button. The application will hide infrequently used menu items, but you may customize the display of menu items using these two checkboxes. If you check the **Always show full menus** checkbox, the application will always show full menus and the **Show full menus after a short delay** checkbox will be disabled. If you do not check the **Always show full menus** checkbox, there will be the option of checking or clearing the **Show full menus after a short delay** checkbox. The **Reset menu and toolbar usage data** button will allow you to delete the record of commands used in the application and

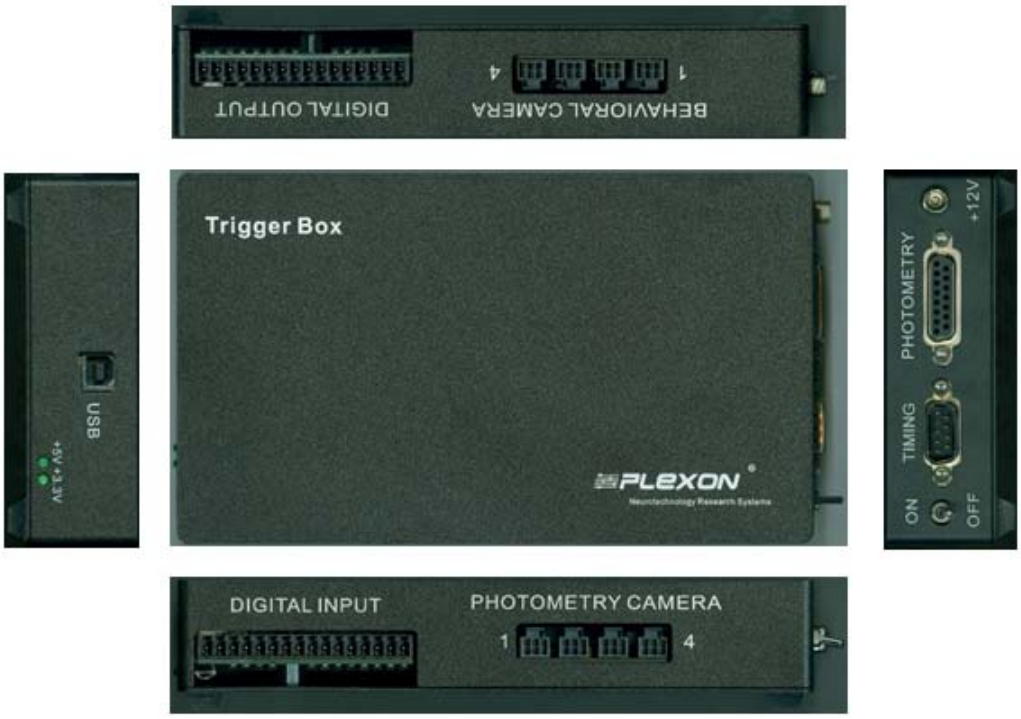
restore the default set of visible commands to the menus and toolbars. A confirmation dialog displays.

- **Other** - This area contains three checkboxes and a dropdown list. If you check the **Large icons** checkbox, the application will use large icons on the toolbar. If you check the **Show Screentips on toolbars** checkbox there will be the option of checking the **Show shortcut keys in Screen Tips** checkbox. The **Menu animations** dropdown list allows you to select the type of animation to be used on menus that have animation.

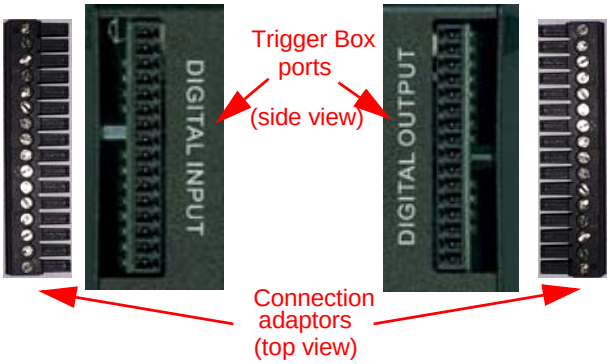
Appendix C
Trigger Box and Digital Input/Output

C.1 Trigger Box Ports and Connectors

The Trigger Box provides timing and synchronization for all system functions. The image below shows the top and side views.



This image shows the connection adapters for the input and output ports.



As required by the user's third party device (for example, Med Associates Inc®, Lafeyette Instrument®, etc.) an adaptor or cable will be required to interface with the Trigger Box. This can be provided by Plexon at the time of system purchase.

	**CAUTION** **Push connectors straight in** To avoid bending or damaging any pins, always use a straight push to attach or detach the interface connectors.

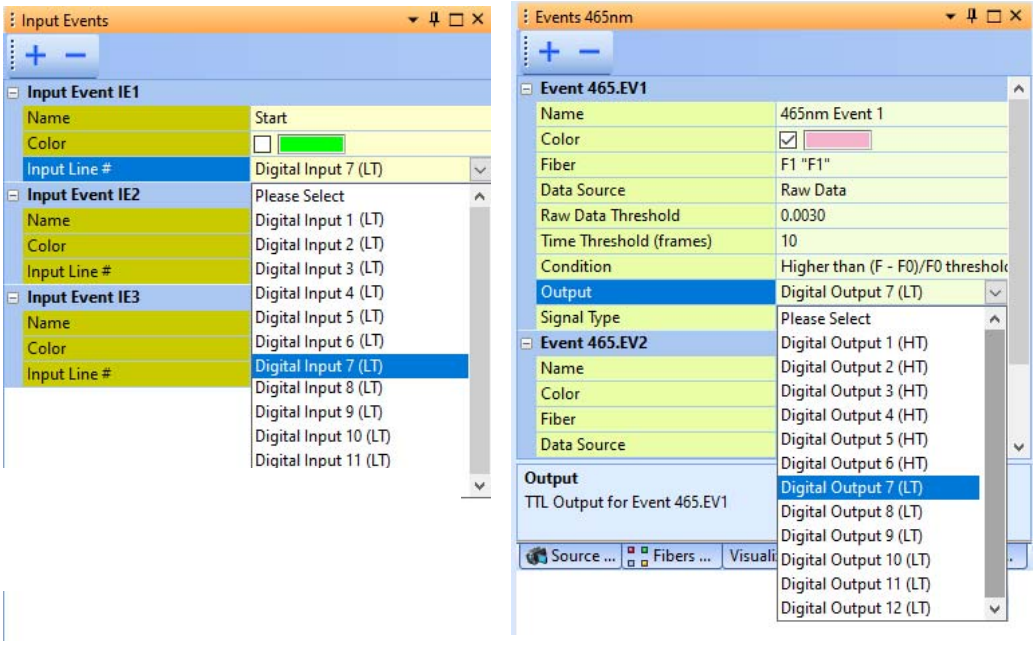
C.2 Digital Input and Output Interfaces—Description

The Trigger Box works with standard TTL input and output signals. It provides lines for 12 different input events and 12 different output events.

Trigger Box Input and Output line configurations

The standard Trigger Box is configured for six high true and six low true lines for both input and output. However, when a system is ordered, the number of high true and low true lines can be adjusted based on the customer's need to interface with external equipment. Consult with Plexon Support or your Plexon sales representative regarding these adjustments. You can see how your existing system is configured by looking at the label on the bottom of the Trigger Box, for example:

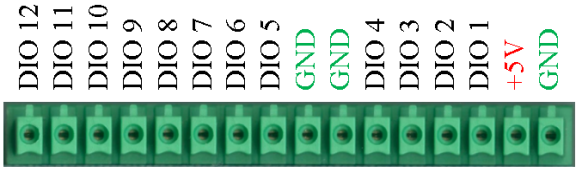
You can also see how your system is set by looking at the Input or Output line dropdown lists in any of the **Event** tabs in the GUI. For example, for the system labeled as shown above you will see these dropdown lists:



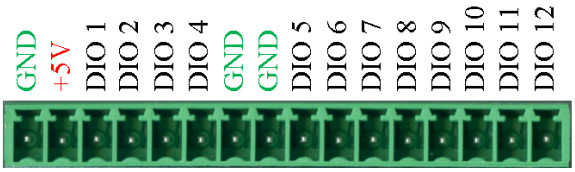
Trigger Box connector pinouts

The pins of the DIGITAL INPUT and DIGITAL OUTPUT connectors are shown in the image below. Note that you can use the +5V pin and any of the GND pins to supply voltage to an external device.

Digital Input/Output device connector



External device connector



IMPORTANT—Trigger Box Voltage Limits

All digital inputs should be kept within the range of 0 to 5V relative to the ground pins during normal operation. The digital inputs are protected against inadvertent exposure to voltages up to ±28V, but voltages beyond ±28V are likely to permanently damage the inputs. Like the digital inputs, the digital outputs are protected against inadvertent exposure to voltages up to ±28V, but voltages beyond ±28V are likely to permanently damage the outputs.

CAUTION

Limit voltage on the Digital Input/Output interface pins

Avoid voltages beyond ±28V, which are likely to permanently damage the inputs/outputs on the Trigger Box.

C.3 Input and Output Logic

Digital Input Logic

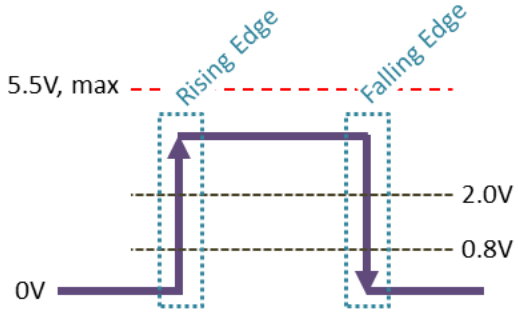
All digital inputs should be kept within the range of 0 to 5V relative to the ground pins during normal operation. The input logic is as follows:

- High True input—The system recognizes voltages greater than 2.0V as asserted and voltages less than 0.8V as de-asserted.
- Low True input—The system recognizes voltages less than 0.8V as asserted and voltages greater than 2.0V as de-asserted.

The system recognizes a digital input event as a rising or falling edge detected on the pin(s) of DIGITAL INPUT port.

Rising edge—A transition of the input signal from less than +0.8V to greater than +2.0V.

Falling edge—A transition of the input signal from greater than +2.0V to less than +0.8V.



If the input is a pulse, the duration of the pulse should be at least as long as the specified camera frame rate (30 frames per second).

Digital Output Logic

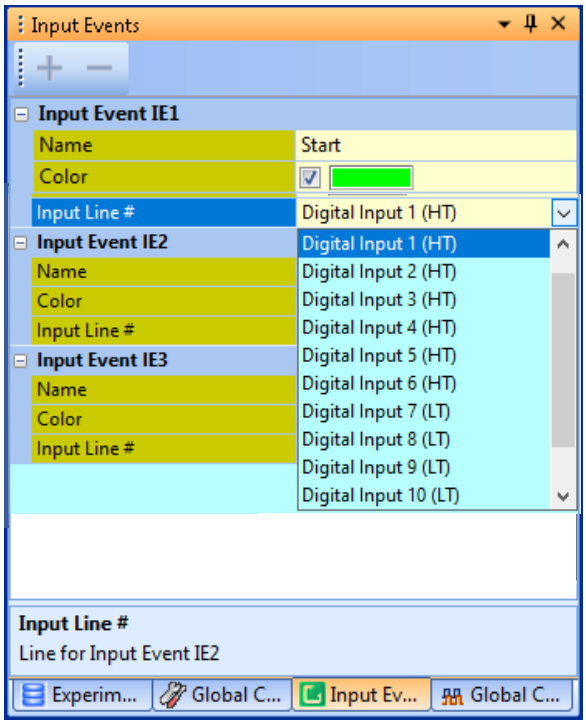
The output signal can be specified in the **Events** tab as a pulse (from 0.1 to 5.0 seconds) or as a level:

- An output signal specified as a pulse indicates that a transition has occurred, for example, fluorescence has risen above a certain value, or an animal has entered a specific zone.
- An output signal specified as a level indicates that a condition is true for some period of time, for example, fluorescence is remaining above a certain value, or an animal is inside a particular zone.
- High True output voltage transitions between 0V (not asserted) and 3.3V (asserted).
- Low True output voltage transitions between 3.3V (not asserted) and 0V (asserted).

C.4 Assigning Digital Inputs and Outputs

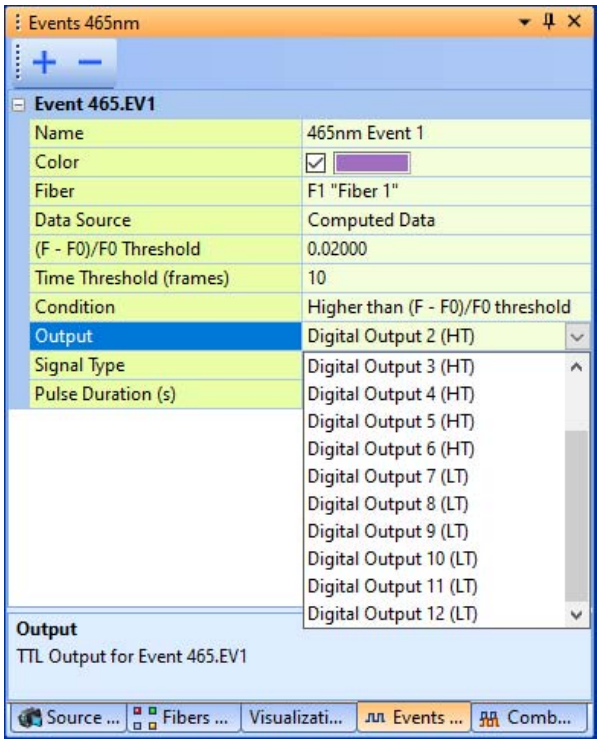
Assigning Digital Inputs

To assign a specific **Input Line #** to an event, use the **Input Events** tab. The software is able to read the DIGITAL INPUT settings of the Trigger Box, and will change the GUI to accurately represent the **Input Line #** menus.



Assigning Digital Outputs

To assign a specific **Output** line to an event, use the **Events** tab (shown below). The software is able to read the DIGITAL OUTPUT settings of the Trigger Box, and will change the GUI to accurately represent the **Output** line menus.



Note: Each digital output line is restricted to one event at a time.

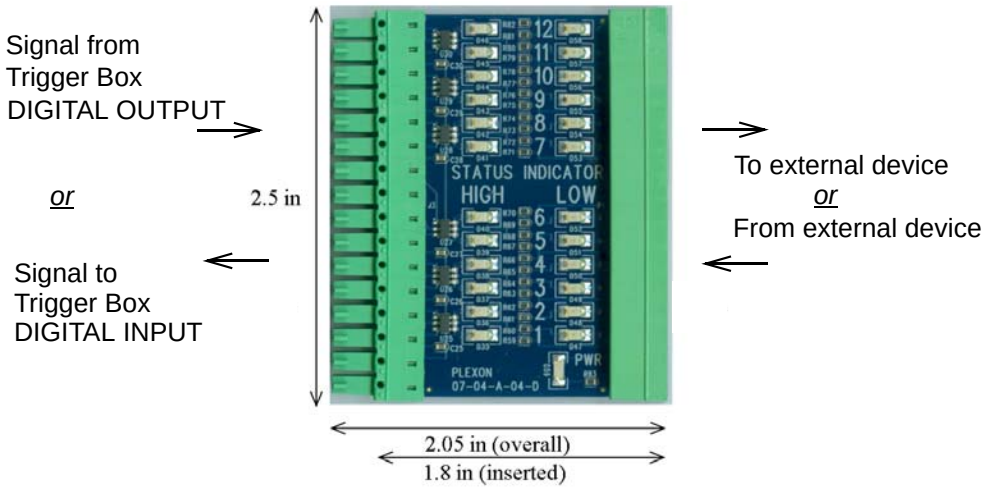
C.5 Status Indicator and Input Generator Boards

The system includes an Input/Output Status Indicator, an Input Generator, and two Connection Adaptors. These parts are shown in the images that follow.

	**CAUTION** **Push the adaptors and connectors straight in** To avoid bending or damaging any pins, always use a straight push to attach or detach the boards.

C.5.1 Input/Output Status Indicator Board

The Status Indicator can be connected to the DIGITAL INPUT or DIGITAL OUTPUT port on the Trigger Box. This board is typically used for testing or debugging, but can be left in place during normal operation, if desired, just to provide visual feedback as to the state of the inputs or outputs. The normal voltage range is 0V to +5V.



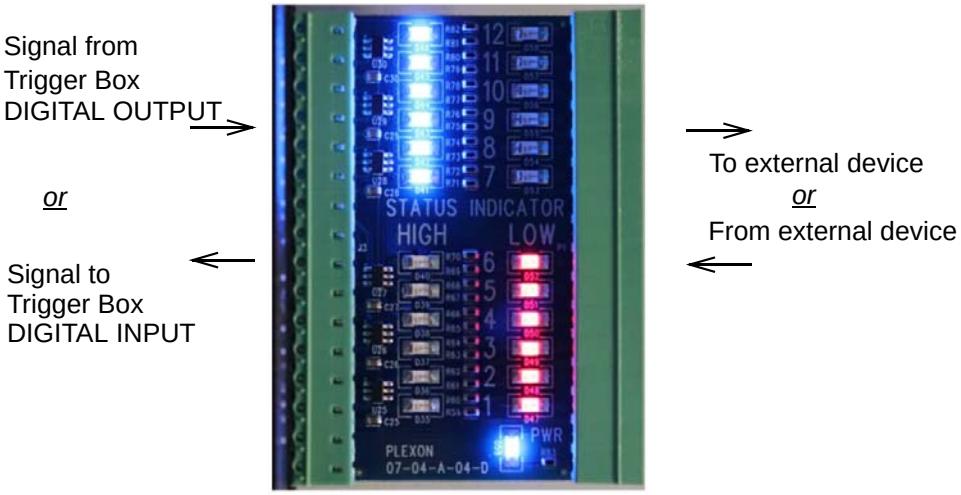
Note that the Status Indicator is ***not*** protected against over/under voltage and will quickly fail if voltages beyond –0.5V to +6.5V are applied.

	**CAUTION** **Do not exceed –0.5V to +6.5V on Status Indicator pins** Do ***not*** apply voltages outside of this range (–0.5V to +6.5V) to any of the pins on the Status Indicator. Voltages outside the range will damage it.

Interpreting the LED Indicator Lights on Status Indicator Board

In the image below, some of the LED lights are on. These lights are interpreted as follows:

- The blue power (PWR) light is on whenever the Status Indicator board is plugged into the Trigger Box (individually or in series), the Trigger Box is connected through the USB cable to a USB port on the PC, and the PC is turned on.
- Blue LEDs illuminate when the signal is greater than 2.0V and red LEDs illuminate when the signal is less than 0.8V. In the image below, the lights indicate the board is detecting voltages less than 0.8V on lines 1 through 6 and voltage greater that 2.0V on lines 7 through 12.

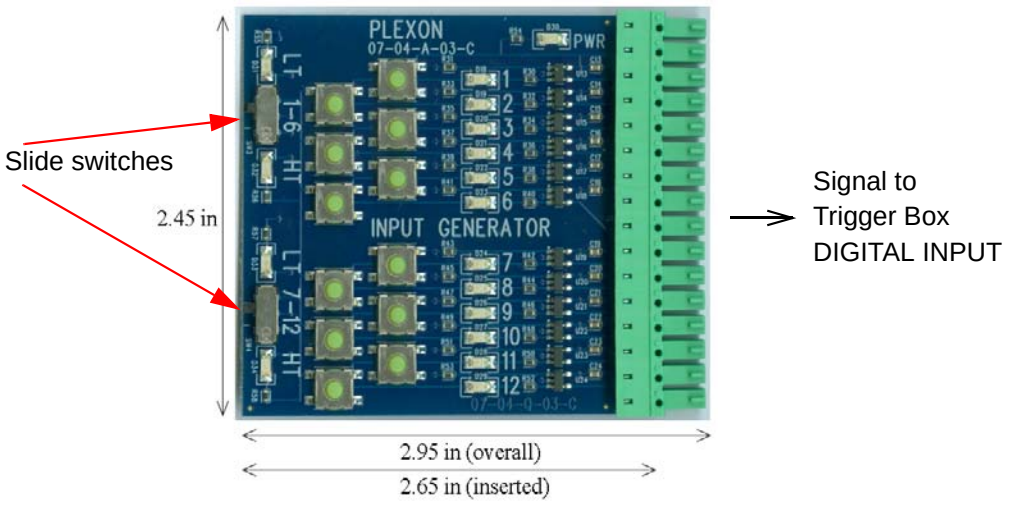


C.5.2 Input Generator Board

The Input Generator board is typically used for testing and debugging, but it is generally not used during normal operation. Use this board when you want to verify that your hardware and software are properly detecting inputs and your software is configured correctly.

Slide Switches on the Input Generator Board

There are two slide switches on the Input Generator board. One switch configures buttons 1–6 for high true or low true operation, and the other switch configures buttons 7–12 for high true or low true operation. You can set these switches according to the signals you want to generate—high true (HT) or low true (LT).



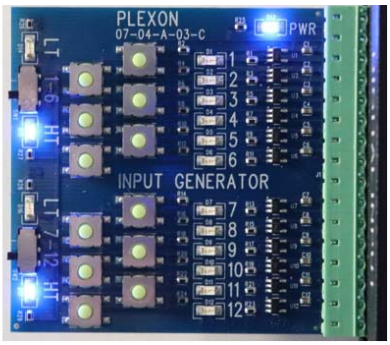
Interpreting the LED Indicator Lights on Input Generator Board

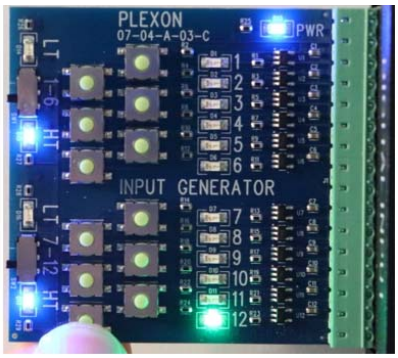
In the images that follow, some of the LED lights are on. These lights are interpreted as follows:

- The blue power (PWR) light is on whenever the Input Generator board is plugged into the Trigger Box (individually or in series), the Trigger Box is connected through the USB cable to a USB port on the PC, and the PC is turned on.
- The green lights turn on when the corresponding buttons are pushed.

Example 1—All lines set to HT
Button for line 12 pushed
Green light for line 12 turns on
Input Generator sends HT signal to Trigger Box on line 12

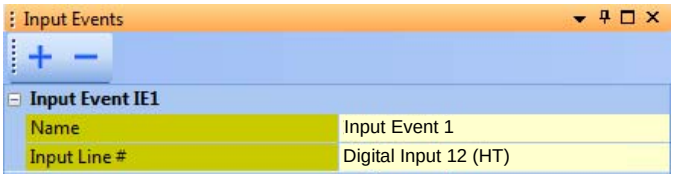
both slides moved to HT



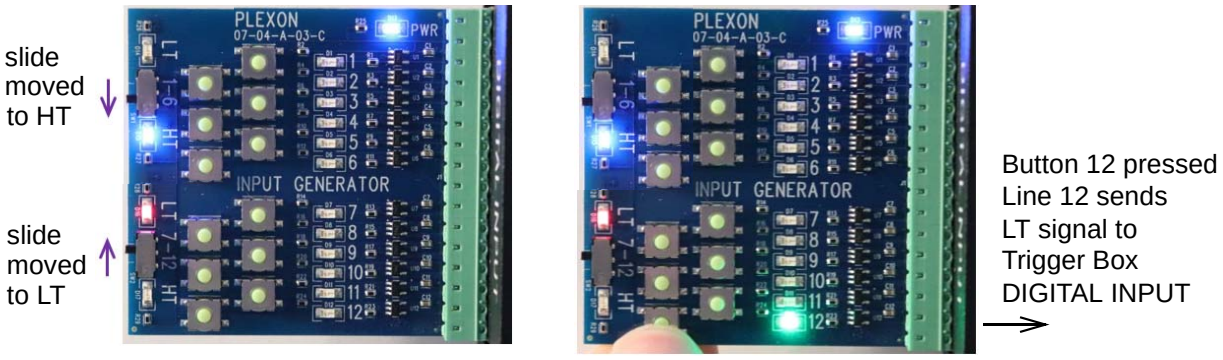


Button 12 pressed
Line 12 sends
HT signal to
Trigger Box
DIGITAL INPUT

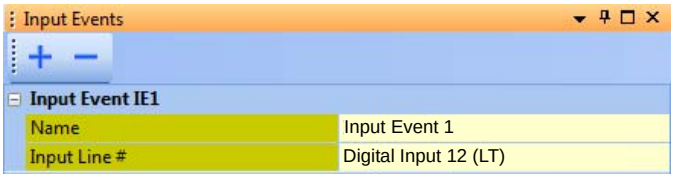
If you have configured **Input Event 1** as **Digital Input 12 (HT),** as shown below, the system should show this event being detected when you press the button for Line 12. (Detection and display of Event Statistics are described in Section 9.4, “Displaying Event Statistics As They Occur” on page 232.)



Example 2—Lines 1–6 set to HT, Lines 7–12 set to LT
Button for line 12 pushed
Green light for line 12 turns on
Input Generator sends LT signal to Trigger Box on line 12



If you have configured **Input Event 1** as **Digital Input 12 (LT),** as shown below, the system should show this event being detected when you press the button for Line 12. (Detection and display of Event Statistics are described in Section 9.4, “Displaying Event Statistics As They Occur” on page 232.)



Appendix D
Modifying Arenas and Zones

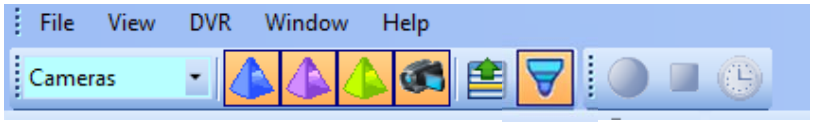
D.1 Introduction

The system provides some flexibility in modifying arenas, zones and calibration parameters in an experiment that already has one or more sessions recorded. The descriptions in this section apply to behavioral camera recordings made in the following modes, except as noted:

- **Cameras** mode
- **Files**/**Analyze Session Data** mode
- **Files/Add New Sessions** mode

Note: No changes to configuration data can be made in **Files/View Sessions As Recorded** mode.

Cameras mode:



Files mode:

D.2 Recalibrating the Arena and Recalculating Tracking Data

The system always preserves uncalibrated tracking coordinates—in pixels—for all sessions. The calibration factor (cm/pixel or inches/pixel) is an optional convenience for the researcher.

Rules the system follows to apply calibration ("Use Calibration" checkbox)

Cameras mode, Files/Add New Sessions mode

If you want tracking coordinates and behavioral event data (speed, trajectory length) to be exported in metric units, please make sure this checkbox is checked before you start recording sessions.

Files/Analyze Session Data mode.

When you check or uncheck this checkbox, it applies to all sessions within the experiment and affect computation results when the  button is pressed.

Rules the system follows for the Calibration factor

Cameras mode and Files/Add New Sessions mode

You can recalibrate the arena dimensions (change calibration factor) at any time during an experiment. The system applies the new calibration factor to all future sessions in the experiment (until you change the setting again). The calibration factor for previously recorded sessions is not affected by recalibration.

In these modes the system always shows the calibration factor the way it is currently set for future recordings, even when you select previously recorded sessions. It doesn't show it on per-session basis.

Files/Analyze Session Data mode

In this mode, you can click on any recorded session in the experiment and change the calibration factor for each session individually. It will affect the session results during computations (when you press the  button), and also will set the calibration factor for all future recordings in this experiment (when you go back to Cameras mode or Files/Add New Sessions mode). It might be needed, for example, if you decide to adjust arena dimensions at the analysis/computation stage (see sections D3 and D4). However, it doesn't change the value of the calibration factor for this session stored at the time when the session was recorded. You will be able to see the value for each session in Files/View Sessions As Recorded mode.

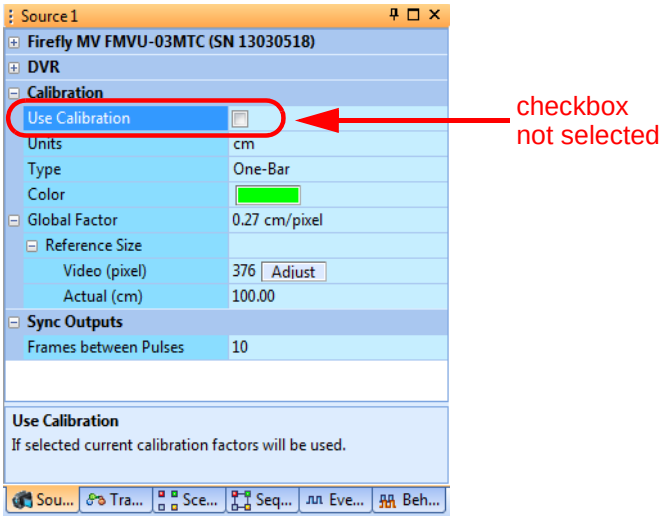
Files/View Sessions As Recorded

Calibration factor value and status of calibration (applied/not applied) stored at the time each session was recorded are displayed in this mode for each session. These values cannot be changed. So, if you want the tracking coordinates and behavioral event data to be extracted in metric units (not in pixels) in Files/View Sessions As Recorded mode, make sure **Use Calibration** is checked before start recording in Cameras or Files mode.

Here are examples that illustrate the rules the system follows for calibration, and how calibration would affect computation results.

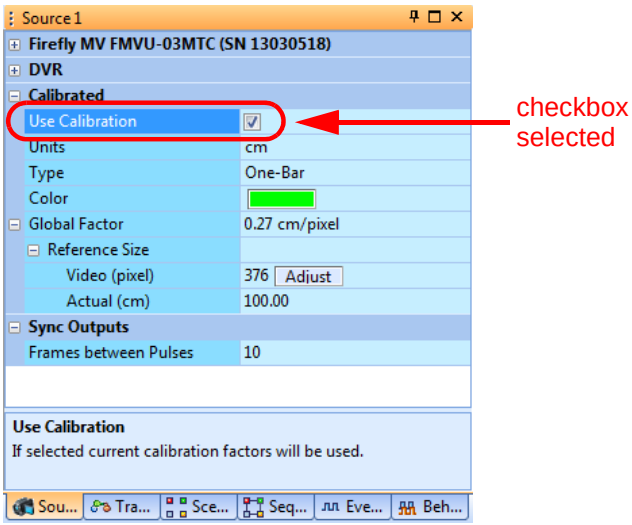
Example A
Several sessions have been recorded without calibration, and then calibration was activated

1 Create a new experiment and record several sessions without any calibration, that is, with the **Use Calibration** checkbox deselected.

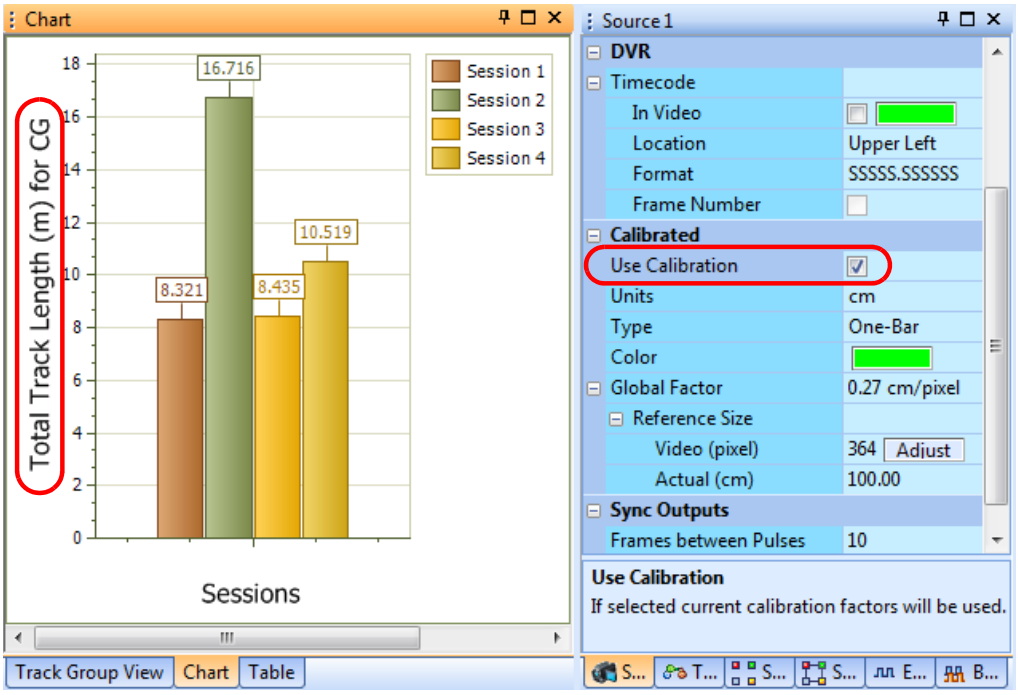


2 Press the **Compute** button . Notice that the track lengths for all the previously recorded sessions are displayed in pixels.

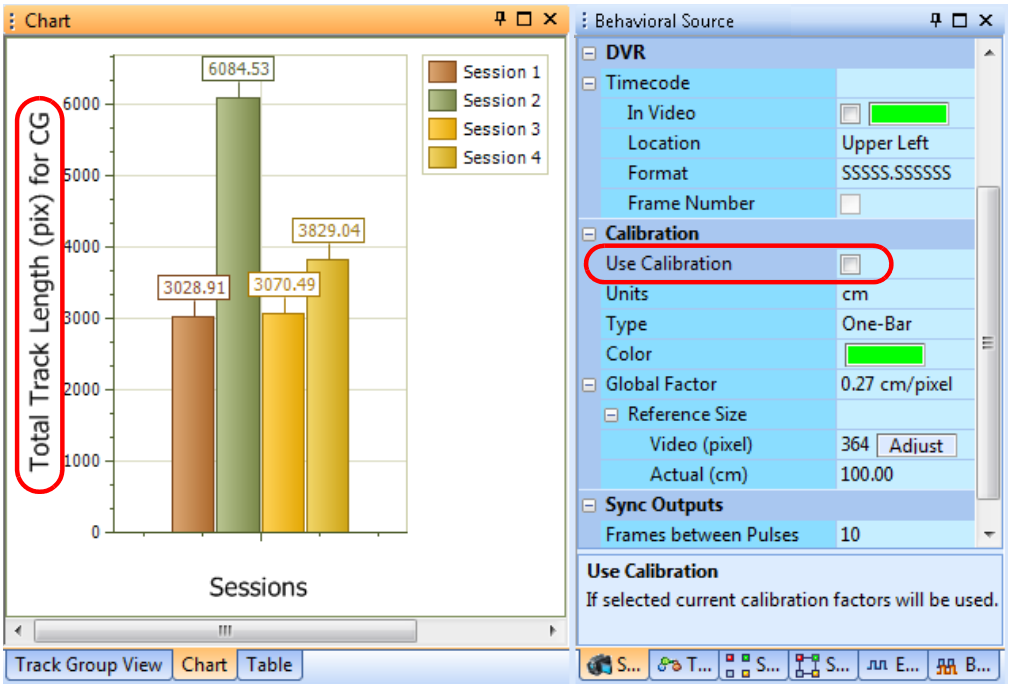
3 Check **Use Calibration**, then press the **Compute** button .



4 Observe the track length data. Notice the system applied the **Global Factor** to all recorded sessions, and the result is in metric units.



5 If you want to compute track length in pixels again, deselect the **Use Calibration** checkbox and click the **Compute** button. You will see the track length data displayed in pixels.



In other words, calibration can be applied to the computations (or removed from computations) any time. However, the calibration factor converting pixels to

metric units will be used on a per session basis—each session can have its own value. It is demonstrated in the example B.

Example B
Several sessions have been recorded with the same calibration value, and then the calibration factor was changed

1. Record several sessions with calibration, that is, with the **Use Calibration** checkbox selected.
2. Press the **Compute** button. Notice that the track lengths for all the previously recorded sessions are displayed in meters.
3. Recalibrate the arena (adjust the **Global Factor** in the Scenes tab as needed but leave the **Use Calibration** checkbox selected), then press the **Compute** button.
4. Observe that the track length data did not change. It means the system did not apply the new calibration factor (the **Global Factor**) to any previously calibrated sessions. It still uses the calibration factor value the system had at the time of recording. The new factor will be applied to future sessions.
5. Now select Session 1, for example, and recalibrate it (adjust the Reference size and thus the Global calibration factor. Please note the calibration value will change only for Session 1; all other recorded sessions will still have their own original value (the same for all sessions except Session 1 in this example). Then press the **Compute** button. Observe the track length changed for Session 1 only, and all other sessions have the track length as in Step 4.

   Note: The new changed value will always show for Session 1 in Analyze mode (until you change it again).

   Note: However, you still can see which calibration value was used for each session at the recording time. To do so, please switch to the View Sessions as Recorded mode and click on Session 1—this originally recorded value will be restored automatically.

   Note: In Cameras mode and Files/Add New Sessions mode the latest used Calibration Factor shows for all sessions.

D.3 Modifying Arena Shapes in Cameras Mode and Files/Add New Sessions Mode

In **Cameras** mode and **Files/Add New Sessions** mode, when you move or resize an arena shape, the changes apply only to future sessions, not to any of the previously recorded sessions. This feature allows you to alter the arena positions and shapes for future recordings in case camera position has shifted. You can also recalibrate the modified arena if necessary, and this change will be applied to future sessions as well. Please note if you select one of recorded sessions in these modes, you still will be seeing the latest arena shapes setup for future recordings.

D.4 Modifying Arena Shapes in Files/Analyze Session Data Mode

1. If you move or change the dimensions of an arena shape for a particular session in Analyze Session data Mode, this change will be applied to this session only, and will not affect all other recorded sessions.
2. In addition, if you move or change the dimensions of an arena shape for a particular session, this change will also be applied to any future sessions that will be recorded when you switch to Files/Add New Sessions mode (until you modify the position or dimensions again).
3. If you modify the shape or position of an arena for recorded sessions, please be aware it might affect the computation results, since only parts of the trajectories inside the arena are used for computations of behavioral data.
4. You can always see the original shape/position of the arena (if it was defined) for each session when it was recorded in Files/View Sessions as Recorded mode.

D.5 Adding and Deleting Arena Shapes

If you add or delete an arena shape during an experiment (that is, after sessions have been recorded), the system applies the addition or deletion to all sessions in the experiment—the ones that have been recorded already and the ones that will be recorded in future.

CAUTION

Adding or deleting an arena shape affects tracking data

Adding an arena shape could cause tracking data that was previously excluded (outside the original arena) in previously recorded sessions to be included in computations.

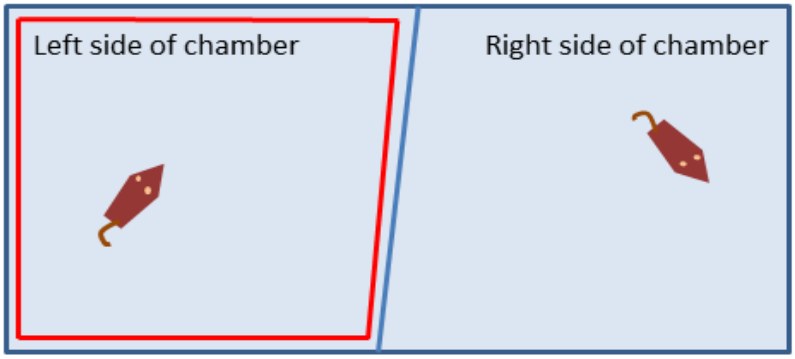
Deleting an arena shape could cause tracking data that was previously included (inside the original arena) in previously recorded sessions to be excluded from computations.

If you delete all arena shapes, the system will include in analysis all tracking coordinates that were previously recorded for each session.

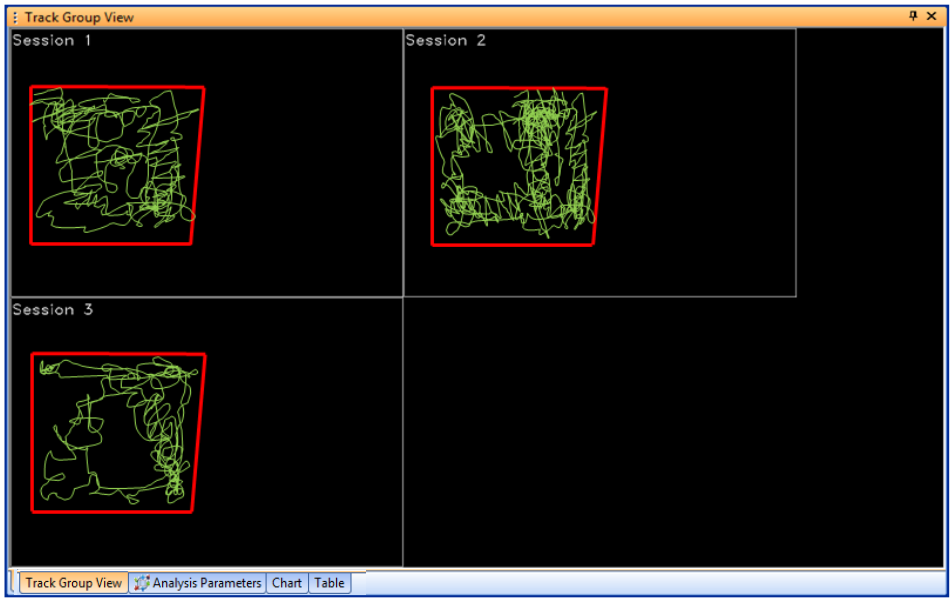
D.6 Examples of Adding, Deleting and Modifying Arena Shapes

Example 1 - Arena shape (polygon) used for recording in a new location

In this example, the researcher uses one camera to track an animal on one side of an experiment chamber, then uses the same camera to record an animal on the other side of the chamber. (The camera has a good view of the entire chamber, so there is no need to move the camera to record sessions in either side.) The chamber is illustrated in this diagram. The red polygon is the arena drawn by the researcher.

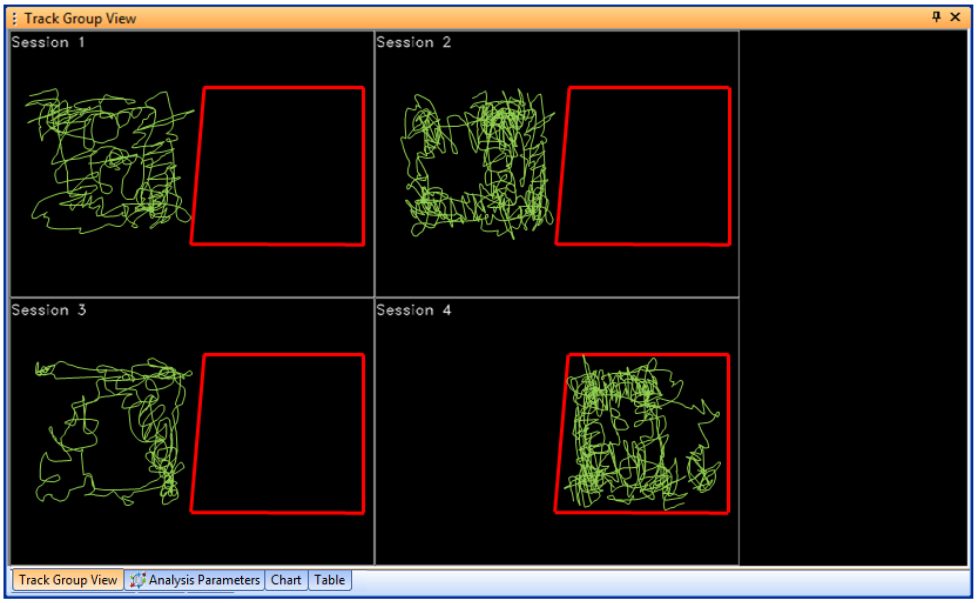


The researcher records three sessions in the left side of the chamber. The **Track Group View** looks like this.



Next, the researcher wants to record an animal in the right side of the chamber.

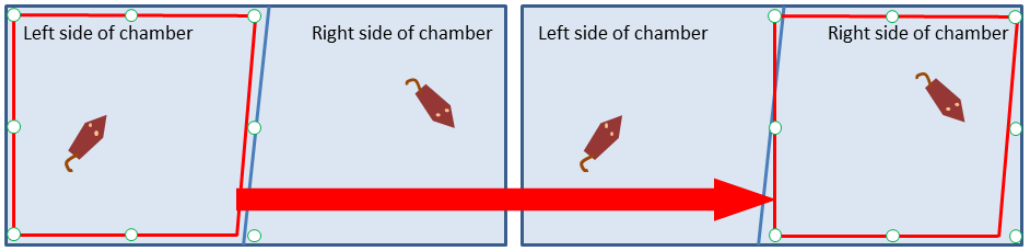
If the researcher simply deletes the existing arena shape and adds a new shape in the right side of the chamber, the **Track Group View** would look like this after recording Session 4.



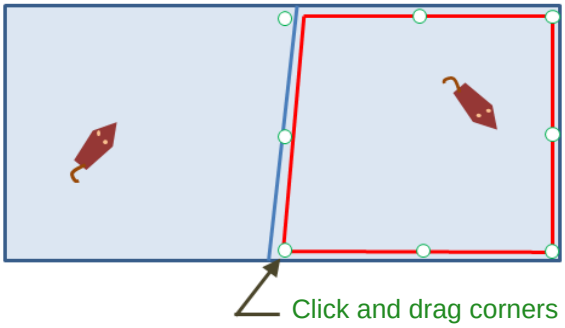
The problem with this procedure, as seen in the above view, is that deleting an arena shape deletes it for all previous sessions as well as all future sessions, and adding a shape adds it for all sessions. In general, the system keeps the same basic configuration of shapes added or deleted for all sessions, but you can move or resize shapes on a per-session basis.

> **IMPORTANT:** Deleting the existing arena and then drawing a new arena for Session 4 would also exclude from analysis all of the tracking data previously recorded in Sessions 1 through 3.
>
> Therefore, in this example, the researcher should have moved and resized the arena shape prior to recording Session 4. In this case, Sessions 1 through 3 keep the arena they had during recording.

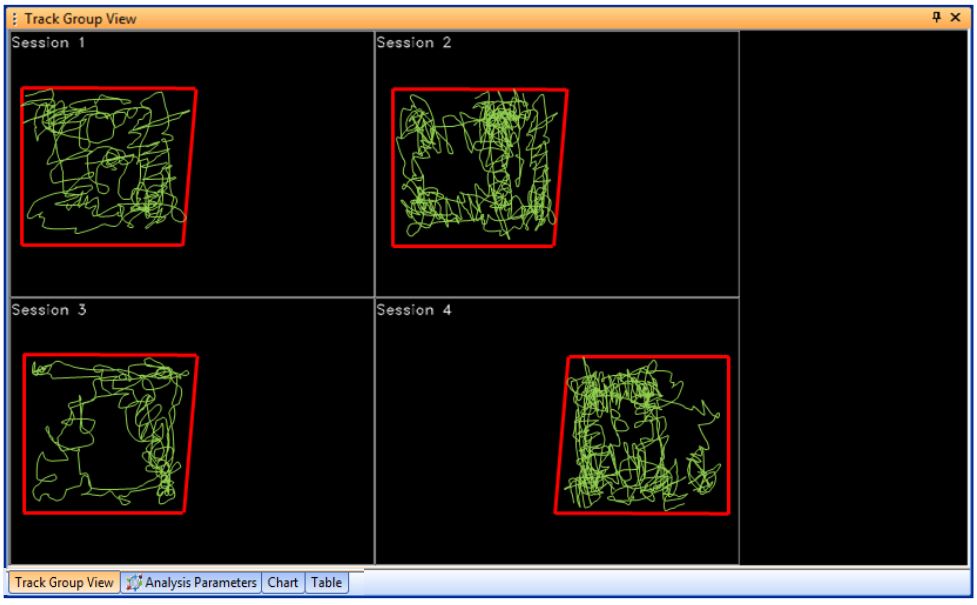
To move an arena shape, left-click somewhere inside the shape to select it. It will get selected on the video with little white dots (similar to how you move shapes in Microsoft® Word, for example). Then drag and drop it to the required position.



To adjust the points of the polygon to match the right side of the chamber, left click on one of the corners (it will display a little white circle), hold the left button of the mouse, drag the corner to the desired position and then drop it.

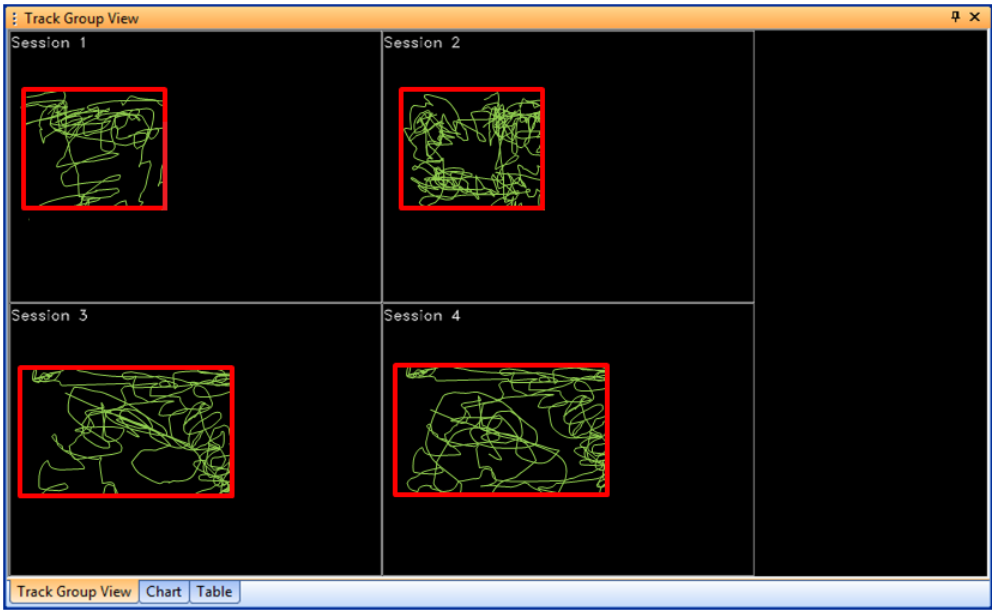


Moving and resizing the arena shape (before recording Session 4) has no effect on the data in Sessions 1 through 3—the position of the arena in those sessions is unchanged. After recording Session 4, the **Track Group View** looks like this.



Example 2 - Arena shape (rectangle) resized after Session 2

Modifications to a shape are reflected in the Track Group View for the experiment. In the example below, the researcher noticed that the original arena (red colored rectangle) was not drawn correctly and didn't cover the whole chamber. Therefore, prior to Session 3, the researcher increased the size of the arena to include the whole chamber. The system continues to use the new (larger) shape in Sessions 4, but does not change the arena shape in the previously recorded sessions (Sessions 1 and 2).



Example 3 - Arena shape (rectangle) deleted after Session 2

Deletion of a shape is reflected in the Track Group View for the experiment. In the example below, the experimental arena consists in two chambers. After Session 2 the researcher decided he/she is not interested in recording trajectory in the second chamber and deleted the right rectangle shape from the arena. So, starting from Session 3 tracking was performed only in the remaining part of the arena.

Example 4 - Adding and resizing an arena shape in Object Contour Mode

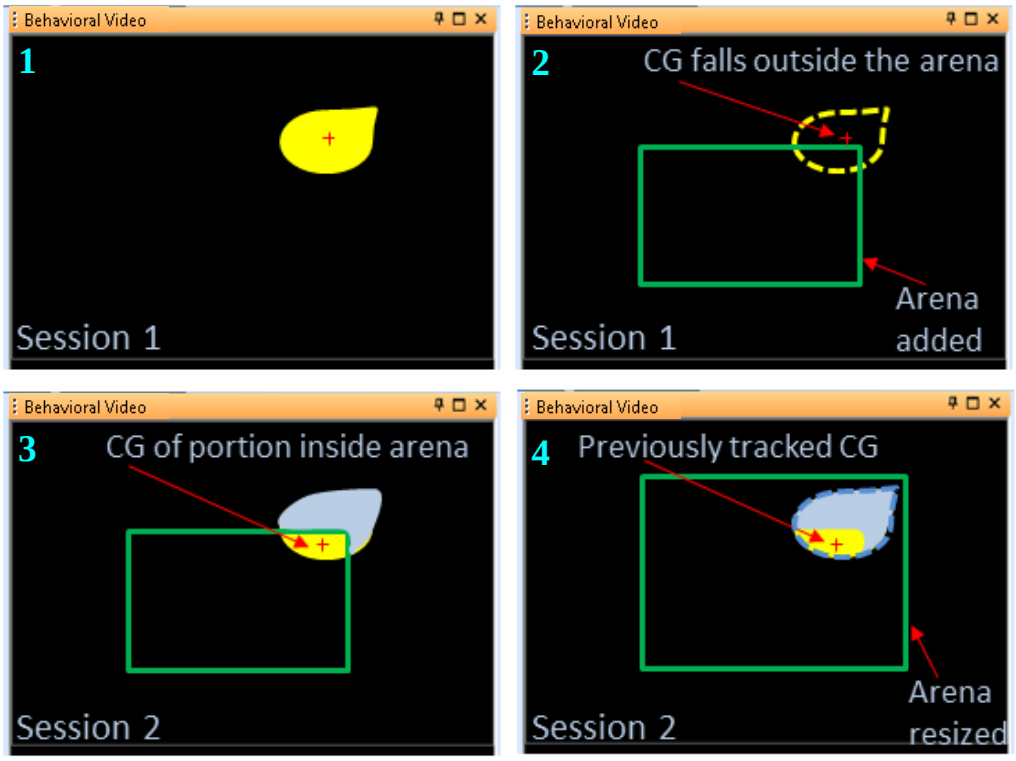
This example illustrates the behavior of the system as it tracks the center of gravity (CG) of the subject in **Object Contour** mode.

Image #1: In Session 1, the researcher did not define an arena, so the CG of the animal was tracked everywhere within the field of view.

Image #2: In the previously recorded Session 1 video file, the researcher applies an arena over the video image. Now the CG falls outside the arena, so the system *ignores* all tracking data for frames in which the CG was outside the arena.

Image #3: The researcher now records Session 2. In each frame, the system only tracks the CG of the portion of the animal's body that falls inside the arena, which in the image shown here, is not the entire body. The system ignores the portion of the body outside the arena.

Image #4: In the previously recorded Session 2 video file, the researcher expands or moves the arena so it now covers the entire shape of the animal in the video image. However, this does not modify the existing tracking data; the data originally recorded (see Image #3) is not changed.



D.7 Modifying Zone Shapes in Cameras Mode and Files/Add New Sessions Mode

In **Cameras** mode and **Files/Add New Sessions** mode, when you move or resize a zone shape, the changes apply only to future sessions, not to any of the previously recorded sessions. This feature allows you to adjust zone positions and shapes for future recordings in case the camera position has shifted.

D.8 Modifying Arena Shapes in Files/Analyze Session Data Mode

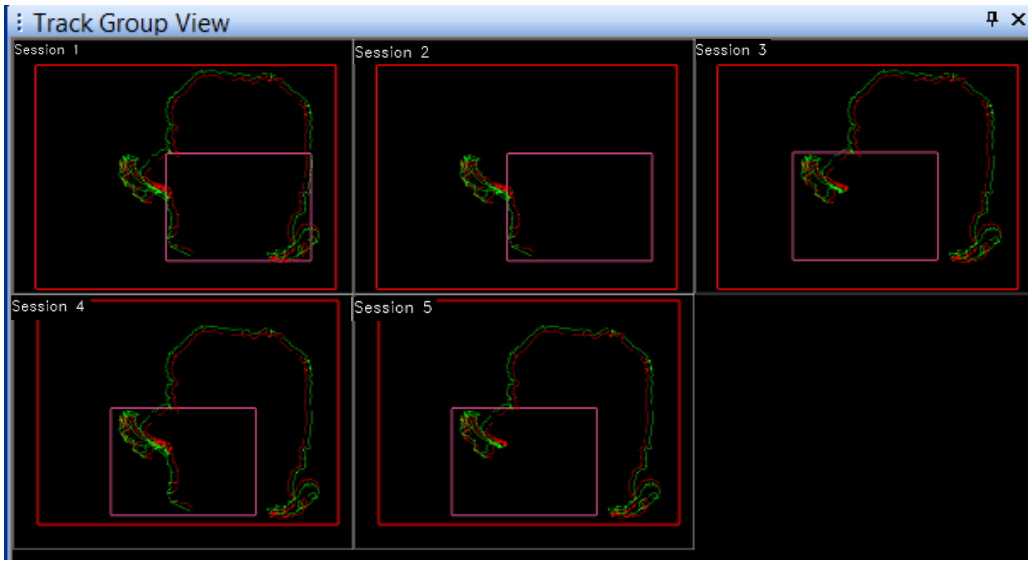
1. If you move or change the dimensions of a zone shape for a particular session in Analyze Session Data mode, this change will be applied to this session only, and will not affect all other recorded sessions.
2. In addition, if you move or change the dimensions of a zone shape for a particular session, this change will also be applied to any future sessions that will be recorded when you switch to Files/Add New Sessions mode (until you modify the position or dimensions again).
3. You can always see the original shape/position of all zones (if they were defined) for each session when it was recorded in Files/View Sessions As Recorded mode.

D.9 Adding and Deleting Zone Shapes

If you add or delete a zone shape during an experiment (that is, after sessions have been recorded), the system will apply the addition or deletion to all sessions in the experiment—the ones that have been recorded already and the ones that will be recorded in future.

Example - Zone moved after Session 2

Additions, deletions and modifications are reflected in the Track Group View for the experiment. In the example below, the researcher noticed that the original zone didn't cover the area where the animal was spending a great deal of time. Therefore, prior to recording Session 3, the researcher moved the zone to include this area. The system continued to use the new location in Sessions 4 and 5, but does not change the zone location in the previously recorded sessions (Sessions 1 and 2).



However, if we want to compute, for example, time the animal spent in this zone, it needs to be moved for Sessions 1 and 2. There are two ways of doing it:

1. Switch to Files/Analyze Session Data, select Session 1 and move this zone over video to the new position matching Sessions 3 and 4. Then do the same for Session 2. However, it would be difficult to make the zone position exactly the same for Sessions 1 and 2 and match it to Sessions 3 and 4.
2. To have this zone exactly in the same position, select any session, select this rectangular shape on Video (or its node in the Scenes tab) and delete it. This shape will be deleted for all four sessions. Now draw a new zone in the video of Session 1(or any other session), and it will be added and have exactly the same size and position for all four sessions.

Now you can press the button and get parameters for this zone for all sessions.

Appendix E
Troubleshooting

Plexon® has compiled this list of possible problems and solutions. In some cases, a problem has a simple cause, such as a cable disconnected or a camera not set correctly. Other issues can involve the configuration of various settings in the user interface.

If the steps in this appendix do not solve the problem, or if the problem is not listed, please contact Plexon Support at support@plexon.com or +1 214-369-4957.

E.1 Software Installation and Startup Issues

Error Messages While Running the Software Installation Package

The installation package adds programs and DLLs to the system. Therefore, the installation software:

1. Must be run from a computer account with administrator privileges.
2. Must be run on a computer with the Windows® 7 or Windows® 10 operating system.
3. Must be downloaded to and run from a local hard drive. Removable drives, including USB flash drives and USB hard drives, are not acceptable. Neither are networked drives.
4. Contact Plexon Support if the problem is not resolved.

Antivirus Software Impacts the Software Functions

Some systems come from Plexon with antivirus software pre-installed. The system has been tested with this antivirus software in place and should operate without problem as long as software and update settings are not changed. If problems occur that can be traced to the pre-installed antivirus software, please contact Plexon Support. It could be that a new set of virus signature files has caused some Plexon components to be flagged as untrustworthy.

Please do not install any other antivirus software on the Plexon supplied computer without consulting Plexon Support first. There are two reasons for this:

- Some antivirus tools might quarantine or remove key components of the Plexon software, rendering it unusable. A reinstallation of the software will be required after the antivirus tool is removed from the computer.
- Periodic downloads of updates or periodic scans for infections can disrupt video streams and processing, causing lost frames and delayed events.

Contact Plexon Support if the problem is not resolved.

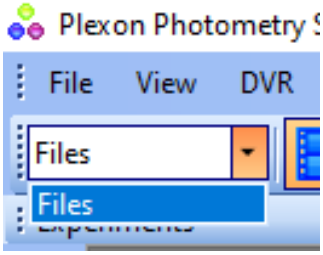
The Software Will Not Start At All

There can be several causes for the software failing to start when its icon is clicked or displaying an error and failing to start when OK is clicked. The most common causes and their solutions are shown below.

1. The appropriate Plexon license key is missing (the corresponding message shows up when you try to start the software). Obtain or find the correct license key, plug it into a USB port on the computer, and start the software.
2. If the software still will not start and reports a missing license key, install the Sentinel drivers (this is done with the installation software or by running the file C:\Program Files (x86)\Plexon Inc\Photometry V1\Common Files\Sentinel System Driver Installer 7.5.8.exe).
3. If the system reports missing DLLs, reinstall the software. All the required DLLs are in the installation package.
4. Contact Plexon Support if the problem is not resolved.

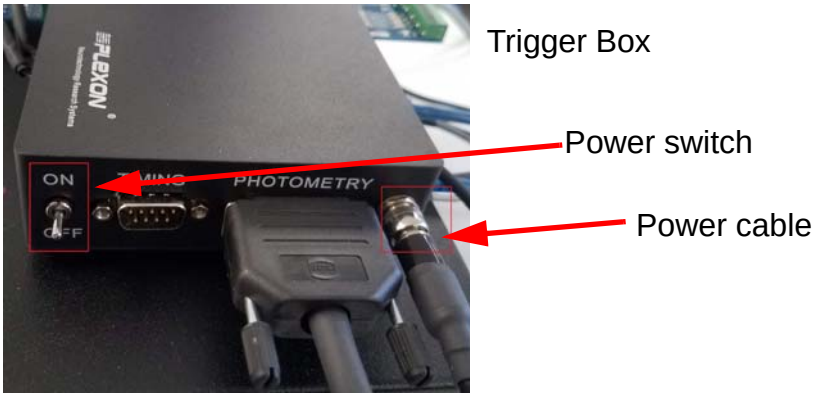
Software starts in Files mode (instead of Cameras mode) and cannot be switched to Cameras mode

In this case “Cameras” mode will not be present in the modes switching drop-down list:



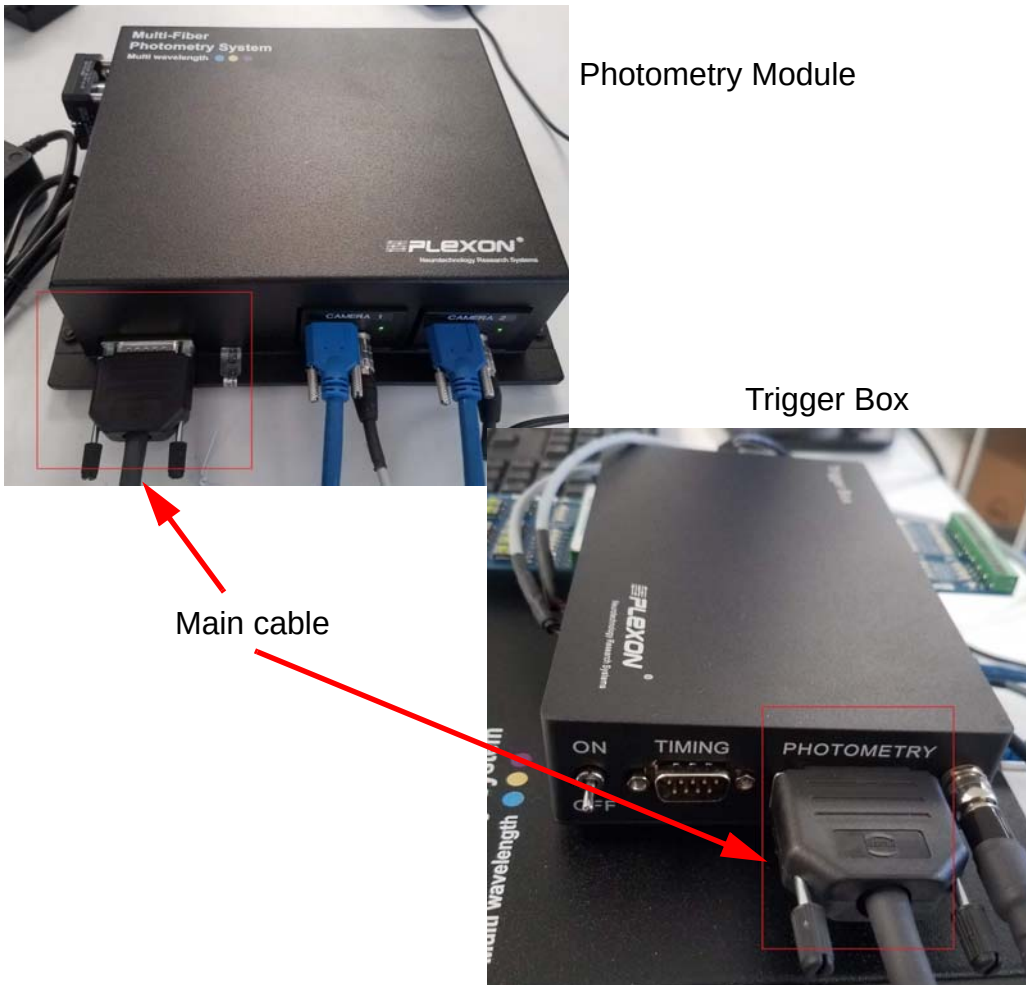
The most common causes and their solutions are shown below.

1. Missing drivers. Reinstall the software package.
2. Cables for Behavioral or Photometry Cameras are loose or unplugged. Close the software, then check and reseat both ends of the cables going to all cameras. Use only the ports on the four-port USB3 card on the back of the PC for the camera connections. Restart the software, and follow the normal startup steps.
3. The Plexon Trigger Box is not connected or not powered on. Check if the Trigger Box power cable is plugged in, and Trigger Box power switch is ON:



Ensure the USB cable from the Trigger Box is connected to the USB port of the computer:

4 The Plexon Photometry Module is not detected. Make sure the Photometry Module is connected to the Trigger Box using the main cable:



Note: If the software was used in Cameras mode before, and the condition(s) described in Step 3 or Step 4 happened, the critical messages described in “Critical Alarm Messages” on page E-9 will show up when you restart the software.

5 Contact Plexon Support if the problem is not resolved.

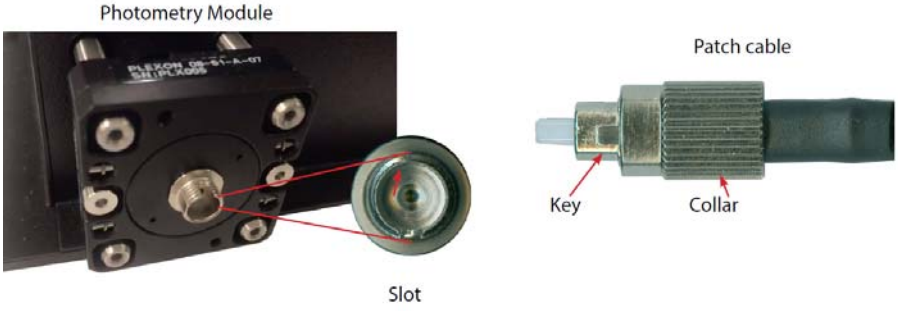
E.2 Photometry Device and Fiber Issues

Fiber Bundle Not Fully Contained Inside the Photometry Video Window

or

Fiber Bundle Appears Out of Focus in the Photometry Video Window

1. Verify the patch cable is attached properly. The patch cable should be “hand-tightened” onto the connector on the side of the Photometry Module.
2. If the above step does not correct the problem, contact Plexon Support for assistance.



Low Excitation Light Levels (or No Light) at Fiber Ends

1. Verify the patch cable is attached properly. The patch cable should be “hand-tightened” onto the connector on the side of the Photometry Module.
2. Verify you have clicked the **Start** button for at least one of the photometry wavelengths. Also note that the light from the 410nm (UV) LED is almost invisible to the naked eye—there is just a low intensity purple glow at the end of the fibers.
3. Ensure you have clicked the **Start** button for all three wavelengths. If there is still no light at the end of the fibers, recheck the connection of the patch cable to the Photometry Module.
4. If some, but not all of the fibers are emitting light, the dark fiber(s) might be broken.You may need to replace the patch cable or fiber stubs.

E.3 Physical Installation Issues with the Behavioral Camera

In general, all of the following should be checked when unexplained problems are occurring. For more detailed installation instructions applicable to the hardware, please refer to the relevant documents.

Video Not Present

1. In Cameras mode—Be sure that all required cable connections are in place and plugged in all the way.
2. Be sure the lens iris is fully open.
3. In Files mode—Be sure that you have opened video files in the Source tabs.

Unstable Video Image

The camera mount must be stable or the resulting video data will shake and not be repeatable. This can be the case, for example, when a tripod is used to mount a camera, instead of the preferred wall or ceiling mount.

Poor Image Related to Camera Focus and Camera Parameter Settings

There are physical settings on the lens that might be incorrectly adjusted, or some of the camera parameters in the user interface (Source tab) might not be set correctly. See also “Cannot Focus the Video Image” below.

Video Field of View Is Distorted or Incorrectly Sized for the Experiment

Depending on the shape and size of the arena and the focal length of the camera, the positioning and distance of the camera relative to the arena might not be optimized. For information on camera positioning, see Appendix A, Optimizing Behavioral Camera Positioning.

E.4 Video Issues with the Behavioral Camera

No Behavioral Video Window

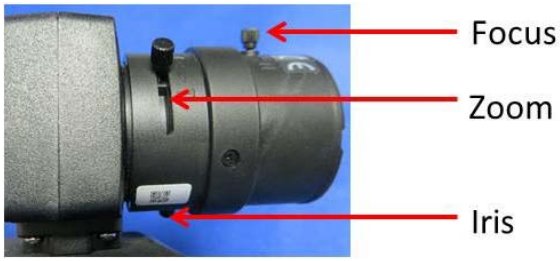
1. Verify the system is in **Cameras** mode.
2. In the **Window** dropdown menu, select **Layout**, then select **Reset to Default Layout**. All the windows for the photometry and behavioral video streams should be displayed.

No video in the Behavioral Video Window

1. Close the software.
2. Verify the USB cable is connected properly between the PC and the camera. Restart the software.
3. If the USB cable seems to be connected properly, but there is still no video image, close the software again and try to use a different port on the USB3 card plugged into to the PC or replace the USB cable for the behavioral camera.
4. If there is still no video, contact Plexon Support.

Image is Out of Focus

1. Check the manual adjustments on the body of the lens. Ensure the following:
   - The cover cap on the camera has been removed from the front of the lens
   - The iris on the camera is fully open
   - The zoom on the camera is set properly
   - The focus is properly adjusted
2. For additional information on focusing the camera and setting camera parameters in the user interface, see Section 5.3, "Configuring Behavioral Source Parameters" on page 105.



3. Contact Plexon Support if the problem is not resolved.

Cannot Focus the Video Image

The video is present, but blurry and cannot be focused at any zoom or focus setting.

It could be a broken lens. Please contact Plexon Support.

Flickering Video

Video is present, but the image is flickering.

1. Turn off any fluorescent lights illuminating the arena and use incandescent lights instead. If the flickering goes away, there may be problems with the fluorescent bulbs themselves, the starters, or the ballast. If fluorescent fixtures must be used, these problems must be fixed. Otherwise, look for alternative lighting sources
2. Contact Plexon Support if the problem is not resolved.

Arena is Too Small or Too Large within the Video Image

Once video has been obtained, the camera and lens must be adjusted so that the maximum dimension of the arena nearly fills the long dimension of the video image while ensuring that all other parts of the arena are within the video image as well.

1. If the arena image is too small, move the camera closer to the arena (outside of the near limit) or adjust the zoom ring towards the T (telephoto). Then adjust the lens focus ring until the video image is clear, if possible.
2. If the arena image is too large, move the camera away from the arena (within the physical limits of the environment) or adjust zoom ring towards the W (Wide Angle). Then adjust the lens focus ring until the image is clear, if possible.
3. Contact Plexon Support if the arena cannot be adjusted to fill the video window.

Video Stream from Behavioral Camera is Freezing Periodically or is Corrupted

1. Use only USB ports in the four-port USB3 card on the back panel of the PC for the USB cable going from both the Behavioral camera and Photometry cameras.
2. If cards have recently been added to the PC bus, remove them to see if the problem is solved.
3. Contact Plexon Support if the problem persists.

E.5 Start Recording Button is Grayed Out

Cameras Mode

1. If the Start button in the main toolbar of the GUI is grayed out, be sure you have performed all of the steps in the applicable start up and configuration procedures, beginning with the steps in Chapter 2, Installing and Starting the System.
2. Take the necessary actions to respond to critical alarm messages (if present) as described in “Critical Alarm Messages” on page E-9.

Files Mode

If you are planning to record in Files/Add New Sessions mode, ensure you have loaded source files for all four video sources.

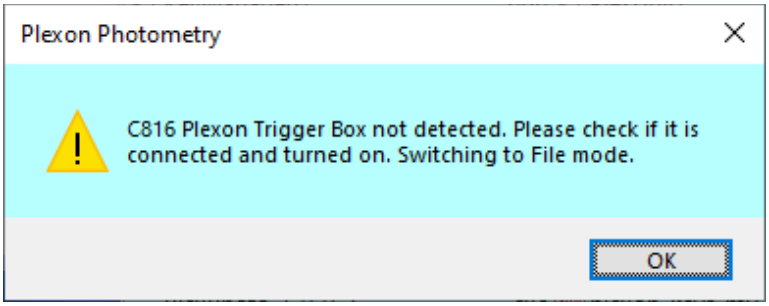
E.6 Critical Alarm Messages

If the photometry system hardware is connected to the computer, and the software was used in Cameras mode before it was closed, it should come up in Cameras mode if you start it again. This section covers critical alarm messages which will show up if there are certain issues with cable connections. These issues need to be resolved to use the system in Cameras mode.

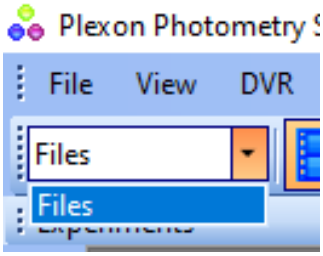
Note: These messages do not show up if the software is restarted after using it in Files mode, even if the system is connected, and there are problems with the cable connections.

C816—Plexon Trigger Box not detected

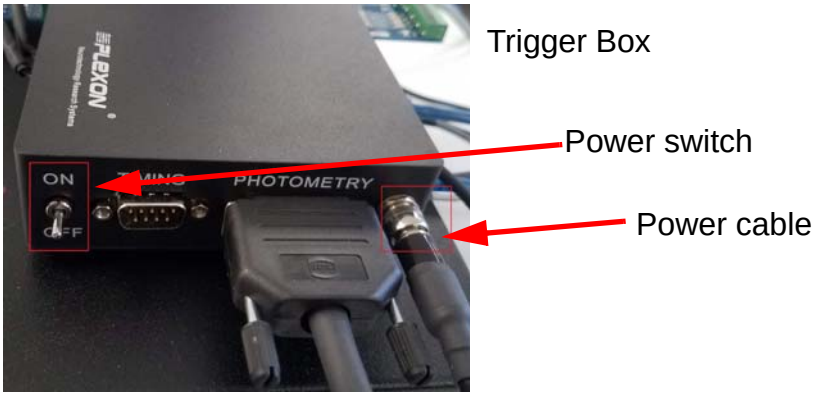
This alarm indicates the power or USB cables to the Trigger Box are not properly connected, or the Trigger Box power switch is not turned ON.



After the **OK** button is clicked, the software will start in Files mode, and “Cameras” mode will not be present in the modes switching drop-down list:



1. Close the software.
2. Ensure the Trigger Box power cable is plugged in and the Trigger Box is turned ON. If the power cable seems to be plugged in, check the connection.



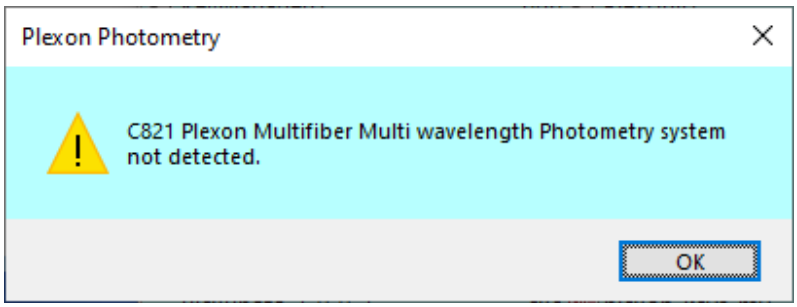
3. Ensure the USB cable from the Trigger Box is connected to the USB port of the computer.

4. After you press **OK** on this message, the system will switch to Files mode.
5. If you exit the software, fix all the issues and start the software again, the system will start in Files mode, so make sure to switch to Cameras mode again if you need to continue in Cameras mode.

Note: When the system is on and the Trigger Box power switch is in the ON position, both green LEDs on the Trigger Box (+5V and +3.3V) should be on. If one or both of them fails to turn on, it might indicate an electrical problem with the Trigger Box. If you have performed the troubleshooting steps, above, and one (or both) light(s) on the Trigger Box is still off, contact Plexon Support.

C821—Photometry System not detected

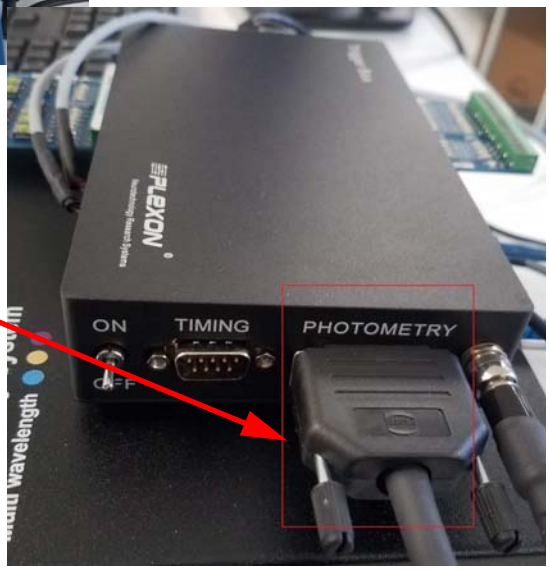
This alarm is displayed when the main cable between the Photometry Module and the Trigger Box is not connected. To fix this problem, ensure that the cable is securely connected at both ends.



Photometry Module

Trigger Box

Main cable



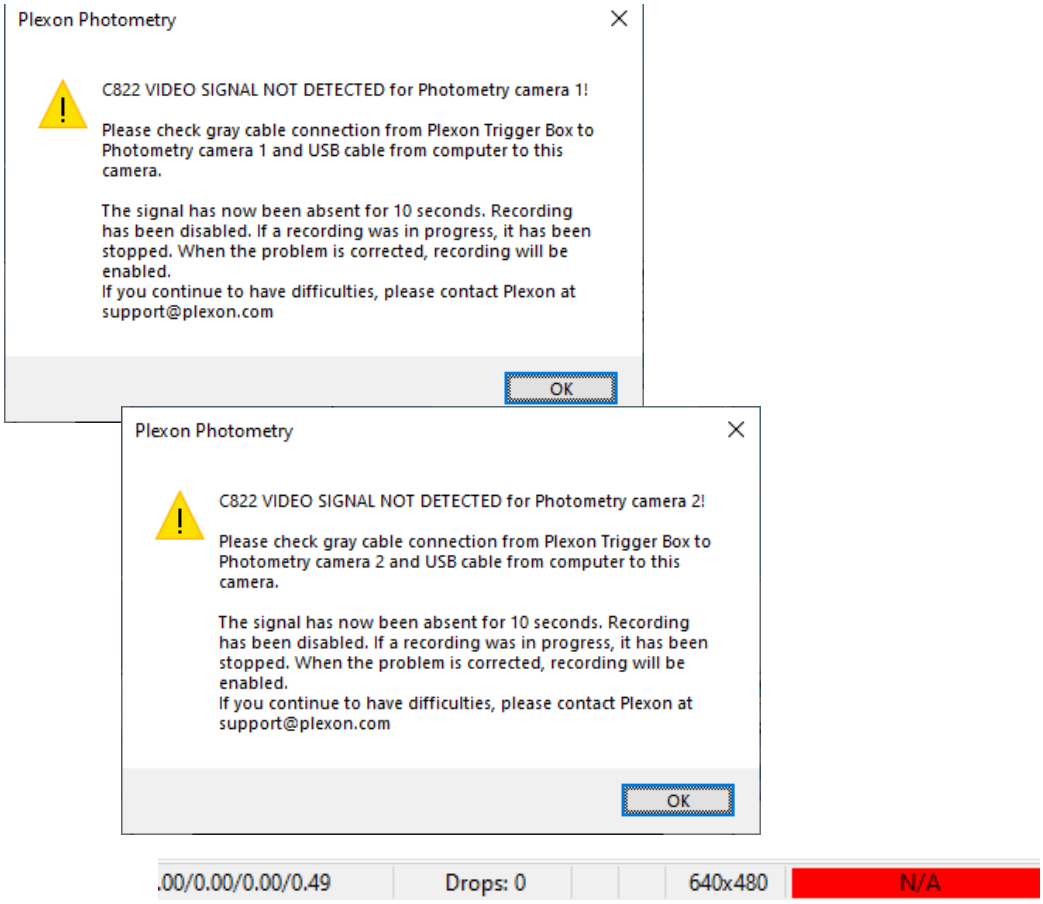
E.7 Critical Alarm Messages—Recording Disabled

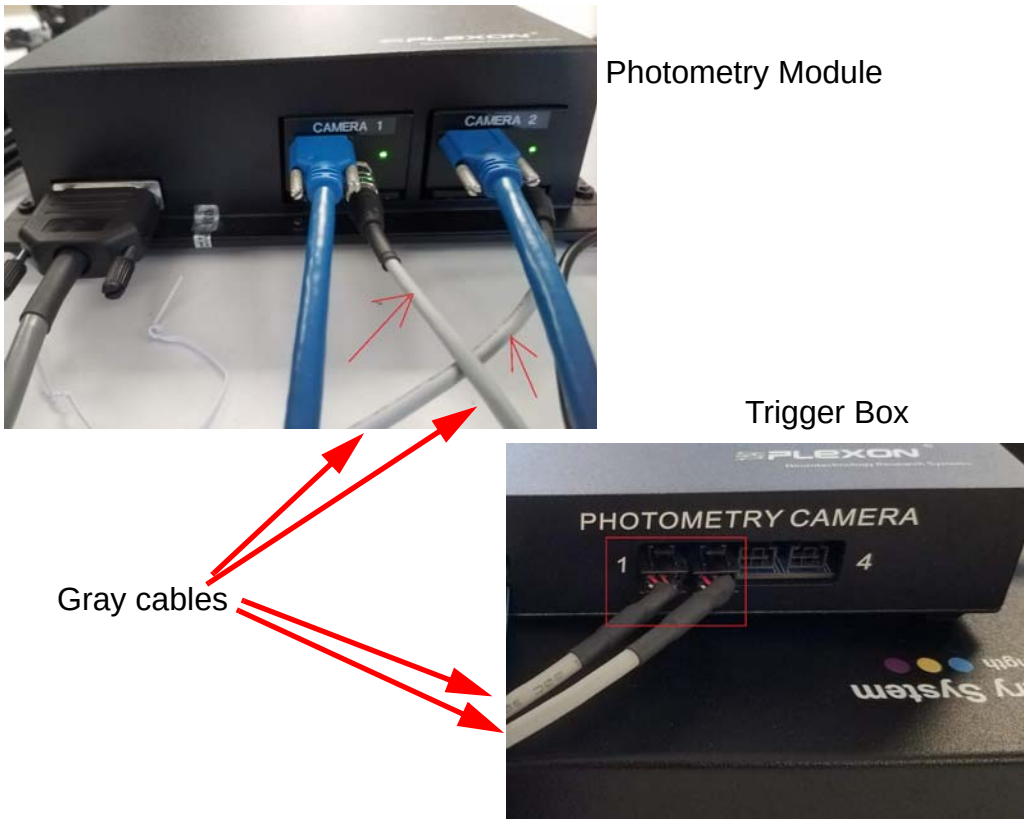
This section describes critical alarms that need to be resolved before recording will be enabled. If the instructions in this section do not resolve the problem, ensure that you have performed all of the steps in the applicable start up and configuration procedures, beginning with the steps in Chapter 2, Installing and Starting the System. If the problem persists, contact Plexon Support for assistance.

Note: Note: these messages do not show up if the software is restarted after using it in Files mode, even if the system is connected, and there are problems with the cable connections. However, these messages will pop up if you are switching from Files mode to Cameras mode, and problems with cable connections are detected by the software.

C822—Video signal not detected for Photometry Camera 1 or 2

This alarm is displayed when the video signal has been disabled for 10 seconds. No image will be displayed in the corresponding Video window(s). Either the gray cable from the Trigger Box to the Photometry camera indicated in the message, or the blue USB3 cable from the computer to the Photometry camera indicated in the message are disconnected or not plugged in all the way. Red “N/A” will be blinking on the right side of the status bar where the Frame Rate normally appears. To fix this problem, ensure the blue and gray cables are connected properly.

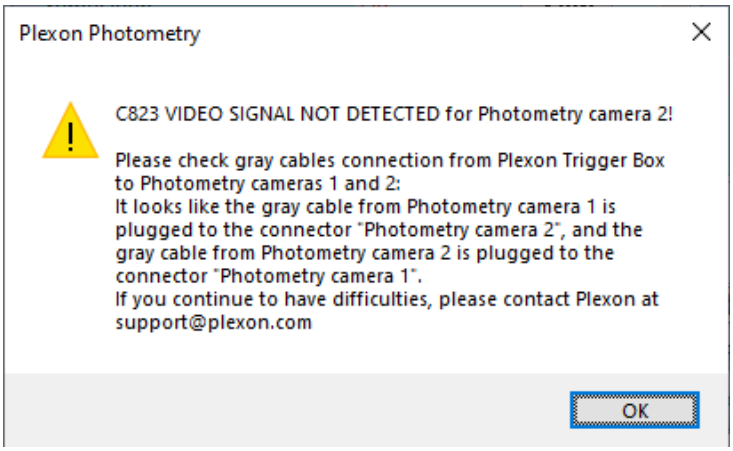




Note: If the issue was related to the gray cable, the software will automatically go back to normal operation after you press the **OK** button. (The red “N/A” stops blinking and the actual frame rate will show up again. The record button will be enabled.) If the issue was with the USB3 cable being loose or not connected, the software will have to be restarted.

C823—Video signal not detected, cables not plugged into correct ports

This alarm is displayed when the gray cable from the CAMERA 1 port on the Photometry Module is plugged into the PHOTOMETRY CAMERA 2 port on the Trigger Box, and vice versa (see the image above). Red “N/A” will be blinking on the right side of the status bar where the Frame Rate normally appears. To fix this problem, connect the cables to the correct corresponding ports. The software will go to normal operation after you press the **OK** button. (The red “N/A” stops blinking and the actual frame rate will show up again. The record button will be enabled.)

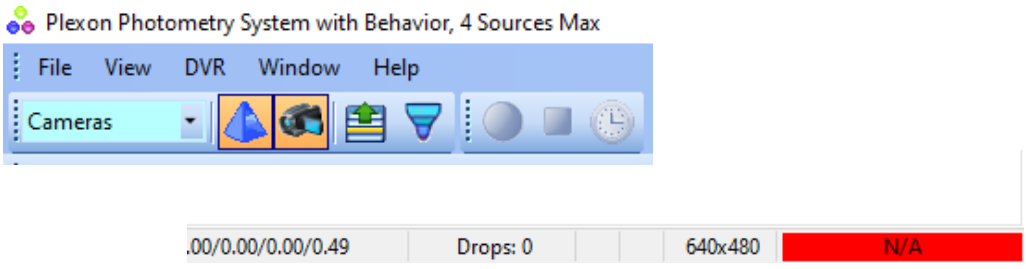


Status Bar displaying red “N/A” alarm

This alarm is displayed when:

- The blue USB cables going from the USB card inside the computer to the Photometry cameras are either not plugged in or not plugged in all the way.
- The gray cables from the Trigger Box to the Photometry cameras are disconnected or not plugged all the way.
- The gray cable from the CAMERA 1 port on the Photometry Module is plugged into the PHOTOMETRY CAMERA 2 port on the Trigger Box, and vice versa.

Red “N/A” will be blinking on the right side of the status bar where the Frame Rate normally appears, and some of the photometry devices will be missing from the toolbar. To fix this problem, ensure the cables are plugged in fully and to the proper ports on the Trigger Box.



E.8 Issues with Dropped Frames and PC Overload

Dropped Frame Count Non-zero While Recording

1. Connect USB cables from the Behavioral camera and Photometry Cameras 1 and 2 only to the USB ports in the four-port USB3 card on the back panel of the PC.
2. Stop running all unnecessary or resource-consuming applications on the computer while recording, for example, Microsoft® Office® programs, Skype®, other videos, games, email applications, scripts, etc.
3. Disable scheduled tasks or tasks that run automatically, such as virus scans, PC backups and Windows updates. These can cause dropped frames when they run. Use the Task Monitor to view all tasks. If necessary, contact your system administrator to disable unwanted tasks.
4. Internet activity, especially that involving heavy downloads such as from YouTube or similar sites, should be prohibited on the computer. To avoid this possibility, Plexon recommends placing the computer on a subnet isolated from intensive internet traffic.
5. Disable automatic power saving features, such as those that put the display or computer to sleep after a specified interval.
6. If you are tracking more than five color markers, ensure the color tracking (colors and color thresholding) is set up correctly, and the markers are actually being tracked as they move in the arena (follow procedures in Chapter 7, Configuring the Tracking Parameters).
7. If dropped frames still persist after these steps, please contact Plexon Support.

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Plexon® Multi-Wavelength Photometry System

User Guide

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