User Guide

Version 4 - December 2018

CineLyzer® with Photometry

Behavioral Research System
with Photometry Imaging

Plexon Inc
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USA

CAUTION

Limit voltage to 0–5V at the DIO Interface pins

Do not apply more than 5V to any of the input/output pins on the USB Digital Input/Output (DIO) Interface unit. Doing so could damage the device.

CAUTION

USB Security Key Damage

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.

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User Guide

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CE Conformity

We declare, under our sole responsibility, that the standard CineLyzer System (excluding the Photometry System option) and its components conform to the directives of the CE. Additional information regarding this conformance is available from Plexon Inc.

Publication History

December 2018

This issue of the user guide is based on CineLyzer® software version 4.4.

You can see a summary of hardware and software features that have been implemented for this first release by accessing the Change Log for this product on the Plexon website, www.plexon.com.

May 2018

This issue reflects the new product name—Plexon® CineLyzer Behavioral Research System with Photometry Imaging.

In this User Guide:

- The term CineLyzer System refers to the standard behavioral research system.
- The term Photometry System refers to the optional additional equipment and software license needed for photometry imaging.

You can see a summary of hardware and software features that have been implemented for this first release by accessing the Change Log for this product on the Plexon website, www.plexon.com.

November 2016

This issue of the user guide incorporates the following information:

- Conformity with European certification (see the title page).
- Updated trademarks for the individual CineLAB® modules: CineCorder® Module, CineTracker® Module and CineLyzer® Module.

October 2016

This issue of the user guide is updated for software Version 4.1, which incorporates substantial enhancements to the user interface.

November 2015

Version 4.0 is the first release of the Plexon CineLAB Behavioral Research System.

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Chapter 1
System Applications and Features

1.1 Scope of this User Guide

The scope of this user guide is the Plexon® CineLyzer® Behavioral Research System (CineLyzer System), a research tool for video capture (recording), tracking and behavioral analysis of laboratory animals. The CineLyzer System consists of hardware and software that are fully integrated and tested in the Plexon factory. (See the Plexon website for examples of multisystem options.)

This user guide also describes the optional photometry imaging function that detects and measures light emitted by certain proteins—genetically encoded calcium indicators (GECI), for example GCaMP, and genetically encoded voltage indicators (GEVI). The optional hardware and software license are referred to as the Photometry System.

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

1.2 Overview of the CineLyzer System

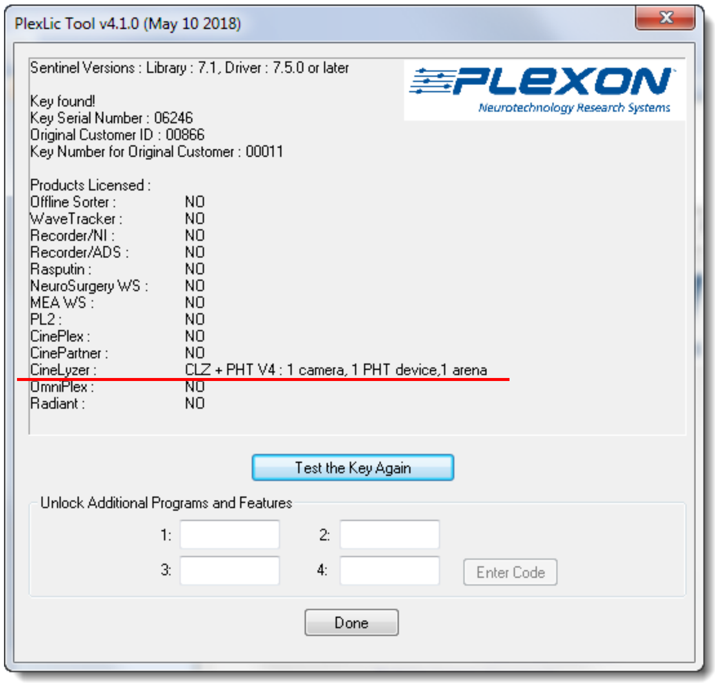
The CineLyzer System provides these features:

- 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—There are two types of signaling supported:
  - Input and output signals related to triggering and detection of behavioral events
  - Output of regular pulses every N frames (configurable)
- Tracking—Arena definition, dimensional calibration and positional tracking features.
- Behavioral analysis—Zone and event definitions, tracking in specific zones and zone sequences, data output capabilities and behavioral analysis tools.
- Photometry (optional feature)—Detection and recording of fluorescence emitted in the brain or nervous system in response to an applied excitation light, and event definitions based on light power levels detected by the photometry system.

The standard CineLyzer hardware and cameras (up to four cameras are supported with each standard CineLyzer license) are designed to support all of the CineLyzer recording, tracking and behavioral analysis functions.
With the photometry option, there is a single behavioral video camera, and the system requires additional hardware plus the CineLyzer photometry software license.

1.3 CineLyzer Licensing Options

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 (CineLyzer with Photometry in this case).



The table below shows the CineLyzer licensing options.

Research Requirements	License Required	Label on USB License Key
<u>Standard CineLyzer System</u> Recording—Video capture and playback Tracking—Positional data Behavioral Analysis—Events and analysis **<u>Note:</u>** Each standard CineLyzer license can be configured to handle up to four cameras.	CineLyzer v4	CLZ V4
Recording—Video capture and playback Tracking—Positional data Behavioral Analysis—Events and analysis Photometry imaging and photometry events **<u>Note:</u>** Each CineLyzer photometry license is configured to handle one behavioral camera and one Plexon Photometry System.	CineLyzer v4 plus Photometry	CLZ V4+PHT

1.4 CineLyzer Features

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

- Recording, Playback and Re-recording Features
- Input/Output Features
- Tracking Features
- Behavioral Analysis Features
- Photometry Features (Optional)

1.4.1 Recording, Playback and Re-recording Features

These features are provided as follows:

- Recording (online) function—The software runs in **Cameras** mode on the Windows® 7 operating system, which is installed on the computer supplied by Plexon as part of the system.
- Playback and re-recording (offline) functions—The software runs in **Files** mode on Windows 7 on the computer supplied by Plexon. It can also run on a standalone customer-supplied computer that meets certain minimum requirements.

Note: The standalone computer must have minimum memory, processor and instruction set to run CineLyzer System efficiently. The recommendations for the computer are summarized in Section 1.8, “Using a Standalone Computer for Data Analysis” on page 12. The appropriate Plexon license key must be plugged into a USB port on the computer.

A specially configured Plexon supplied computer is required for supporting the four-camera license.

The video functions are as follows:

- Provides a digital video capture and recording capability
- 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 CineLyzer System are compatible with Windows Media Player® and many other media players.

- Enables the user to select image quality for recordings by changing the nominal bit rate (lower bit rates results in smaller file sizes)
- Provides the capability to start and stop recording by means of external signals
- Records directly to an internal hard drive

- Supports digital imaging with up to four cameras simultaneously (see Section 1.5, “System Hardware, Software and Cameras” on page 10 for the camera specifications)
- Supports near IR (infrared) recording, optionally available (see Section 1.5, “System Hardware, Software and Cameras” on page 10 regarding the IR capable camera)

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

The system supports offline playback and re-recording from certain types of previously recorded video files:

- Reads MPEG files previously created by a CineLyzer System (the appropriate Plexon license key must be plugged into the computer)
- Reads MPEG files created by CinePlex® System Version 3
- Reads MJPEG files created by CinePlex System Version 1 or 2
- Reads certain MJPEG files that were originally created by tools not supplied by Plexon, for example, files with either “mjpg” or “ffds” signatures
- Allows re-recording of MJPEG files in the more efficient MPEG-4 format resulting in file size reductions of up to 90%

1.4.2 Input/Output Features

The USB Digital Input/Output Interface provides lines for 12 different input events (six high true and six low true inputs) and 12 different output events. See Appendix C, USB Digital Input/Output Interface.

The Camera Trigger Board is mounted on the behavioral camera and allows the system to output a pulse to an external device every N frames, where N is configurable from 1 to 16 frames between pulses. See Section 1.5, “System Hardware, Software and Cameras” on page 10.

1.4.3 Tracking Features

The tracking feature set includes arena definition and automated tracking of an animal's movement and position over time. This capability supports both real-time and offline tracking functions and includes the following features and functions.

- Provides the option of calibrating the video and tracking data in inches or centimeters.
- Allows you to define an arena (one for each camera) with simple or complex geometries for tracking in particular zones of interest (for example, open versus closed arms in an elevated "plus" maze).
- Provides options for LED tracking, object contour tracking, and color marker tracking.
- Allows tracking of up to 12 different color markers on each of the cameras (up to four cameras are supported per CineLyzer System).
- 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 extracted to comma separated values (CSV) files on the host PC.
- Allows adjusting of 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 arena settings and calibrations from a previously-recorded experiment onto a new experiment. Overlays can also be loaded onto an existing experiment as long as the tracking mode, frame rate and frame resolution of the overlay match that of the existing experiment.

Note: For more information about flexible geometry and overlays, see Section 10.14, "Using the Overlay Feature during Analysis" on page 305

Special Conditions

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 that they cannot be reliably tracked at the maximum frame rate (60 frames per second for some CineLyzer Systems).
- 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, "System Hardware, Software and Cameras" on page 10 regarding IR requirements

If your experiment involves a combination of high frame rate combined with an extremely large number of simultaneously tracked objects and simultaneous behavioral events, or other factors involving high CPU usage (for example, running browser applications or software other than Plexon software while recording), it is possible to overload the system and drop frames. If this occurs, refer to the discussion in Section 9.9, "Managing CPU Demand and Avoiding Dropped Frames" on page 255.

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

1.4.4 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.

- Supports creation of static and dynamic zones of interest within an experimental arena
- Allows you to define zone sequences useful for learning tasks such as the water maze, radial maze and T-maze
- Allows you to define combination events on each camera as well as combination events across multiple cameras, or, in the case of CineLyzer System with the photometry option, between the behavioral camera and the photometry system
- Monitors animals traversing zones and sequences of zone entries, and generates logical and digital events
- Tracks each animal as it enters and exits zones of interest that you have defined within the experimental arena
- 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 (six high true and six low true) and output up to 12 digital signals (six high true and six low true) when specific behavioral events occur in the arena
- Allows you to export data from the CineLyzer System in comma-separated values (CSV) format for further analyses by other programs
- Allows adjusting of the arena and zone geometries and calibration on a per-session basis. You can change the position, dimensions and dimensional calibration of arenas and zones 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 arena settings, zone settings and calibrations from a previously-recorded experiment onto a new experiment. Overlays can also be loaded onto an existing experiment as long as the tracking mode, frame rate and frame resolution of the overlay match that of the existing experiment.

Note: For more information about flexible geometry and overlays, see Section 10.14, "Using the Overlay Feature during Analysis" on page 305.

1.4.5 Photometry Features (Optional)

The photometry feature set (optional) includes hardware that emits the excitation light and detects and measures light emitted by GECI or GEVI cells. It also provides several tools that you can use for photometry analysis.

With the photometry features and functions you can

- Record from up to four fibers—Simultaneously investigate fluorescence in discrete 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
- Visualize changes in fluorescence with a CCD-based sensor
- 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
- Combine real-time tracking of behavioral events and photometry signals to trigger external devices via TTL output

1.5 System Hardware, Software and Cameras

This section briefly describes the hardware and cameras supplied with the standard CineLyzer System.

Note: The Photometry option is sold separately and includes all of the photometry hardware and a software license. See Section 1.6, "Photometry Hardware, Software and License" on page 12).

The standard CineLyzer System includes the following hardware:

- A host PC, specially prepared by Plexon, including the Windows 7 operating system, and the CineLyzer software
- One or (optionally) two monitors to display the CineLyzer user interface
- A keyboard and mouse
- One camera kit, consisting of the camera, lens, Manfrotto® quick release mounting plate, and Camera Trigger Board

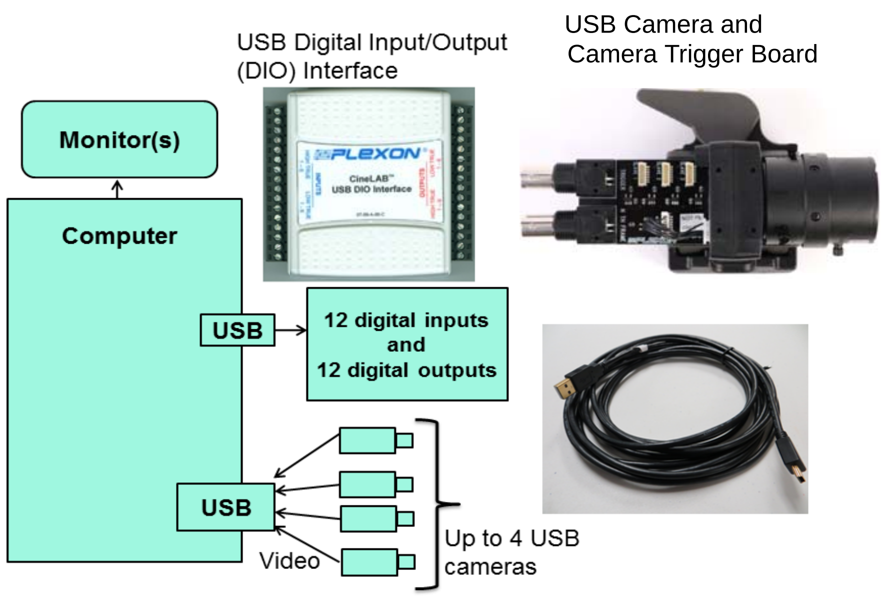
Note: The standard CineLyzer System can operate with up to four cameras. Additional camera kits can be purchased separately.

- A digital input/output (DIO) interface unit

Note: The DIO unit is supplied with an IO Status Indicator and Input Generator, as shown in Appendix C, USB Digital Input/Output Interface.

- All necessary cables and other specialized CineLyzer System hardware items
- License key

The diagram below shows this equipment.



Software

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

Input/Output Interface

The Plexon CineLyzer USB Digital Input/Output Interface (DIO Interface) included with the CineLyzer System connects to a USB port on the host PC. It permits the system to transmit digital outputs to external devices and receive digital inputs from external devices.

For additional details about this interface, see Appendix C, USB Digital Input/ Output Interface.

Note: The DIO interface pins are rated at 5V maximum. The allowed voltage range is 0–5V. If you will be connecting external devices to the DIO Interface, ensure that appropriate voltage adaptors are in the circuits. For example, if your external device inputs and outputs are up to 28V, use adaptors that reduce the voltage to 5V or lower before the attachment to the DIO Interface. In many cases, adaptors are available from the suppliers of the original equipment.

CAUTION

Limit voltage to 0–5V at the DIO Interface pins

Do not apply more than 5V to any of the input/output pins on the DIO Interface. Doing so could damage the device.

CineLyzer Behavioral Camera Kit Supplied with Standard System

The standard CineLyzer System can be ordered with one to four camera kits of the models listed below. These cameras are used to record the movement and behaviors of the subjects within the experimental arena.

- Point Grey Research® Inc. Firefly® USB 2.0 camera, 752 x 480 pixels or 376 x 240 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 Manfrotto 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 with a dedicated power/signal cable that provides power to the board and a trigger signal from the camera to the board

1.6 Photometry Hardware, Software and License

The photometry option is enabled by additional hardware and a license available for purchase from Plexon. If your experiment includes photometry, see Chapter 8, Configuring the Photometry Parameters, for information on connecting the additional equipment and setting the parameters for photometry.

1.7 Camera Repositioning

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

1.8 Using a Standalone Computer for Data Analysis

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

Alternatively, you can transfer the files to a different computer for analysis. The standalone computer must have sufficient memory, processor and instruction set to run CineLyzer software efficiently, and the CineLyzer software and license must be present.

We recommend the Dell™ OptiPlex™ 5050 or 7050 computer with the following specifications (see below), as they have been used for our testing.

- Processor: Intel® i7-6700 (Quad core), 3.4 GHz
- RAM: 8 GB
- Hard drive: 1TB
- USB1, USB2 or USB3 port for CineLyzer license key
- Display adaptor: The processor we recommend has a built-in display adaptor Intel HD Graphics 530. If your processor does not have a built-in display adaptor, NVIDIA® GeForce® GT730, NVIDIA GeForce GT74CS or more powerful adaptor is recommended. The recommended resolution is 1920x1200.
- Windows® 7 operating system

Note: A specially configured Plexon supplied computer is required for supporting the four-camera license.

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 System

In most cases, the system software has already been installed on the computer by Plexon®.

One or more cameras (with cables and mounting kits) are provided with each system. See Section 1.5, "System Hardware, Software and Cameras" on page 10 for a description of the cameras and a list of items included in the mounting kit.

Note: The use of cameras other than those listed in Section 1.5, "System Hardware, Software and Cameras" on page 10 might not produce acceptable results in your experiments, and is not supported.

If assistance is needed, contact Plexon Support at +1 214-369-4957 or support@plexon.com.

2.2 Startup and Operational Testing

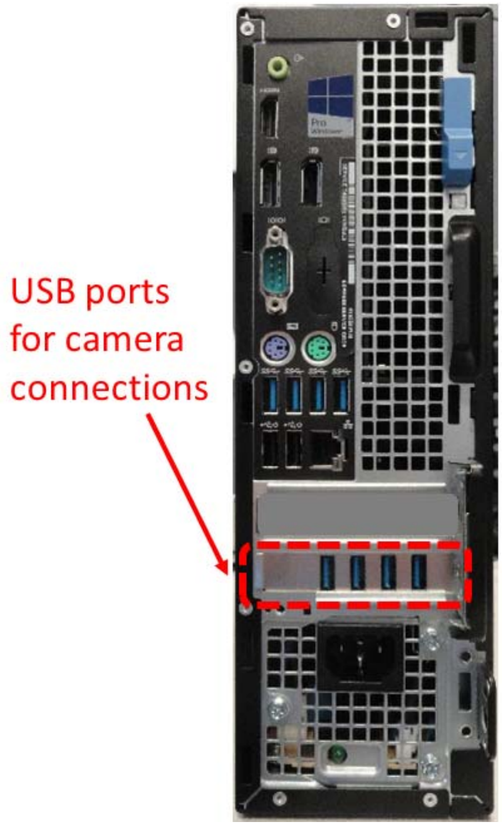
Note: If your experiment includes photometry, refer also to Chapter 8, Configuring the Photometry Parameters regarding additional equipment and settings.

Follow these steps to start the system and perform an operational test. For this operational test, it is not necessary to have the camera(s) mounted in their final location. The purpose of this test is to ensure that the computer and camera(s) are functioning correctly.

1 With the computer off (powered down), use the supplied USB cable(s) to connect each of the camera(s) to a USB port in the expansion card on the back panel of the PC. It is important to connect the camera(s) to these ports 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. If you have any questions regarding cable connections, please contact Plexon Support.

Note: You can connect and disconnect cameras in the future, but you *must* power down the computer before connecting or disconnecting cameras.



2 Power up the computer (host PC) and log in.

Note: When the PC power is turned on, each of the cameras should display a green LED indicating that power is connected. (This applies to most camera models.)

3 Plug the SafeNet® Sentinel™ license (USB key drive) into any available USB port on the PC.

4 (Optional) Plug the USB Digital Input/Output (DIO) Interface into any other available USB port on the PC.

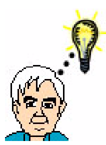
Note: The use of the DIO Interface is described in Section 2.4.6, “Input Events Tab” on page 27.

5 Start the CineLyzer program. The easiest way is to double-click the **CineLyzer** desktop icon.

6 When the CineLyzer® user interface opens. select **Cameras** mode (from the dropdown menu shown below), if not already selected. Video should now be displayed on the Video 1 window.

Note: The system automatically activates all available cameras when you start the CineLyzer application and were last in **Cameras** mode. It also activates all available cameras whenever you switch from **Files** mode to **Cameras** mode.

7 Verify that Camera 1 is active and that the Camera 1 video is displayed in the Video 1 window.



TIP

Check that your camera(s) have been activated

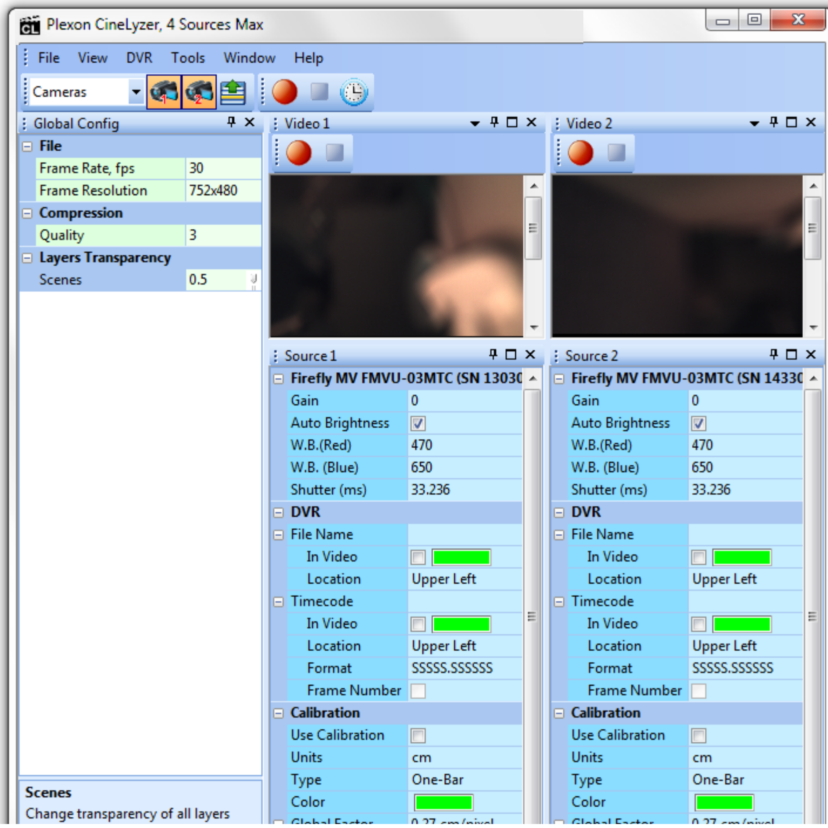
The icon for a camera turns orange if the camera is active. In the example below, Camera 1 is active and Camera 2 is inactive:

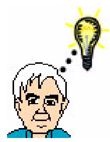
If the icon for Camera 1 is not already orange (active), do the following:

a Click on the icon to activate Camera 1.

b In the **Window** dropdown menu, select **Layout**, then select **Reset to Default Layout**. All normal windows for all of the connected and activated cameras will be displayed.

It is acceptable if multiple cameras are active. For example, in the image below, Camera 1 and Camera 2 are both active.





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 list in the main menu.

8 If the CineLyzer user interface displays video, as shown in the example in Step 7 (and Camera 1 is active), skip to Step 10. Otherwise, perform Step 9.

9 If there is no video displayed in the Video window, it probably means that one of the following problems has occurred:

- The camera icon is inactive because it has not been selected.
- The USB cable is not connected between the host PC and the camera.
- The camera is not functioning correctly.

Take the necessary action:

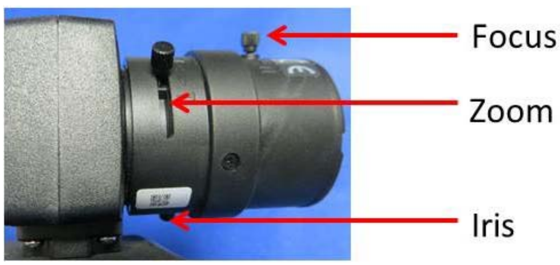
- If the **Camera 1** icon background (to the right of the **Cameras** dropdown) is *not* orange, it is not selected. Click on this icon now to select it. Ensure that the background changes to orange.

- If the USB cable is not connected between the PC and the camera, or a connection is loose, close the CineLyzer application, shut down the PC and perform the steps in this section starting from Step 1 again.

- A USB cable could be defective, or a USB port on the PC might not be working correctly. Close the CineLyzer application, shut down the PC and try using a different USB port or a different USB cable (a cable that was shipped with another camera of the same model for example).
  Then perform the steps in this section starting from Step 1 again.

- Check the manual adjustments on the body of the lens. The iris on the camera could be shut, the zoom on the camera could be set improperly, or the focus could be set so that everything is a blur.

- If the image is displayed in the Video window but it cannot be focused by adjusting the focus ring on the camera, the camera as delivered might have a CS-to-C mounting adaptor ring around the aperture. If so, remove this adaptor ring and reattach the lens. (The lens is a CS mount, and will not be able to focus if the adaptor is left in place.)



- If additional troubleshooting is necessary, see Appendix G, Troubleshooting.

10 If proper video is observed at the monitor, it is reasonable to assume the camera, computer, and monitor are working correctly.

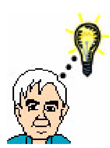
2.3 Setting Up Cameras and the User Interface

Follow this procedure to position the cameras and verify video quality.

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. For example, if two cameras are installed, the toolbar will display two camera icons, as shown in the image below. (Notice that Camera 1 is active (selected) and Camera 2 is inactive in this example.)

Note: If the **Cameras** option is not present in the menu, it means that the system has not detected any attached cameras. If this occurs, turn off (power down) the computers, then verify that each of the cameras is 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.

Note: If there are two or more cameras, the system will automatically assign the camera with the lowest serial number to be Camera 1, the camera with the second-lowest serial number to be Camera 2, and so forth. If there is only one camera, it is Camera 1**.**



TIP

Consider calibration requirements

Plan the mounting of cameras with calibration in mind. Calibration of linear dimensions (in inches or centimeters) works most accurately when the camera is orthogonal to the arena.

Each of the arenas (that is, the arenas for Cameras 1, 2, 3 and/or 4) can be calibrated independently.

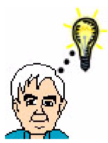
The calibration procedure is explained in Chapter 4, Calibrating the Arena Dimensions.

2 Select the icon(s) for the camera(s) to be used in the experiment (up to four cameras). A camera is active only if the corresponding icon is selected. The orange color in the icon indicates a camera is selected.

In the image in Step 1 above, the orange color on the **Camera 1** icon shows that Camera 1 is active, and it can also be seen that **Camera 2** is inactive. Clicking on the **Camera 2** icon will make it active also, as shown in the image below.

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

4 Set up one of the cameras as described in Appendix A. It is typical to set up Camera 1 first and mount it orthogonally to the arena.



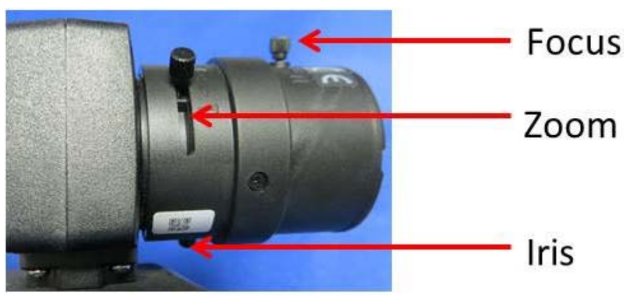
TIP

Ensure that cameras are 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 Camera Positioning for the Experiment explains how to compute the distance from the camera to the arena so that the whole arena is visible.

5 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).

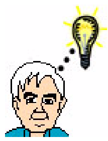


6 If any attached camera experiences either of the following problems, take the action recommended here:

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

b If there is a Source window corresponding to the camera, but no video is displayed there, select the camera icon in the toolbar to make the camera active. (For example, see the camera icons in the image below. In that image, the icon for **Camera 2** has not been selected, and therefore **Camera 2** is not yet active.)

c If there is still no video, go back to Step 9 on page 17 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.

7 If the previous steps do not result in a good image on the camera, change values of the camera parameters in the 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 G, Troubleshooting.

8 Repeat Step 1 through Step 7 for each additional camera that will be used in the experiment.)

9 Optional—Calibrate the dimensions in the video field for each camera. (Calibration works most accurately when the camera is positioned orthogonally to the arena.)

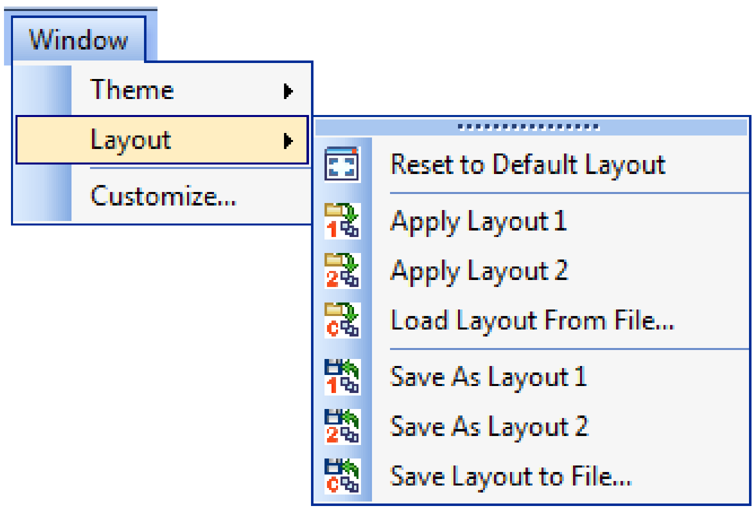
Note: In many experiments, it is useful to calibrate the video image dimensions so that the ratio of centimeters to pixels (or inches to pixels) is known. For the procedure, see Chapter 4, Calibrating the Arena Dimensions.

2.4 Navigating the CineLyzer User Interface

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

2.4.1 Arranging CineLyzer Windows

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**.



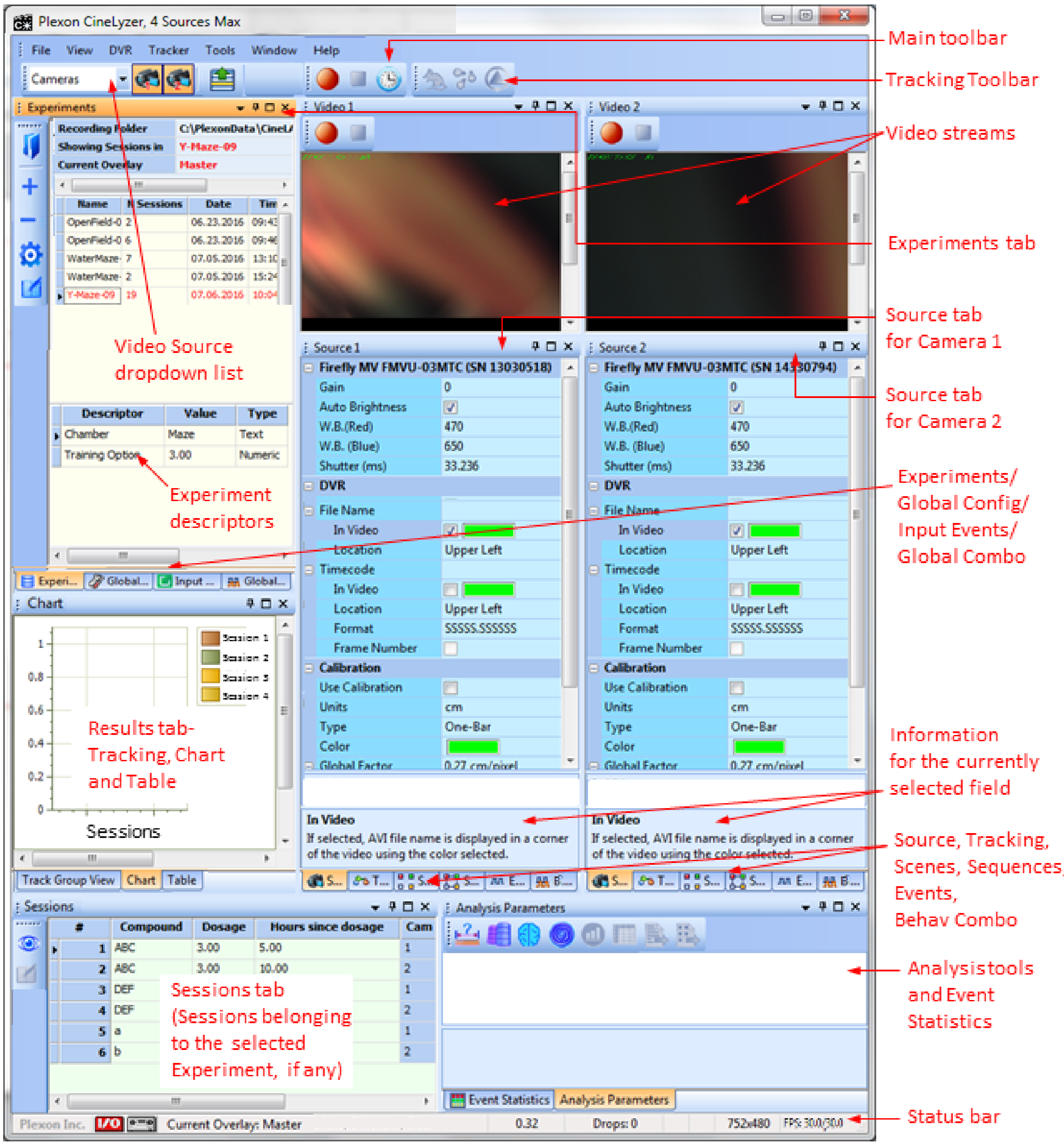
To save an existing layout select **Save Layout to File...** and follow the prompts.

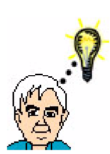
To load a previously saved layout select **Load Layout from File...** and follow the prompts.

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

2.4.2 Identifying the Areas of the User Interface

This image shows the location of the windows in the default layout of the CineLyzer user interface. In this example, there are two cameras connected; up to four cameras can be licensed and connected (optional).





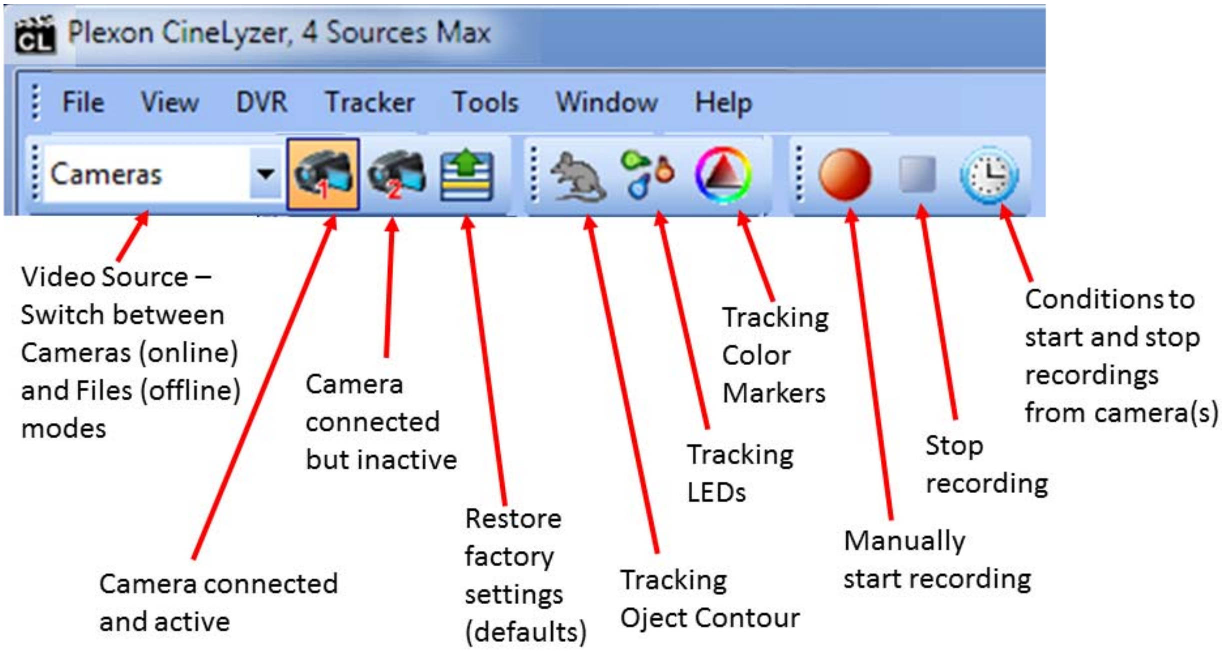
TIP

Verify “Cameras” Is Selected

The parameters for the camera optics do not display in the user interface unless **Cameras** is set in the main toolbar.

2.4.3 Main Toolbar Icons in Cameras Mode

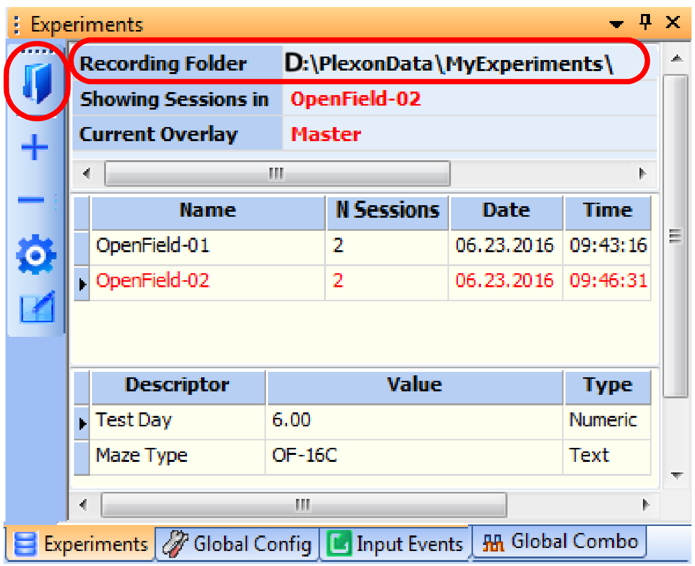
The image below shows a close-up view of the CineLyzer main toolbar. In this example, there are two cameras connected and one of them is active.



2.4.4 Experiments Tab

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.

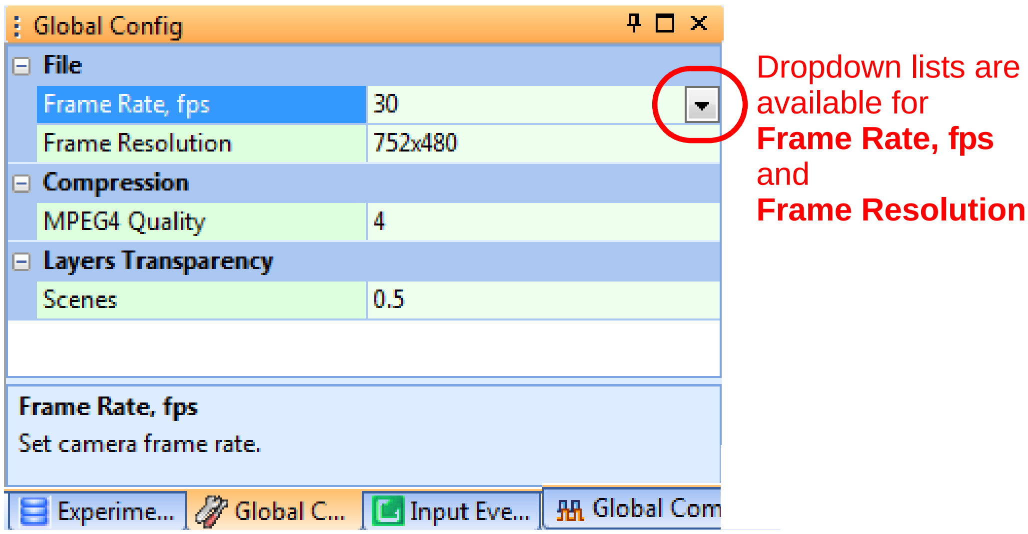
Note: If your PC does not have a D: drive, the **Recording Folder** location defaults to C:\PlexonData.



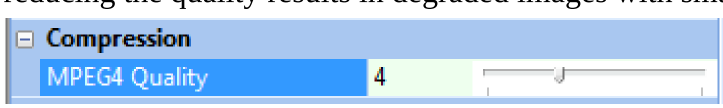
The various features and procedures related to this tab are provided in more detail in Chapter 3, Preparing Your Experiment Database.

2.4.5 Global Configuration Tab

This tab (see the image below) contains parameter values that pertain to the frame rate to use when recording, image resolution (quality of recordings to make), compression bit rate (quality), and image transparency.

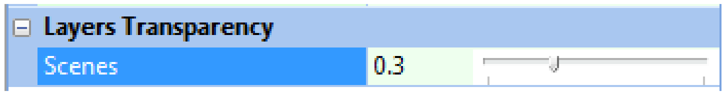


- **Frame Rate, fps**—Frame rate (frames per second), selectable as 10, 15, 20, 30, 40, 50 or 60 fps from the dropdown list. Once you have recorded sessions in an experiment, this value cannot be changed for that experiment.
- **Frame Resolution**—Selectable as 752x480 or 376x240 from the dropdown list. Once you have recorded sessions in an experiment, this value cannot be changed for that experiment.
- **Compression - MPEG4 Quality**—Adjusts the image quality in the recorded MPEG AVI file between 1 and 10 (bitrate/500,000). The default value (4) is suitable for most typical experiments, but you can use the slider to find the lowest setting (from 1 to 10) that is acceptable for your application. Note that increasing the quality results in better video images but larger file sizes; reducing the quality results in degraded images with smaller file sizes.



Note: If you have the Photometry System, please note the following:
The **MPEG4 Quality** parameter is applicable only to the behavioral camera, not the photometry device. When you change compression quality, it affects only videos recorded from the behavioral camera. The videos recorded from the photometry device are preset to a high compression quality. If you have the Photometry System, see Chapter 8, Configuring the Photometry Parameters.

- **Layers Transparency - Scenes**—Adjusts the transparency (from 0.0 to 0.9) of all layers displayed in the video window, for example, arena, zones, tracking visualizations, etc. The default value is 0.5; you can use the slider to change the transparency from the lowest value (0.0) to the highest (0.9).



Note: If you activate the Tracking function, the **Global Config** tab displays additional parameters. For those details, see Section 6.7, “Setting Parameters In the Global Config Tab” on page 93.

2.4.6 Input Events Tab

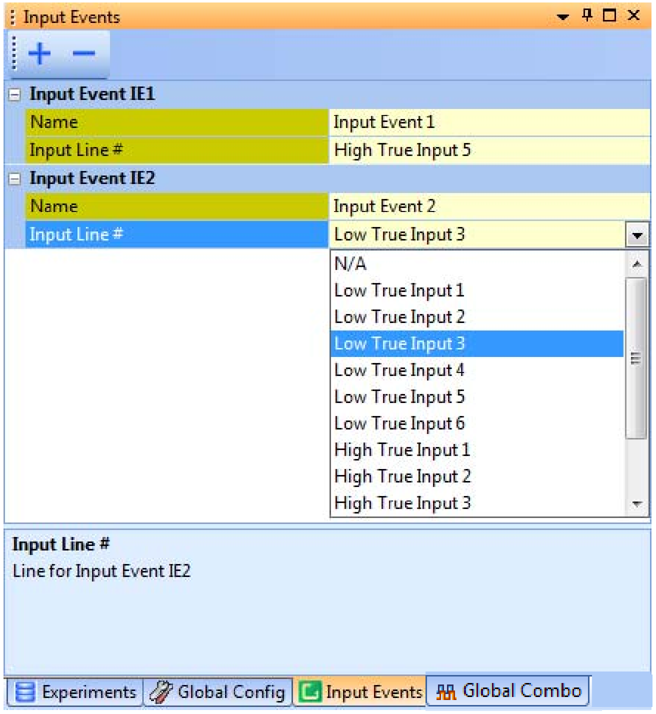
The parameters configured in the Input Events tab allow the system to receive an input event (trigger) from an external source and use that trigger to start/stop a recording session. The system receives the event through the CineLyzer USB Digital Input/Output (DIO) Interface.

Note: As an example of using an input event, you can connect a cable from the Plexon PlexBright® 4 Channel Controller (PlexBright Controller) to the CineLyzer System Digital Input/Output (DIO) Interface and configure the CineLyzer System to start video recording when a stimulation pattern begins playing on the PlexBright controller.

The cable and PlexBright Controller are sold separately.
For details, see these sections:

- Section 11.5, “Transmitting PlexBright Output Signals to the CineLyzer System” on page 317
- “Connecting CineLyzer System to Optogenetic Stimulation Device” on page C-9
- Appendix C, USB Digital Input/Output Interface

The Input Events tab and DIO Interface are shown below.

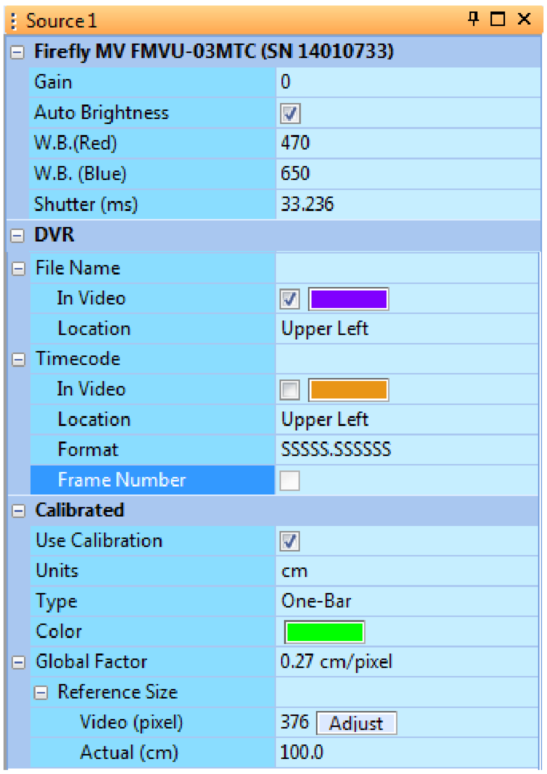


The **Input Line #** parameter in the Input Events dialog box allows you to specify an input line on the DIO Interface. To configure the input lines see Section 5.6, “Configuring the Input Events Parameters” on page 74.

For additional details about the DIO Interface, see Appendix C, USB Digital Input/Output Interface.

2.4.7 Source Tab

This tab is used to set parameters for the video on each camera or video file. There will be multiple Source tabs if there are multiple cameras on the system. This tab contains parameters that relate to the optics and video displays for each camera (such as gain, brightness, white balance and shutter speed), labeling/ timestamping of frames and calibration.



2.5 Where to Go Next

Go to Chapter 3, Preparing Your Experiment Database.

Chapter 3
Preparing Your Experiment Database

3.1 Data Storage and Organization

The general plan for data storage and organization in the CineLyzer® System is as follows:

- 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 camera in each session
    - Extracted and computed data for the experiment

3.2 Planning the Database

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

1. Select a disk with sufficient space for all contemplated recordings.

   For reference, recordings require approximately 3MB of disk space per minute of recording at 30 frames per second (fps). Therefore, if you expect ultimately to conduct 10 experiments of 200 sessions each at 10 minutes per session and 30fps, your selected database disk will need to be capable of storing 60,000MB (60GB) of data. Note that the database can be split over multiple disks if necessary.
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 3.3, “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 3.4, “Setting the Recording Folder Location” on page 36, for the procedure.

3.3 Setting Parameters for an Experiment with Multiple Sessions

Note: If your experiment includes photometry, set the parameters for photometry as described in Chapter 8, Configuring the Photometry Parameters.

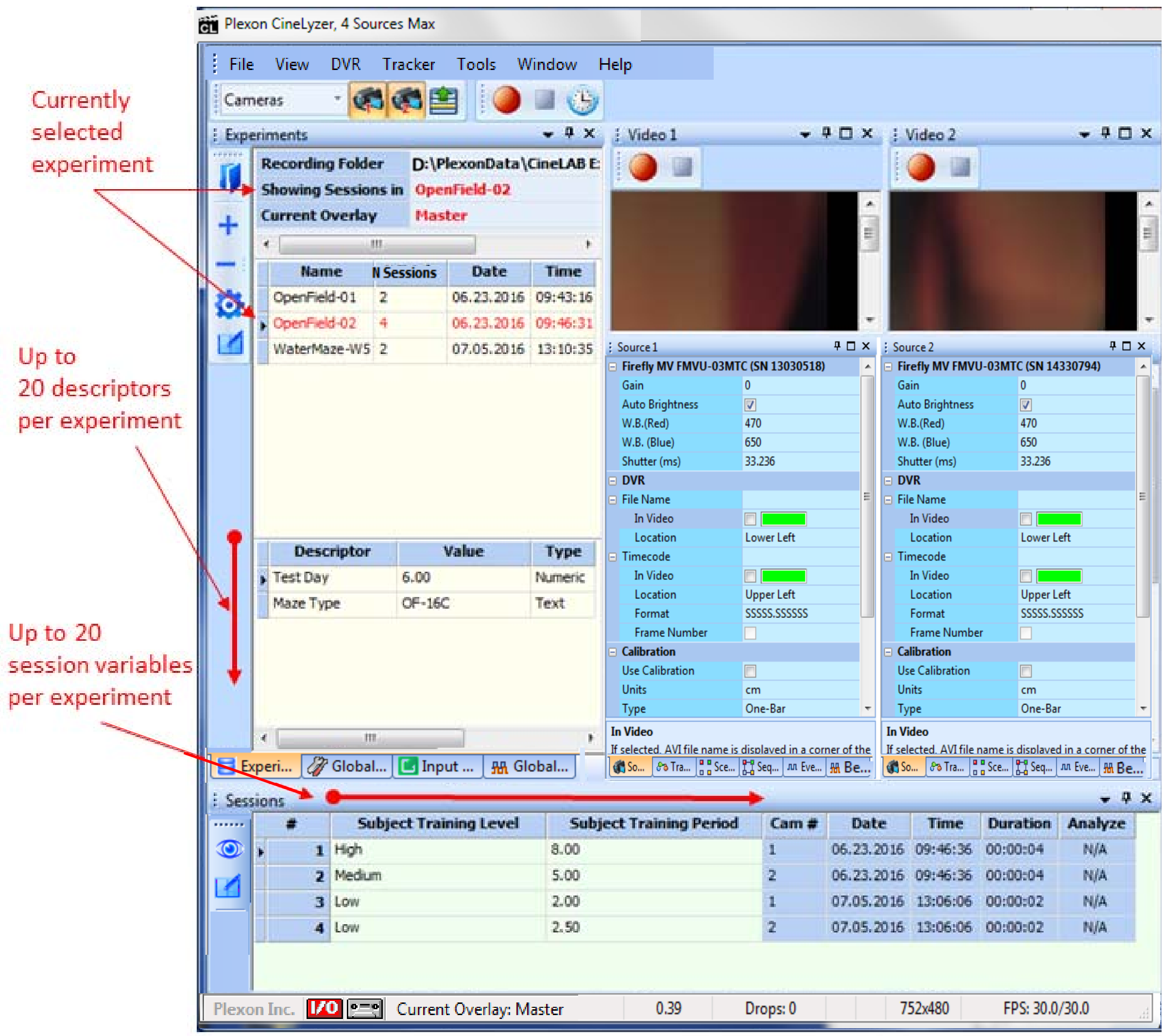
The following diagram shows an example of the setup for an experiment with multiple sessions.

TIP

Reset to Default Layout

It is often helpful to reset the 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**.

Note: You must create or select the **Recording Folder** and create or select an experiment (in the **Experiments** tab) to enable all other CineLyzer functionality.



The CineLyzer 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 9, Recording and Monitoring Video.

You can select from among the session variables to group and filter session results when you perform your analysis with the CineLyzer tools.

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 also keeps a record of behavioral events and facilitates analysis of data from the sessions.

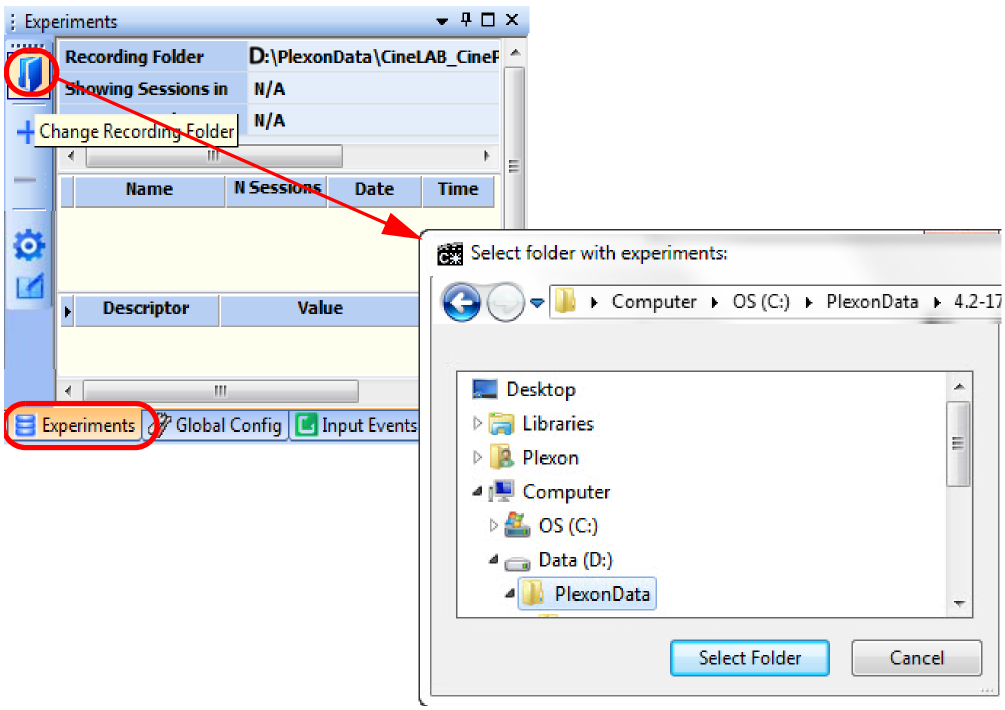
TIP
Use CineLyzer session variables to streamline data analysis

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

3.4 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 CineLyzer 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.

3.5 Creating a New Experiment

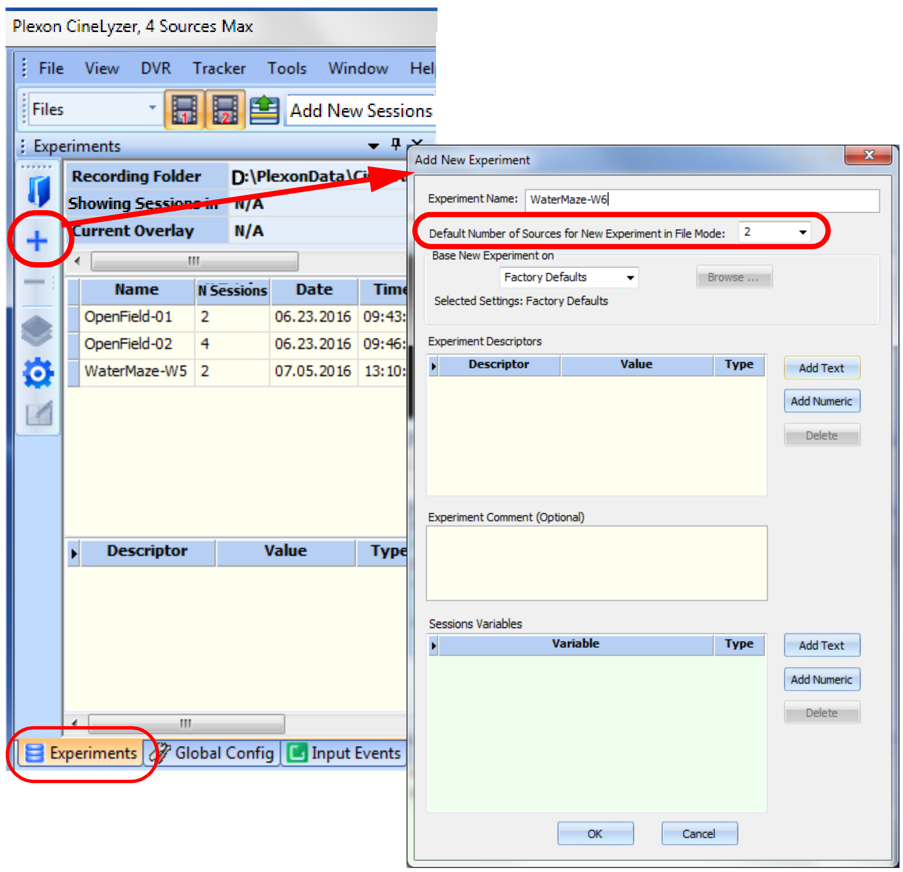
This section explains how to create a new Experiment and add sessions to the experiment, which you can do in either mode (**Cameras** mode or **Files** mode).

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 32.

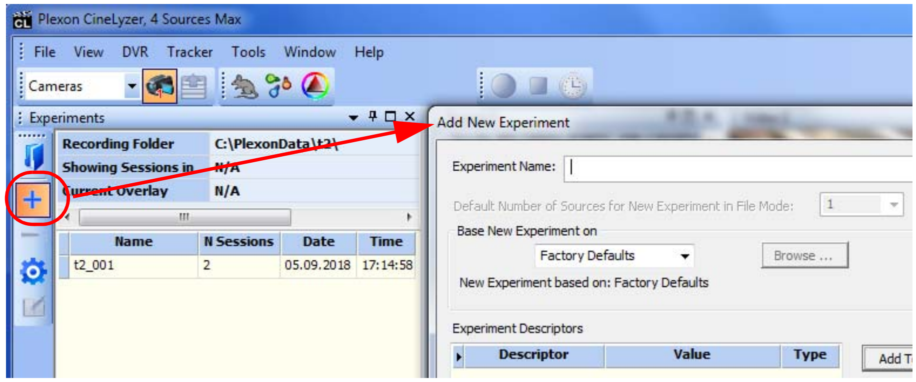
Note: If you prefer to add sessions to an existing experiment, see Section 3.6, “Selecting a Previously Saved Experiment” on page 45.

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

In **Files** mode, the dialog looks like this. The system queries you to specify the default number of sources to activate for any new experiment you create in **Files** mode.

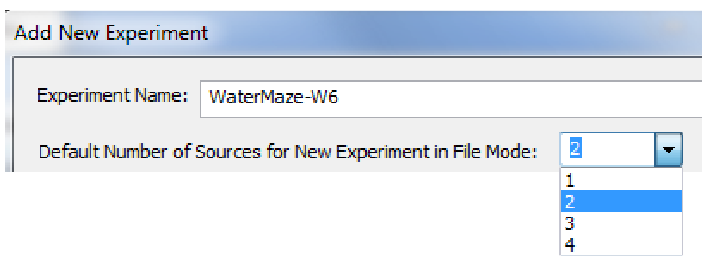


In **Cameras** mode, the dialog looks like this. The system does not query you for the number of sources, because the default number of sources is automatically set equal to the number of cameras currently available in the system and licensed.

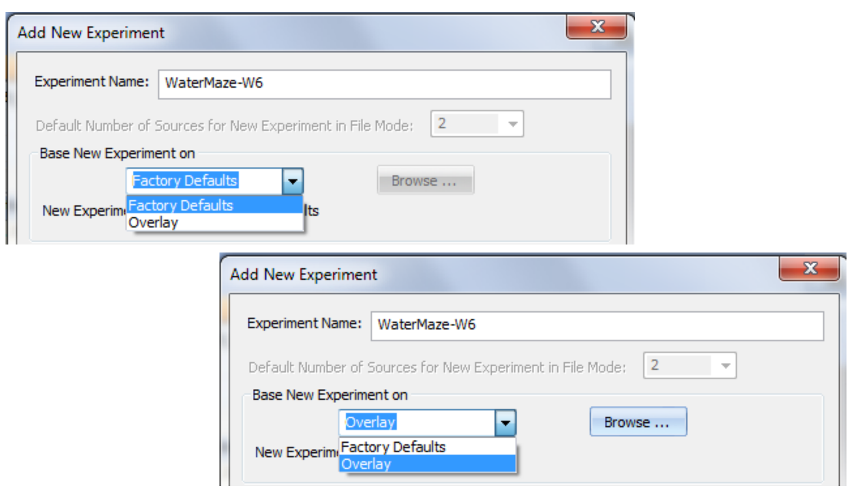


2 Enter a name for the experiment in the top line of the dialog box.

3 In **Files** mode, you can select the desired default number of sources (from 1 to 4) from the dropdown list. (In **Cameras** mode, the default number of video stream sources is equal to the number of cameras that are connected and turned on.)



4 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.)



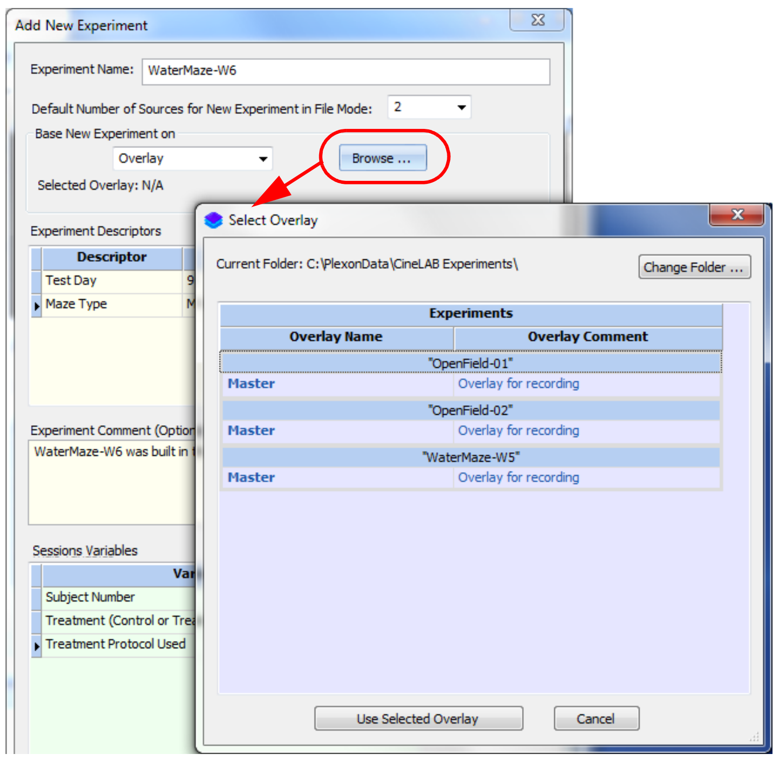
Understanding defaults and overlays

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.

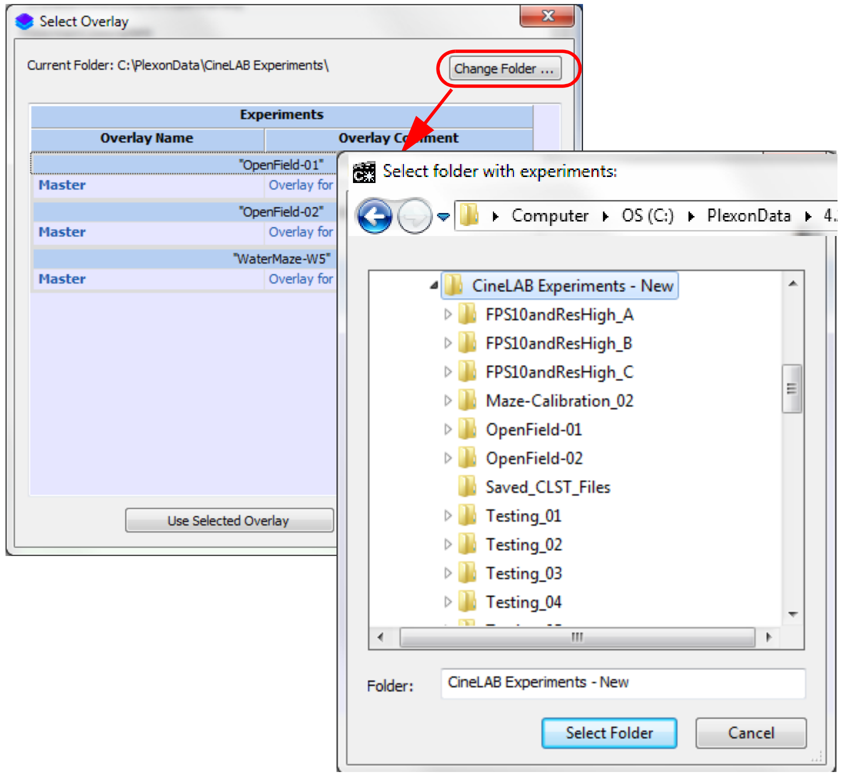
If you select the **Overlay** option, you will be able to apply settings that were created for a previous experiment, that is, to *overlay* those settings on this new experiment.

Selecting an Overlay

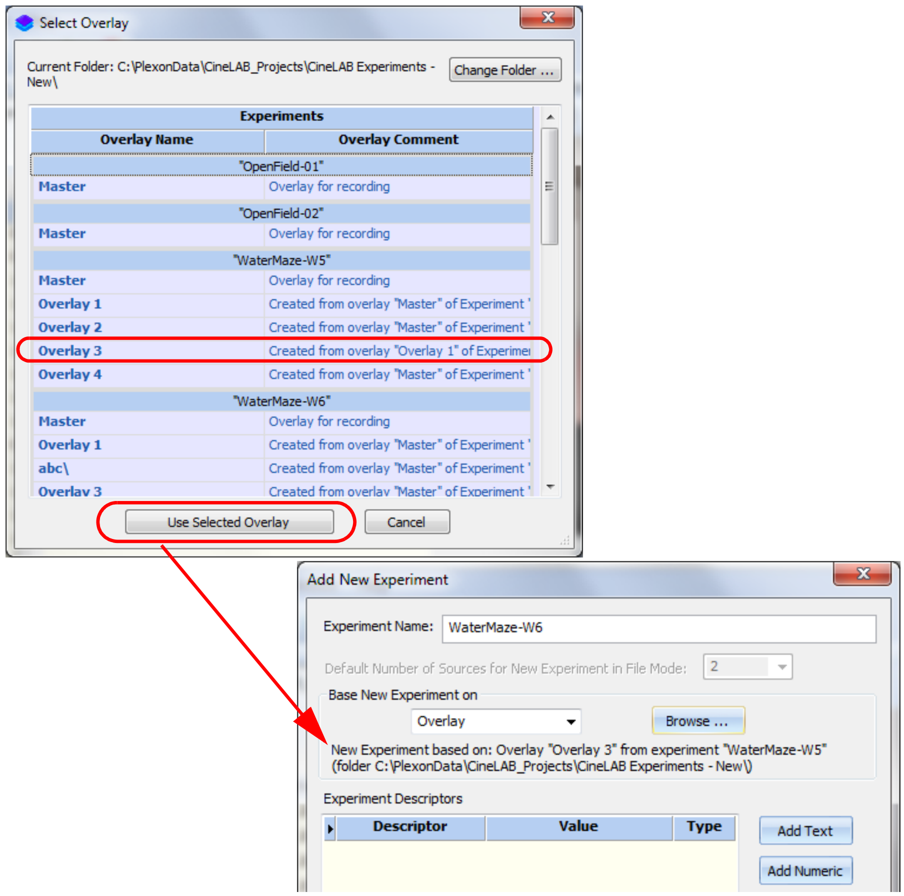
5 If you select **Overlay**, the **Browse** button becomes active; click on this button to open the **Select Overlay** dialog box.



6 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.

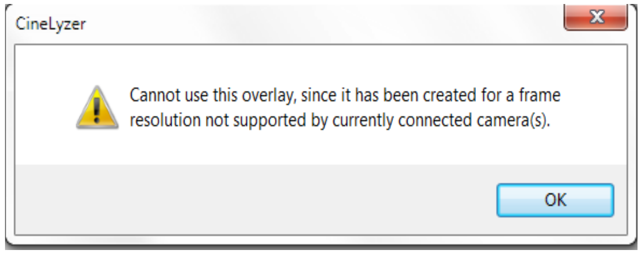


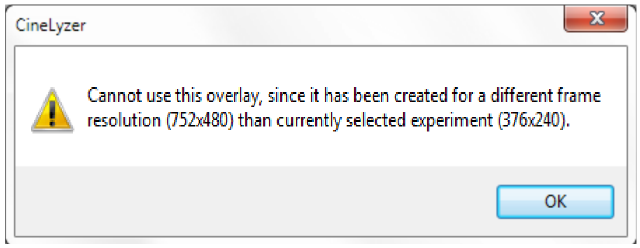
7 Click on an overlay (in this example, click on Overlay 3 from the experiment WaterMaze-W5) and then click the **Use Selected Overlay** button. This action will load the geometry and parameter values from the selected overlay (Overlay 3 of WaterMaze-W5) onto the new experiment (WaterMaze-W6). Notice 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.

Note: In **Cameras** mode, the system does not allow you to select an overlay containing an incompatible frame resolution, that is, a resolution that is not supported by your connected cameras, or an overlay created with cameras set to a different frame rate.



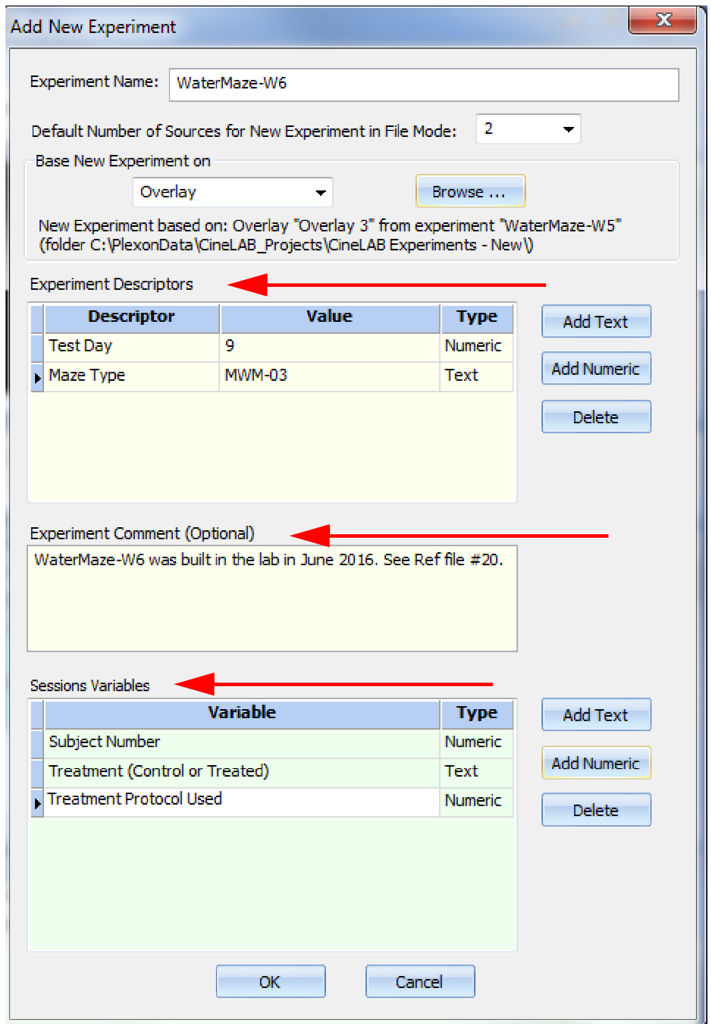


8 If desired, you can modify the parameters in the **Source** tab, such as the camera settings and the **DVR** and **Calibration** settings. In the other tabs, you can modify arena geometries and other settings. The system automatically saves these settings changes as they are made.

Add descriptors and variables

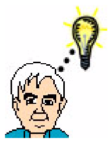
9 Create **Descriptors** for the experiment and **Variables** for the sessions. (See the example below.) You can create up to 20 **Descriptors** for the experiment and 20 **Variables** for the sessions.

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, 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 video stream. You can modify session variable values later as described elsewhere in this section.

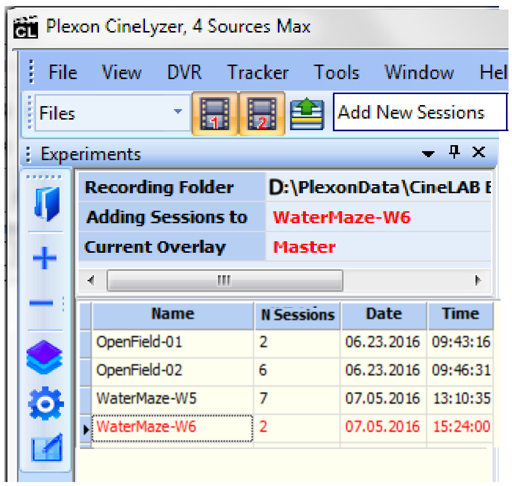


TIP

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.

10 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 3.6, “Selecting a Previously Saved Experiment” on page 45,
- Section 3.7, “Editing the Experiment Name, Descriptor and Variable Values” on page 46
- Section 3.8, “Adding and Editing Comments in Experiments and Sessions” on page 49.

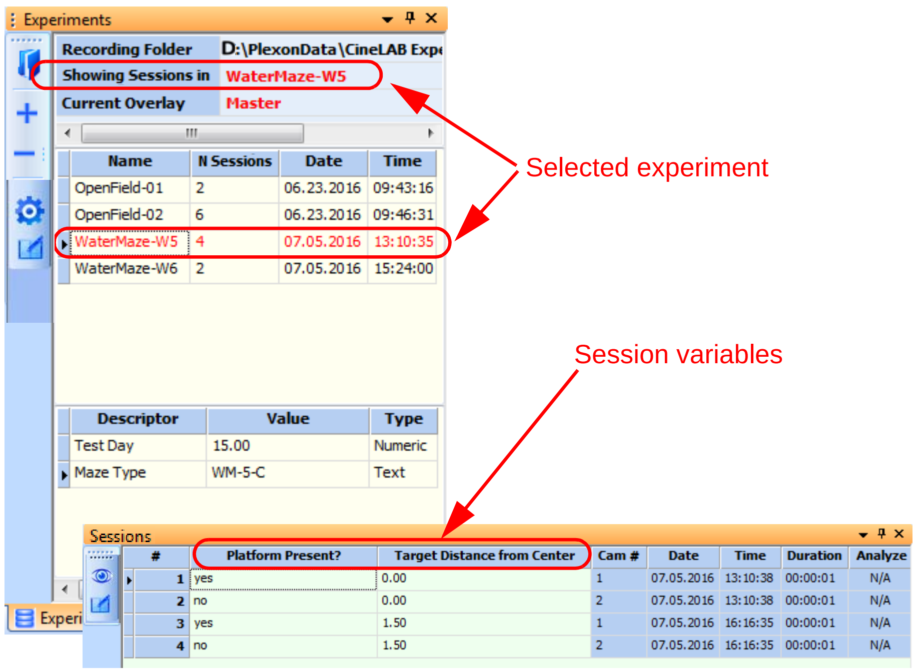
3.6 Selecting a Previously Saved Experiment

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

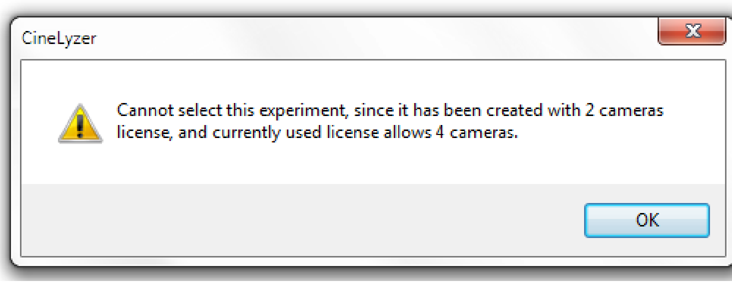
2 In the **Experiments** tab, click on a row to select a specific experiment.

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 session variables for this experiment are displayed also.



Note: You cannot load an experiment that was created under a license valid for a different number of cameras than the license currently plugged into the PC. The system will provide a dialog similar to this:



Display of video streams for existing experiments

In **Cameras** mode, the system displays the video stream from each of the active cameras. In **Files** mode, the system changes the layout automatically to the default layout specific to the number of cameras that were used to record sessions in the selected experiment.

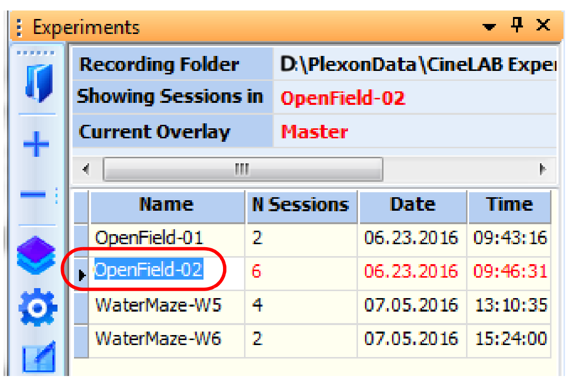
3.7 Editing the Experiment Name, Descriptor and Variable Values

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 CineLyzer interface, not in the Windows 7 interface. (Do *not* change an experiment name by means of the Windows file-naming function, because the CineLyzer System will not be able to locate that experiment or file automatically in the future.)

In the CineLyzer GUI, 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 7 environment. Also note that

the CineLyzer GUI does not allow certain characters in an experiment name. If you enter such a character, it will not be displayed.

CAUTION

Do not change Experiment names outside of the CineLyzer interface

If you want to change the name of an experiment, do *not* change the name in the Windows 7 environment by changing the folder name for the experiment. The CineLyzer System will not be able to locate the file automatically. Instead, make the change by double clicking the name of the experiment in the CineLyzer **Experiments** tab (as shown above) and typing in the new name.

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, 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.

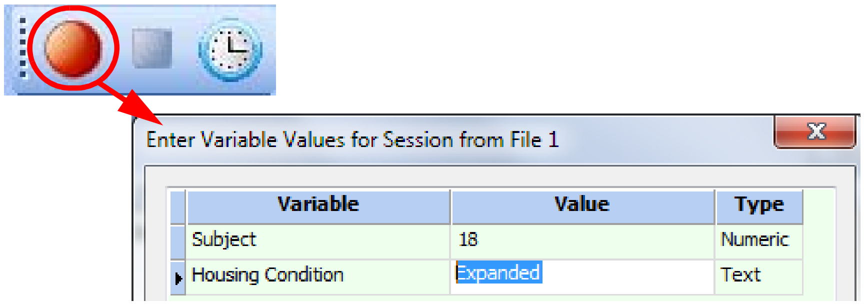
Sessions

#	Subject Number	Treatment (Control or Treated)	Treatment Protocol Used	Cam #	Date
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

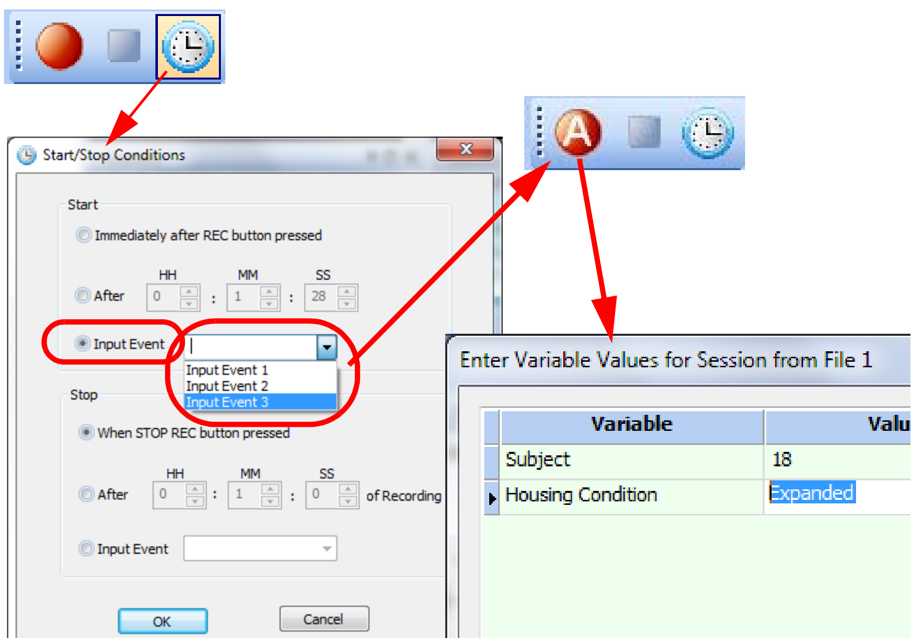
When a new recording (session) is about to start, the system prompts you to enter values for each session variable for each video stream. The prompt is displayed when the starting condition occurs. See the examples below.

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



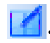
Input Event triggered recording—when the Arm button is pressed for the first session

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

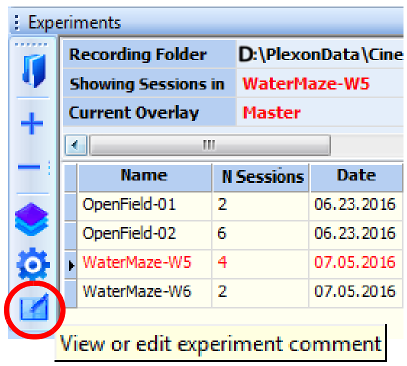


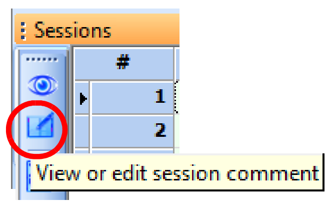
For more information on timed and Event triggered starting of sessions, see Chapter 9, Recording and Monitoring Video.

3.8 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.

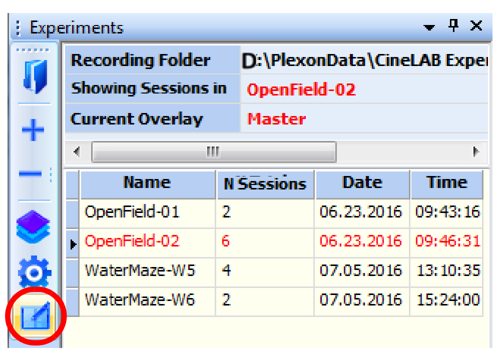
Note: The specific set of icons displayed in the sidebar depend on which CineLyzer license is installed and also whether you have selected Cameras mode or Files mode.



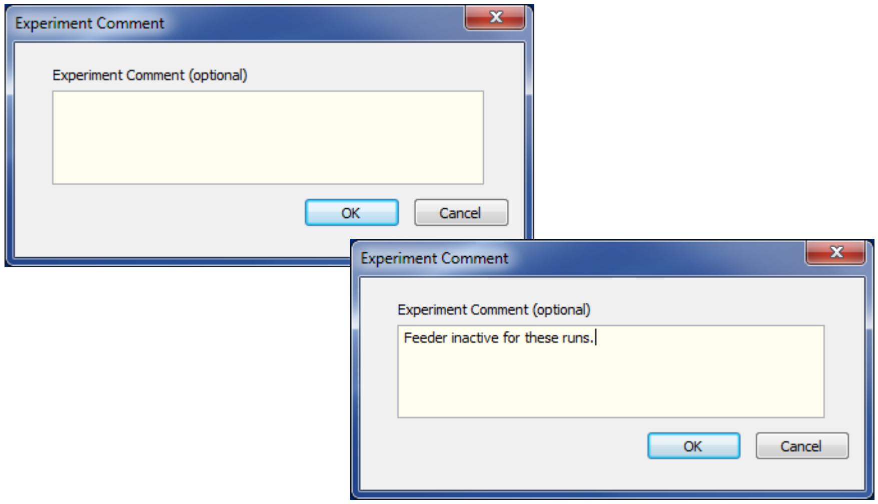


Note: If you are viewing an experiment that was created with a *previous version* of the CineLyzer software, the Comment field might not have been present in the file. the **View or edit experiment comment** icon for this experiment is disabled (greyed out). If the previous version allowed multiple comment fields, only the first Comment field is preserved.

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

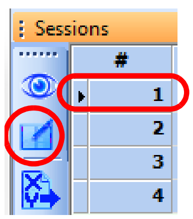


The system displays the **Experiment Comment** dialog box. The comment field might be empty (if no comment has been added yet) or it might contain previous comments.

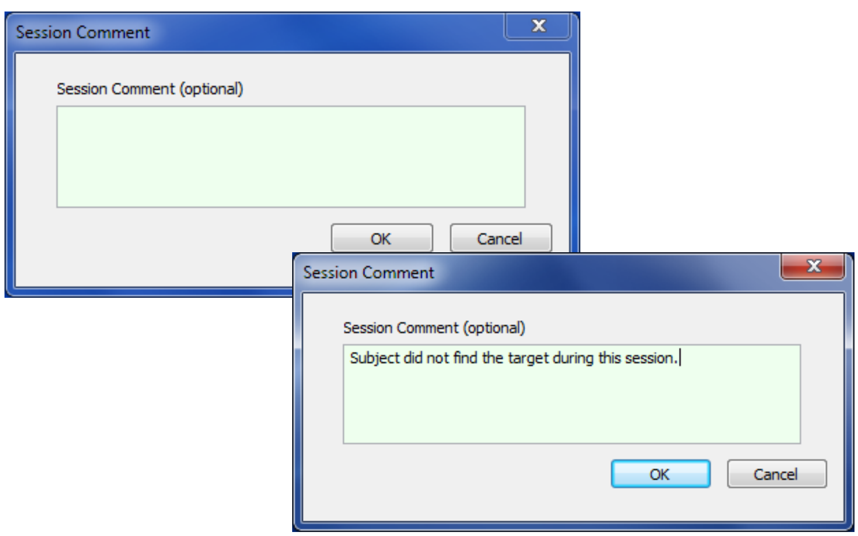


2 Add comments as needed and click **OK**.

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**.

3.9 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 run with consistent parameters. The disabled parameter settings include:

- **Frame Rate (fps)** and **Frame Resolution** parameters in the **Global Config** tab
- 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)

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.

3.10 Where to Go Next

Go to Chapter 4, Calibrating the Arena Dimensions if you want to calibrate the physical dimensions of the arenas in centimeters or inches. If you do not calibrate an arena, the system reports physical dimensions in pixels.

To configure the basic video recording parameters, go to Chapter 5, Configuring the Recording Parameters.

Chapter 4
Calibrating the Arena Dimensions

4.1 Before You Start

Verify that you have completed the procedures in Chapter 3, Preparing Your Experiment Database.

4.2 Preparing for Calibration

In many experiments, it is useful to calibrate the video images from the cameras 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. The calibration procedure can be repeated for any or all active cameras, and in general, you should calibrate all cameras.

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

Setting Up the Camera

The camera should be set up according to the instructions in Section 2.3, “Setting Up Cameras and the User Interface” on page 19.

When to Perform the Calibration Procedure

For any experiment, you can perform the calibration procedure for each of the cameras at any time, even between sessions. The system will apply the new calibration settings to all future sessions. This feature is useful if a 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).

4.3 Calibration Procedure

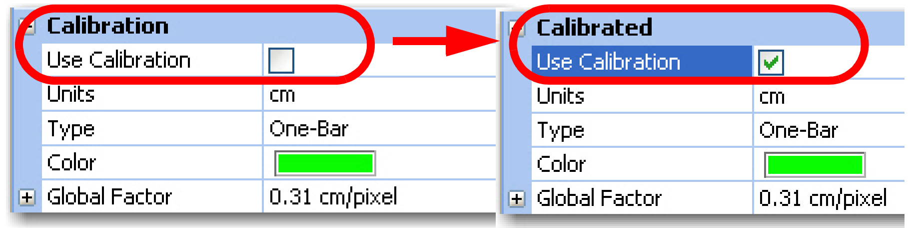
Follow these steps to calibrate the dimensions in the arena. You can set the values for the calibration of any one of the cameras in the system, then copy those values to the other cameras, as explained at the end of this procedure.

Selecting Calibration Units and Type

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

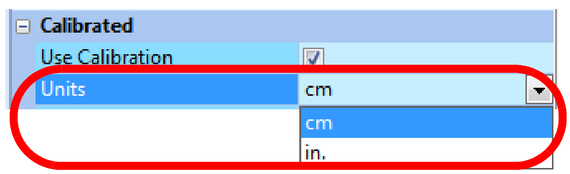
1. Open up the **Calibration** section (if it is not already open) by clicking on the “**+**” sign.

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.



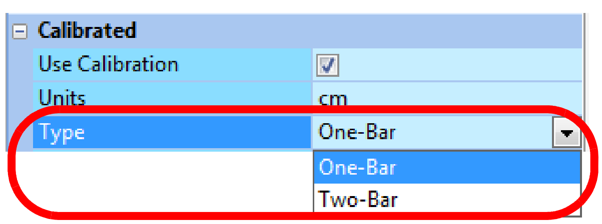
Note: The calibration parameters are applied only if the **Use Calibration** checkbox is checked. If the box is unchecked, the system ignores the calibration parameters.

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**.

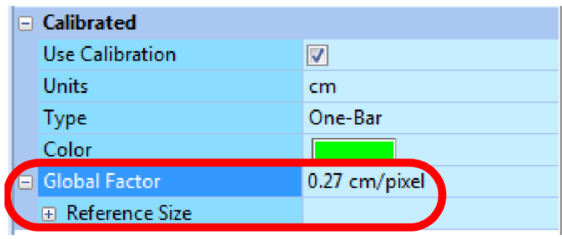
Note: There are two methods of calibration—Single axis (also called One-Bar) and dual axis (also called Two-Bar). Select the method that is consistent with the geometry of your arena.

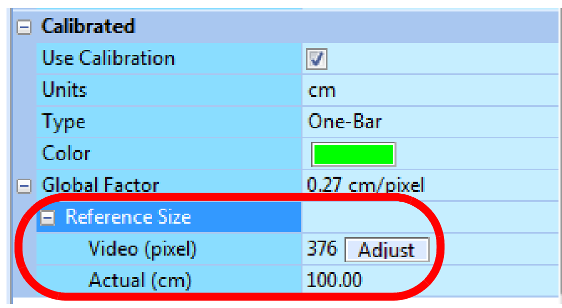


Setting Calibration Parameters with One-Bar Method

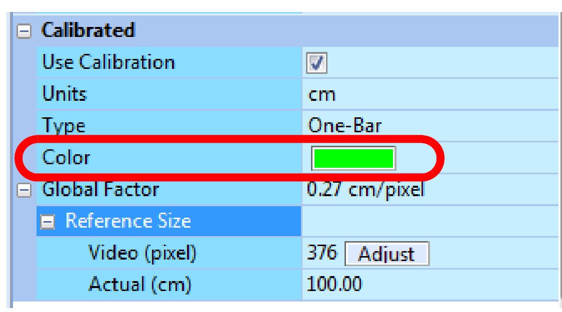
5 When **One-Bar** has been selected:

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.





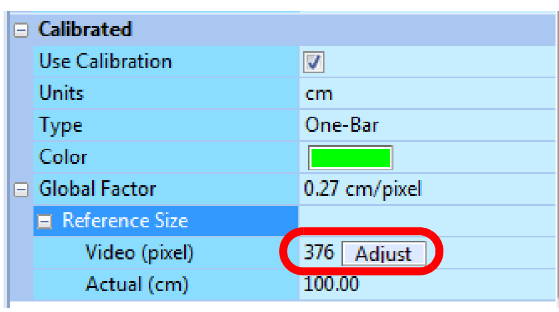
b If the current color in the box next to the **Color** item will not provide good contrast with the video image, click in the box and select a suitable color. The color selected is the color of the measurement bar used for calibration.



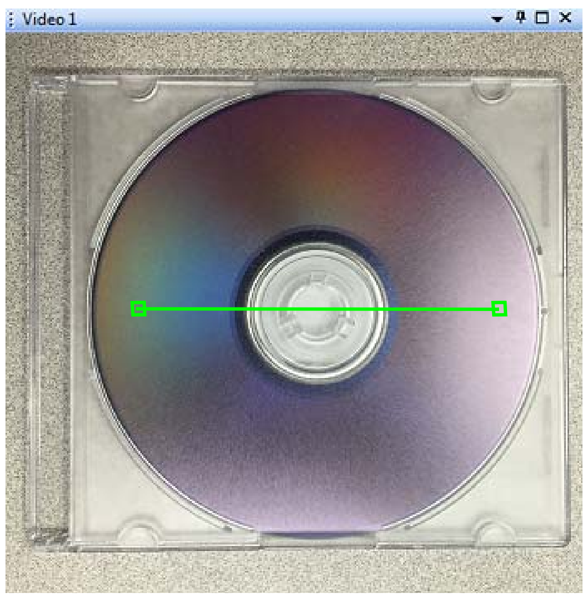
c Choose a feature in the experimental area whose video image extends over much of the field of view. As an illustration, the outside diameter of the disk in the image below will be used as the desired dimension.

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.

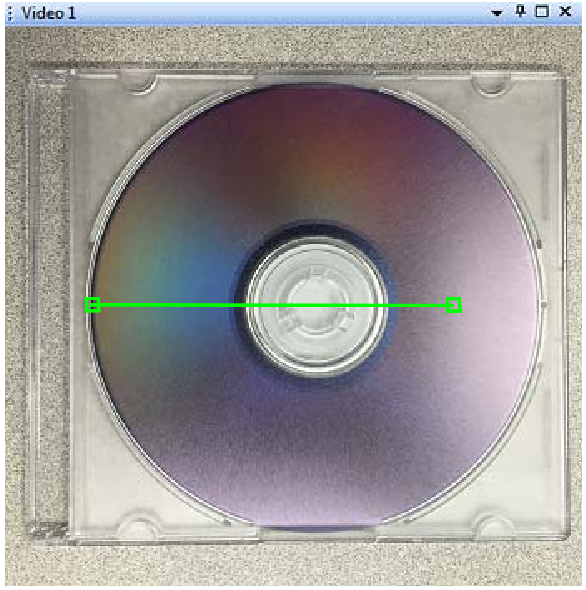
e Click the **Adjust** button. The cursor will go to the video image and a line will appear in the color selected. Select one end of the colored line and move it to one end of the feature just measured. Select the other end of the line and move it to the other end of the feature.



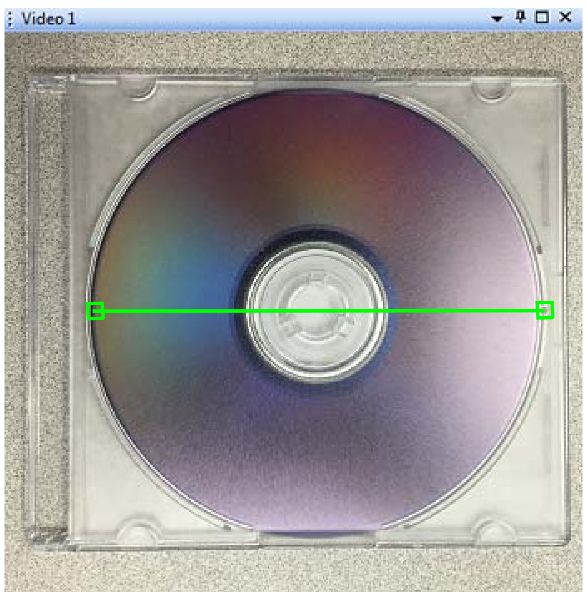
In the image above note the green line with the box on each end. This is the sizing bar. Move the sizing bar vertically to the desired location, in this case, the center of the disk.



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



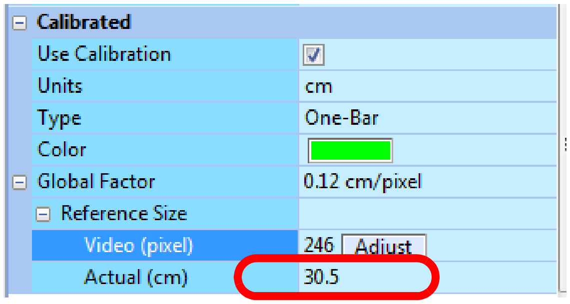
Select the right end of the sizing bar and move it to the opposite edge of the disk and ensure the sizing bar crosses the center of the disk.



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).



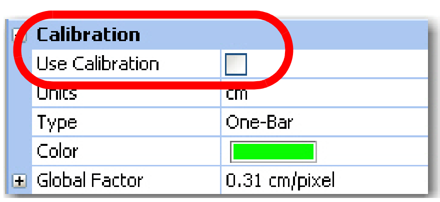
In the image above note that the **Actual** setting has been changed to the measured diameter of the disk (30.5 cm) and the system displays the new Global Factor; in this example, it is 0.12 cm/pixel, which is 30.5 / 246.

The system is now calibrated to the measured distance.

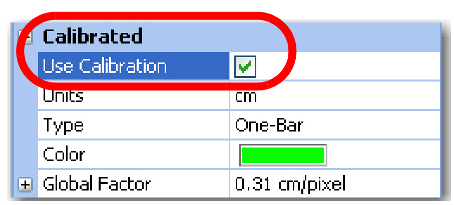
h Click all three "**-**" signs once the adjustment is satisfactory if you want to hide the adjustments.

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.

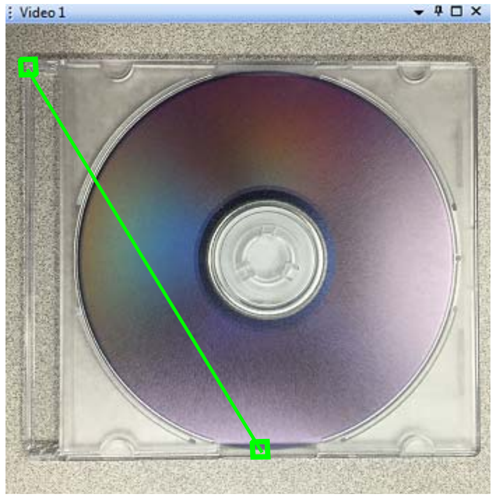
Use Calibration unchecked
when recording started -
Extracted coordinates will be in pixels



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



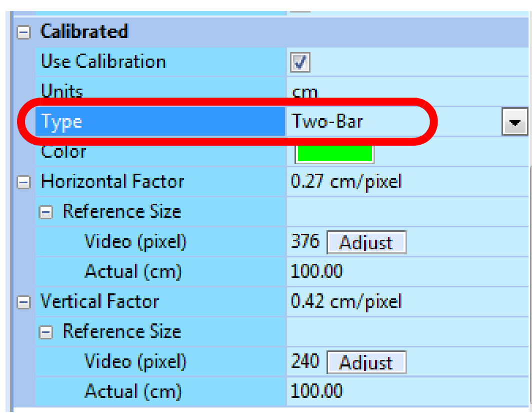
The example above assumes that the known (or most convenient) dimension on the object was oriented in the horizontal direction. In some experiments, the most convenient dimension might be in some other direction. The system allows you to orient your calibration line in any direction. For example, you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

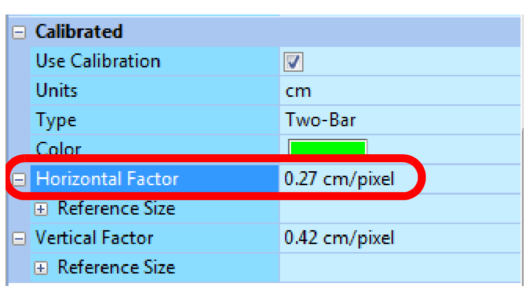


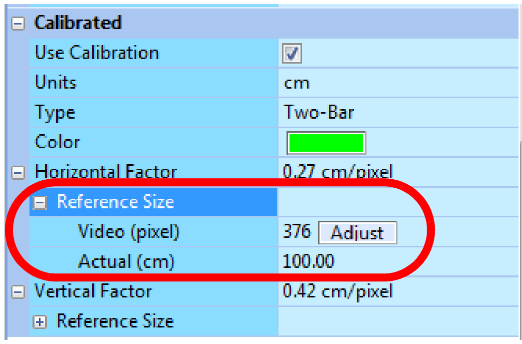
Setting Calibration Parameters with Two-Bar Method

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

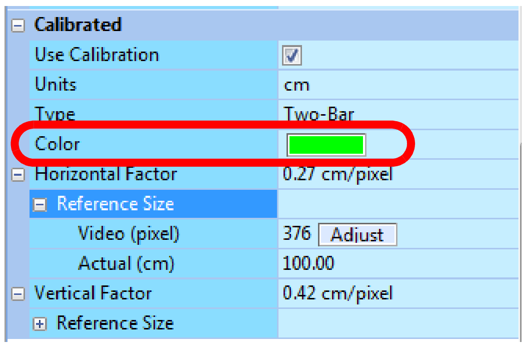
a Click the "**+**" sign next to **Horizontal Factor** and then the "**+**" next to **Reference Size** to expand their subsections, if needed. If they are already shown as "**-**" signs, this is not needed.



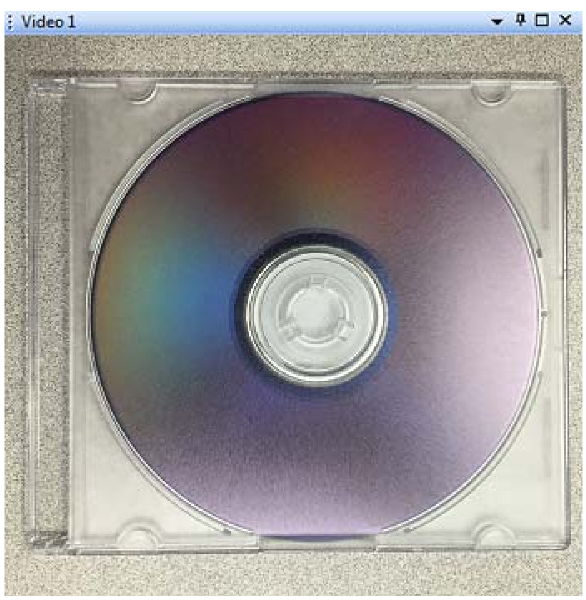




b If the current color in the box next to the **Color** item will not provide good contrast with the video image, click in the box and select a suitable color.



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.

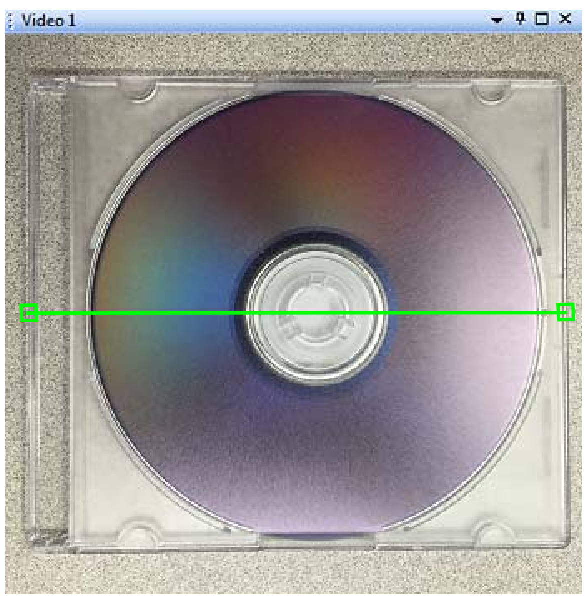


d Measure the feature’s horizontal dimension in the units desired, and record it for reference. In this example, the width is 41.0 cm.

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.

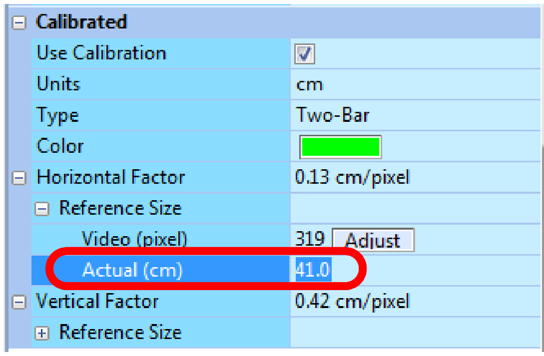
f Select the horizontal sizing bar and move it vertically so that it rests on the desired feature in the frame (in this example, it will be moved to the center of the disk). Select one end of the sizing bar and move it over the desired feature in the image. Repeat this process for the other end of the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



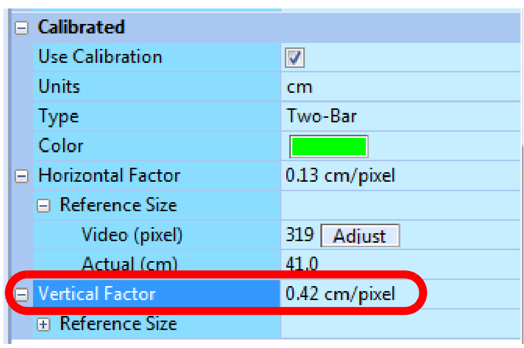
In the above image note that the sizing bar now measures the horizontal width of the plastic case.

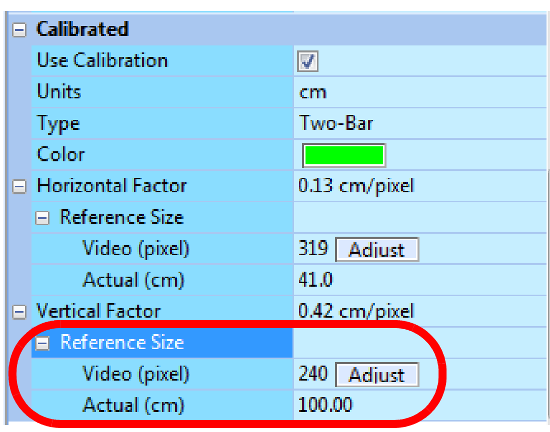
g **Right-click** to record the length of the bar in pixels.

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).

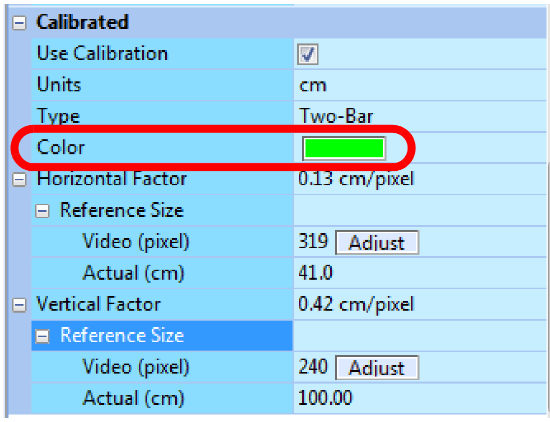


i Click the "**+**" sign next to the **Vertical Factor** and then the "**+**" next to **Reference Size** to expand their subsections, if needed. If they are already shown as "**-**" signs, this is not needed.





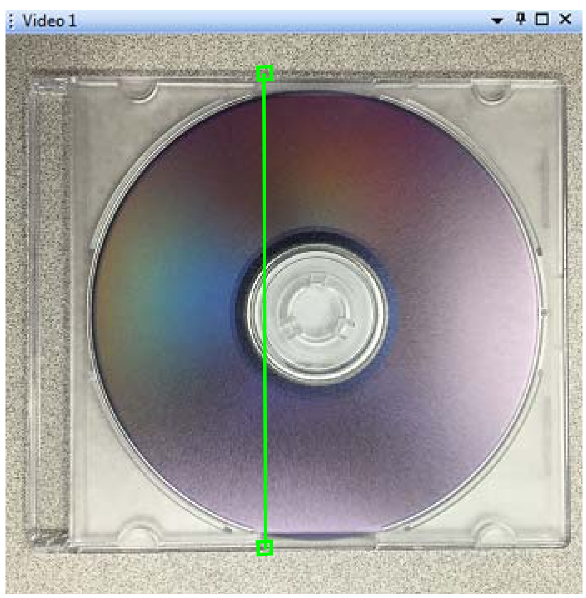
j If the current color in the box next to the **Color** item will not provide good contrast with the video image, click in the box and select a suitable color.



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.

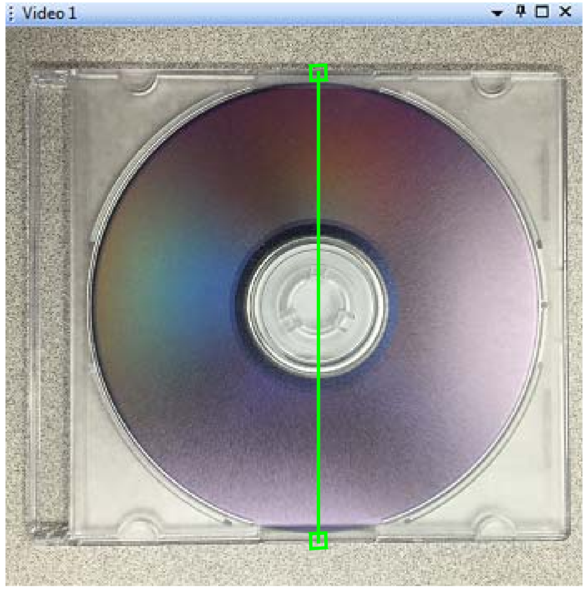
l Measure the feature’s vertical dimension in the units desired, and record it for reference. In this example, the height of the plastic case is 31.9 cm.

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.



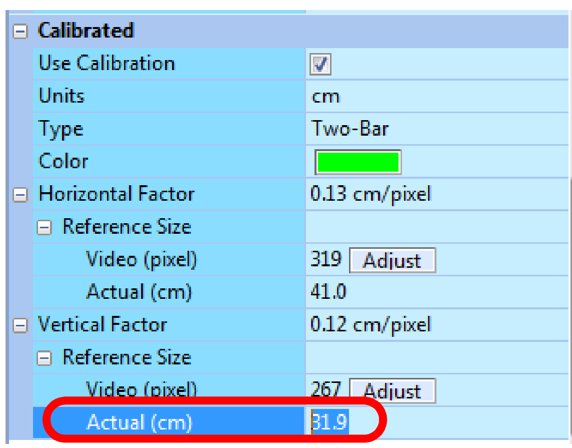
In the image above note the vertical green line with a box at each end. This line is the sizing bar.

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).

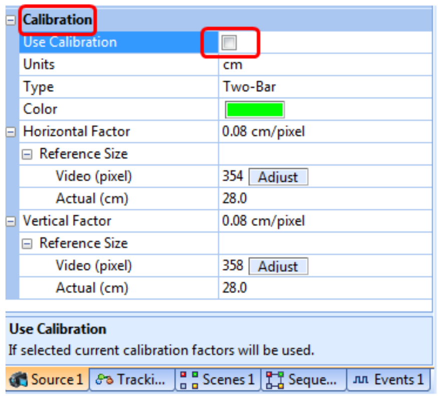


Now the system has been calibrated in both horizontal and vertical directions. Note that the horizontal and vertical factors are slightly different in this example. This situation typically occurs if the camera is not orthogonal to the plane of the object that was measured during the calibration. The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

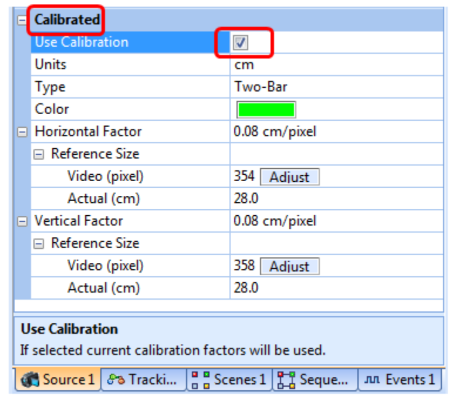
q Click all five “**-**” signs once the adjustments are satisfactory if you want to hide the adjustments.

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

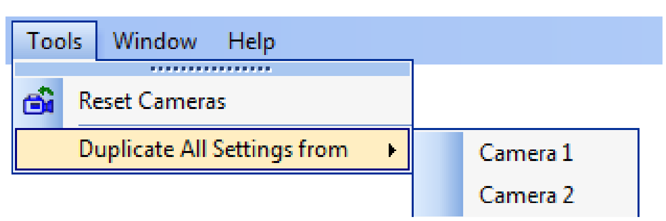


4.4 How to Duplicate Parameter Settings to All Cameras

Some characteristics of all video windows (assuming you are using two or more video streams) are duplicated automatically across all windows as you set them, regardless of the video window in which you configured the characteristic. These settings include, for example, the creation or deletion of arena and zone shapes. timecode display format, certain LED and Color Marker selections, the **Use Calibration** feature, and the calibration units (inches or cm). For these parameters, configuring a value in one video window causes the same value to be assigned automatically in all video windows.

In addition, some parameter settings can be copied from one camera to all the other cameras if you choose to do so. These include, for example, modifications to arena and zone dimensions and locations, the calibration **Global Factor** setting, and the display colors that appear in the video windows.

1 To duplicate settings, from the **Tools** menu, select **Duplicate All Settings from** and then choose one of the cameras in the dropdown list. The system will immediately copy the applicable parameter settings from the camera you select to all the other cameras.



Note: It is highly likely that some of the duplicated settings will need to be modified for the other cameras.

2 Before you start recording any sessions, review all the values you have set for all cameras and ensure that they are set appropriately. It is recommended that calibration be individually adjusted for each camera. This is because it is unlikely that all cameras are positioned at the same distance, viewing angle and magnification from their respective arenas.

4.5 Modifying Arenas and Calibration During an Experiment

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

4.6 Where to Go Next

Go to Chapter 5, Configuring the Recording Parameters.

Chapter 5
Configuring the Recording Parameters

5.1 Before You Start

Verify that you have completed the procedures in Chapter 3, Preparing Your Experiment Database.

5.2 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 run with consistent parameters. The disabled parameter settings are listed in Section 3.9, "Ensuring Consistent Parameter Settings in an Experiment" on page 51.

5.3 Flexible Geometry Feature

You can 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 before you record and as you 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.)

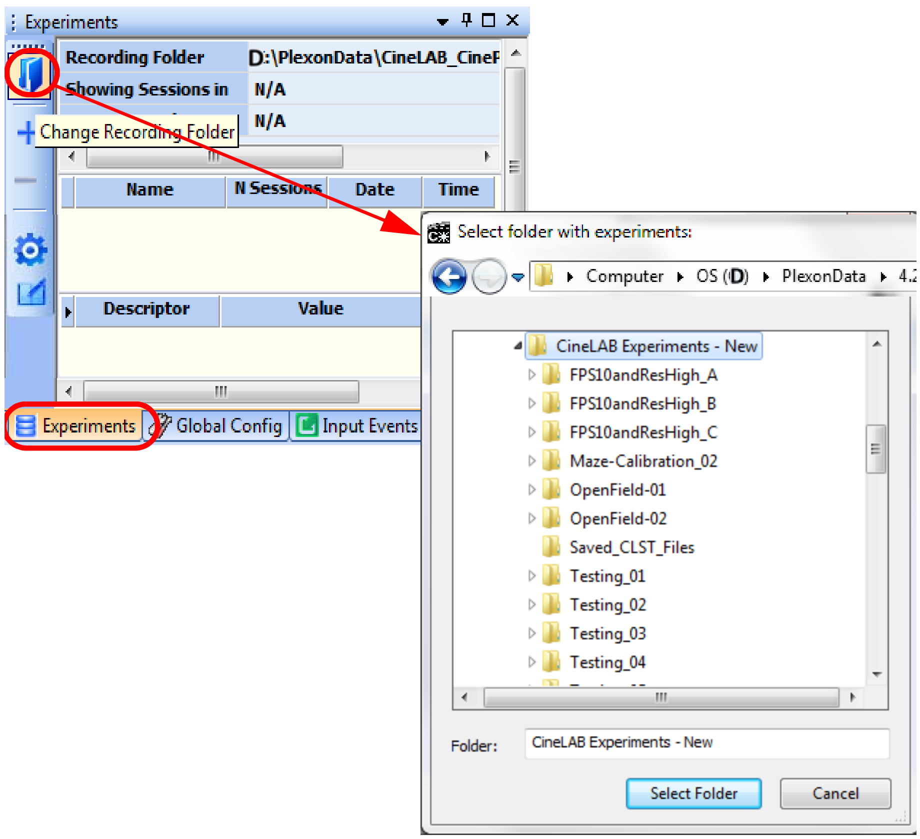
Note: For more information about flexible geometry, see these sections:
— Section 10.14, "Using the Overlay Feature during Analysis" on page 305
— Appendix E, Modifying Arenas and Zones

5.4 Select Cameras Mode and Recording Folder

1 Verify that **Cameras** is selected in the dropdown list and that a camera image is displayed for each of the camera(s) that will be used in the experiment. The example below shows that both Camera 1 and Camera 2 are selected (indicated by the orange color of the icons), so the live images for both of those cameras should appear in the video windows (Video 1 and Video 2).

(If you have already set the **Recording Folder** location, you can skip to the procedure on the next page.)

2 Click the **Experiments** tab and then the **Change Recording Folder** icon. A browsing window opens.



3 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.

You can create folders and subfolders according to the needs of your experiments. Every time you create a new Experiment in the CineLyzer® 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.

4 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.

5.5 Configuring the Basic Global Configuration Parameters

The **Global Config** tab contains settings that pertain to the frame rate and frame resolution to use when recording. Those two parameter settings cannot be changed for an experiment once sessions have been recorded for that experiment. You can adjust the **MPEG4 Quality** and **Scenes** (Transparency) per session. For a description of these parameters, see Section 2.4.5, “Global Configuration Tab” on page 26.

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**.

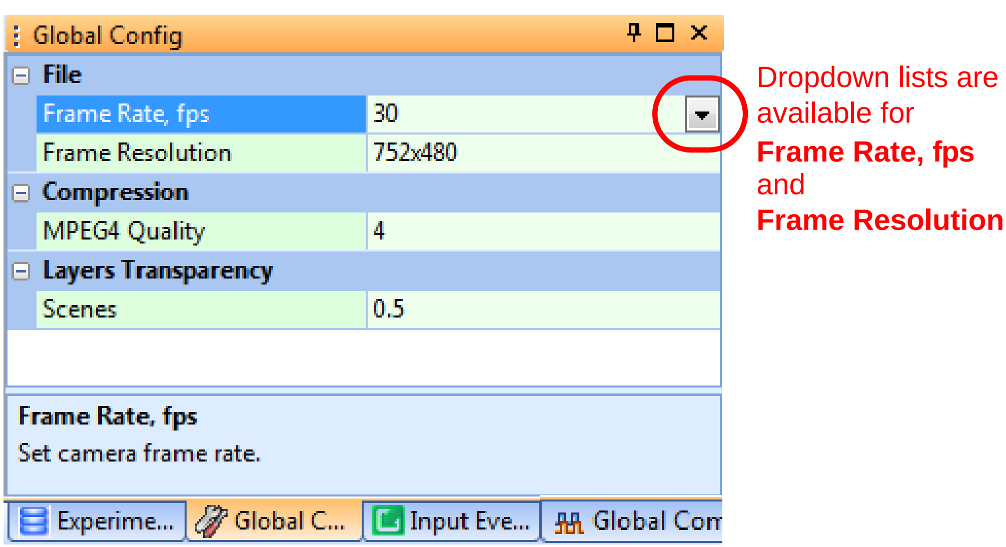
Requirements for frame rate and resolution

All sessions within a single experiment must have the same frame rate in frames per second (**Frame Rate, fps**) and **Frame Resolution**. Therefore, if you want to add one or more video files (sessions) to an existing experiment, the **Frame Rate, fps** and **Frame Resolution** settings of the added files must match those of the existing experiment. If you want to record with a different frame rate or resolution, you will need to create a new experiment.

TIP

Frame Rate and Frame Resolution

The **Frame Rate, fps** and **Frame Resolution** parameters *cannot* be adjusted unless there is an experiment selected *and* that experiment has no sessions recorded.



Tracking parameters in the Global Config tab

If you have selected one of the Tracking modes, you will see additional parameters displayed in the **Global Config** tab. Details on setting these parameters are provided in Section 6.7, “Setting Parameters In the Global Config Tab” on page 93.

5.6 Configuring the Input Events Parameters

The parameters configured in the Input Events tab allow the system to receive an input event (trigger) from an external source and use it to trigger the start or stop of a session. The system receives the event through the CineLyzer USB Digital Input/Output (DIO) Interface. Up to six low true and six high true inputs can be received. (Installation and/or use of the DIO Interface is optional. The use of Input Events is also optional.)

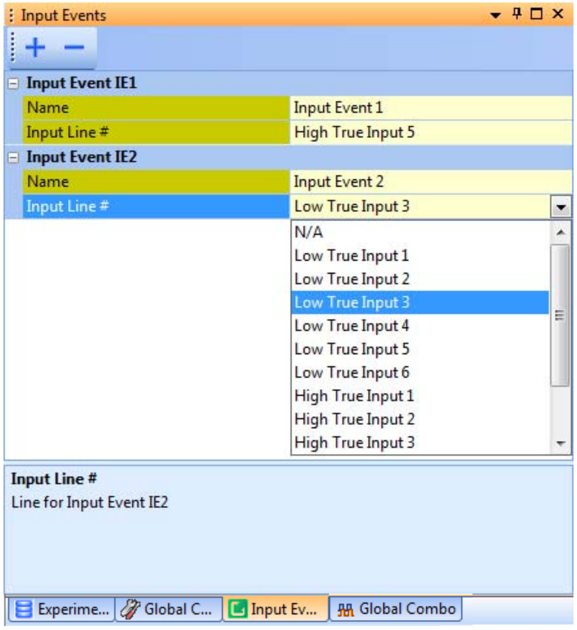
Input signal voltages can vary from 0V to 5V. The DIO Interface input logic is as follows:

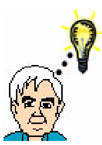
- High True input—The system recognizes voltages greater than 2.0V as asserted and voltages less than 0.8V as de-asserted.
- Low True input—The system recognizes voltages less than 0.8V as asserted and voltages greater than 2.0V as de-asserted.

If the input is a pulse, the duration of the pulse should be at least as long as the time between frames for the specified camera frame rate (the setting for the **Frame Rate, fps** parameter in the Global Config tab).

For additional details about the DIO Interface, see Appendix C, USB Digital Input/Output Interface.

The Input Events tab and the DIO Interface are shown below.





TIP

Plug in the USB cable for the DIO Interface and then start the CineLyzer application

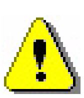
To ensure that the dropdown list with the 12 input lines is populated, you must connect the USB cable from the DIO Interface to a USB port on the host PC and then start CineLyzer application.

Wiring the DIO Interface and configuring Input Events

1 Attach a wire to an input line you want to use on the DIO Interface. 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 DIO Interface and the external equipment.

Note: See Appendix C, USB Digital Input/Output Interface, for an explanation of the voltage and current limits for the input signals from external equipment. If the signals from the external source device are not within those limits, adaptors or converters may be required. The external equipment vendor may supply these devices.



CAUTION

Limit voltage to 0–5V at the DIO Interface pins

Do not apply more than 5V to any of the input/output pins on the DIO Interface. Doing so could damage the device.

3 Click on the **Input Events** tab to view the Input Events parameters.

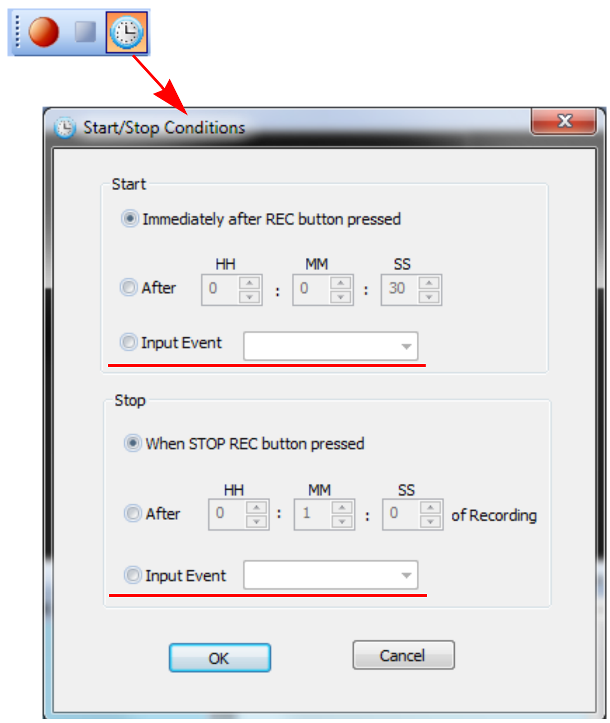
4 Double click in the cell on the right side of the **Name** row to enter a name for the input event.

5 Use the dropdown list to select the input line that matches the line to which you attached the wire in Step 1.

6 Repeat Step 1 through Step 5 for each input line you want to use.

Using an Input Event as a trigger

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 DIO 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).

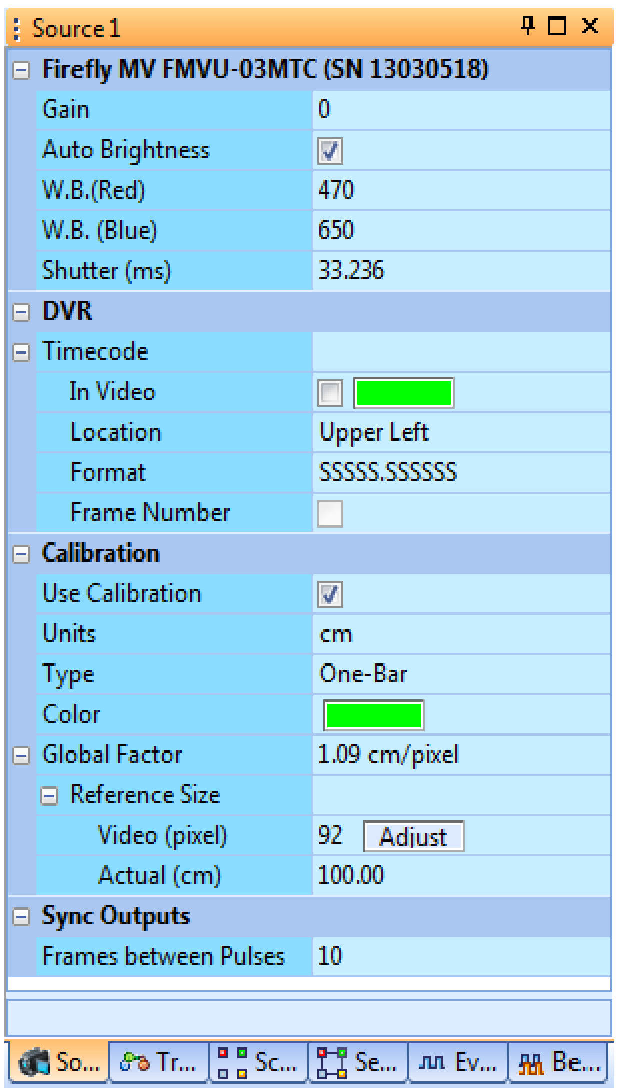
5.7 Configuring the Source Parameters

Note: If your experiment includes photometry, set the parameters for photometry as described in Chapter 8, Configuring the Photometry Parameters.

The **Source** tab is used to set parameters for the video on each camera or video file. There will be multiple **Source** tabs if there are multiple cameras on the system. This tab contains parameters that relate to optical settings for each camera (such as gain, brightness, white balance and shutter speed), labeling/timestamping of frames and calibration.

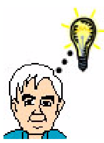
1 With the video image properly set up and focused in the Video window, click on the **Source** tab under the Video window to view the parameters. The following steps configure these settings.

The image below shows the Source 1 tab, which appears under the Video 1 window for Camera 1. Similar tabs appear for each of the cameras you have activated in the previous steps.



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)**).

Note: You can also use other image quality settings. To evaluate image quality settings, manually collect a set of AVI files at different settings and use the file mode to determine a minimum acceptable image quality setting for the experiment. (For information about video formats and the video **Quality** setting, see Section 5.9.2, “MPEG-4 Quality and File Size” on page 83.)



TIP

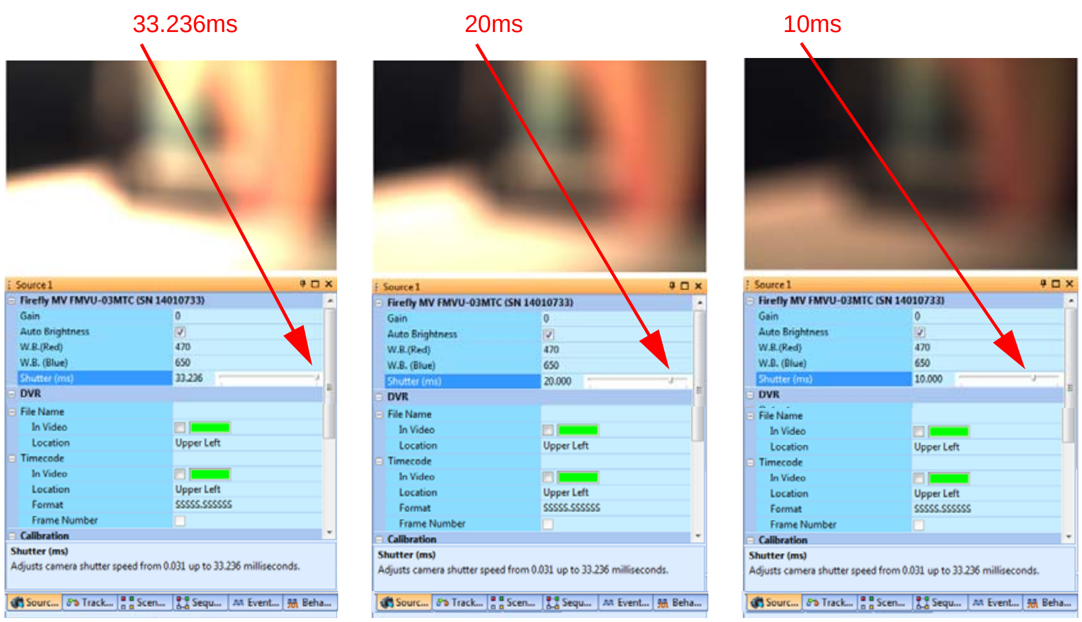
Reducing blurs and color streaks in the video

If the experimental subject moves quickly, blurs and color streaks can appear in the video. Reduce or eliminate these problems by increasing the shutter speed. See the shutter speed adjustment—**Shutter (ms)**—as shown in the images below.

Notes regarding shutter speed

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

If an image is too bright, you can make the image darker by reducing the amount of time that the shutter is open. In all the images shown in the example below, the frame rate is 30 frames per second (one frame per 33.236ms). By default, the shutter will stay open the entire 33.236ms per frame. You can reduce this opening time, **Shutter (ms)**, using the slider to set a new value. The images below show the effects on brightness for shutter speeds of 33.236ms, 20ms and 10ms.



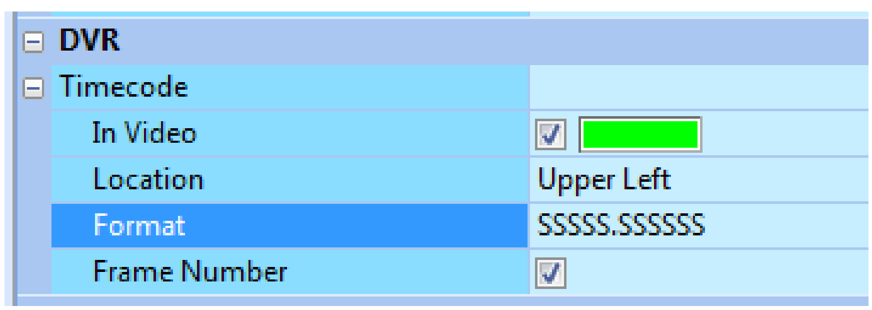
It might be useful, for example, to adjust the **Shutter (ms)** value in the following situations:

- The image is too bright, so the video gets oversaturated:
  You might need to reduce the shutter open time (reduce the **Shutter (ms)** value) to make the image darker. You can reach a similar effect by closing the lens iris, but that means touching the cameras, and you will not be able to have the iris set exactly the same on all cameras.

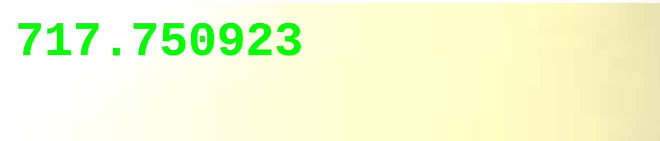
- You will be recording animals in subdued lighting, but prefer to perform dimensional calibration in full lighting:
  In this case, It is useful to have the iris opened all the time when you set up cameras, and then reduce the shutter open time (reduce the **Shutter (ms)** value) slightly when you do the calibration. Then, increase the shutter open time to the maximum prior to performing experiments.

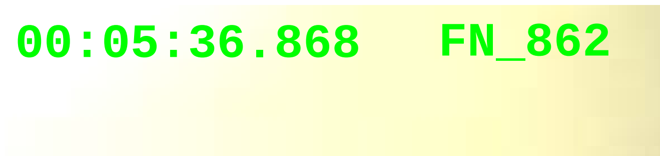
- You are tracking an animal at a certain frame rate, for example 30 fps, but your animal moves very quickly; so you might get a blurry image:
  You can improve the image by keeping the shutter closed most time within each frame. Alternatively, you might simply prefer to use a faster frame rate, which has the effect of reducing the maximum time the shutter can be open.

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.)



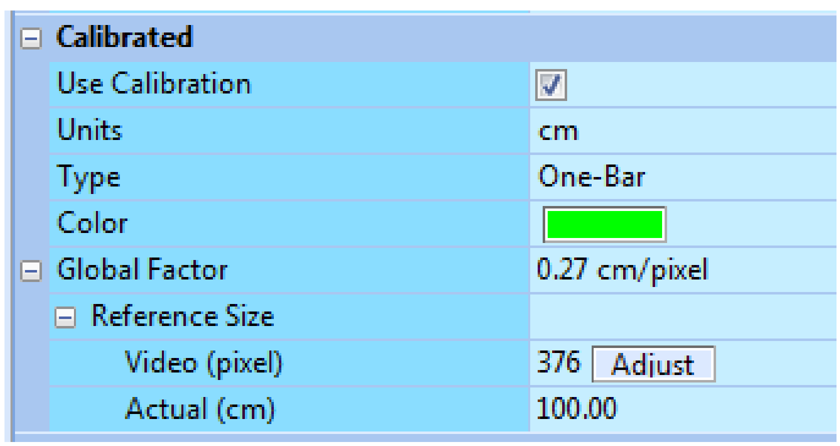
For example, the first image (below) shows the time code in the upper left location in **SSSSS.SSSSSS** format. The second image shows the time code in **HH:MM:SS.SSS** format along with the frame number.



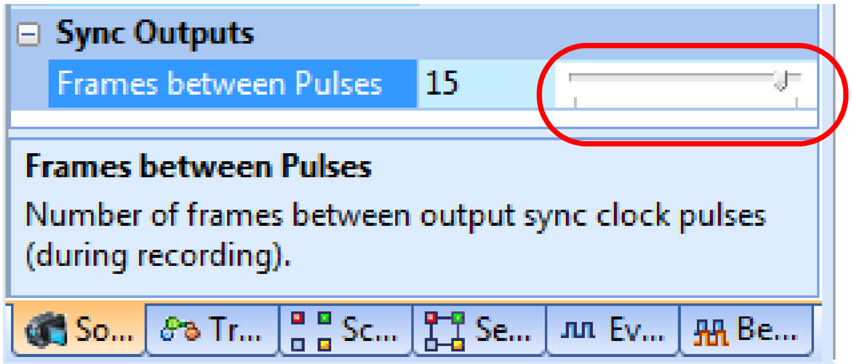


4 (Optional) If not already done, calibrate the image for each camera as explained in Chapter 4, Calibrating the Arena Dimensions. You must check the **Use Calibration** checkbox to enable the calibration function. Note that the heading in this section of the Source tab changes from “Calibration” to “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 If you want to transmit TTL pulses from the Camera Trigger Board (mounted on the behavioral camera) to an external device, use the **Frames between Pulses** slider. Valid values are from 1 to 16.



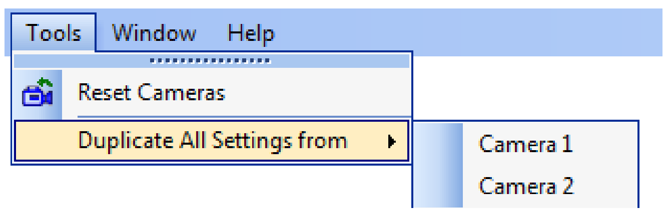
For example, if you set this value to 15, and you are recording at 30 frames per second, the trigger board will transmit a TTL signal every half second. If you connect a cable from the trigger board to your external device, the external device can make use of this timing signal.

5.8 How to Duplicate Parameter Settings to All Cameras

Some characteristics of all video windows (assuming you are using two or more video streams) are duplicated automatically across all windows as you set them, regardless of the video window in which you configured the characteristic. These settings include, for example, the creation or deletion of arena and zone shapes. timecode display format, certain LED and Color Marker selections, the **Use Calibration** feature, and the calibration units (inches or cm). For these parameters, configuring a value in one video window causes the same value to be assigned automatically in all video windows.

In addition, some parameter settings can be copied from one camera to all the other cameras if you choose to do so. These include, for example, modifications to arena and zone dimensions and locations, the calibration **Global Factor** setting, and the display colors that appear in the video windows.

1 To duplicate settings, from the **Tools** menu, select **Duplicate All Settings from** and then choose one of the cameras in the dropdown list. The system will immediately copy the applicable parameter settings from the camera you select to all the other cameras.



Note: It is highly likely that some of the duplicated settings will need to be modified for the other cameras.

2 Before you start recording any sessions, review all the values you have set for all cameras and ensure that they are set appropriately. It is recommended that calibration be individually adjusted for each camera. This is because it is unlikely that all cameras are positioned at the same distance, viewing angle and magnification from their respective arenas.

5.9 General Information on Video Formats and Timestamps

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

5.9.1 Reference Information - Digital Video Recorder AVI Files

The system stores the video recording from the cameras in the database on an internal hard drive. You may start, stop, and time stamp these AVI files in a way that enables you to subsequently correlate them offline with other data.

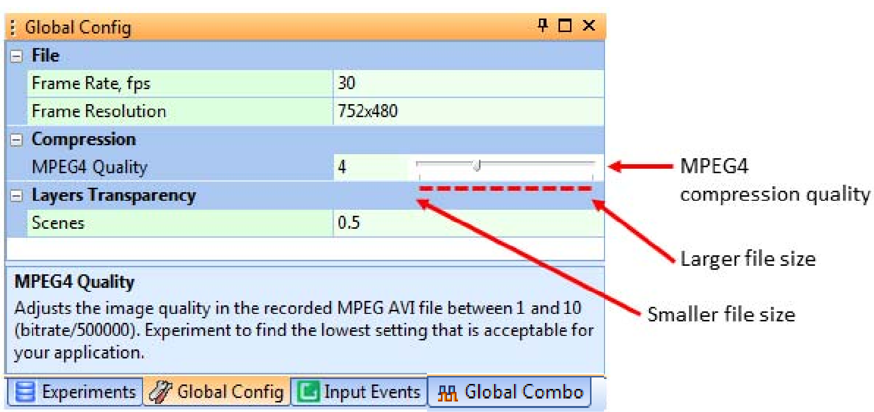
The system records video into AVI files at a resolution of 752x480 or 376x240 pixels at up to 60 frames per second, which implies a raw data rate of over 200 GB/hour. To reduce disk usage, the system compresses the video using standard MPEG algorithms before it writes it to the AVI file. You can vary the file compression to find a suitable balance between AVI file size and image quality.

To save even more disk space, you can also reduce frame resolution to 376x240 pixels (an option in the **Frame Resolution** dropdown list) if that resolution is sufficient for your experiment.

5.9.2 MPEG-4 Quality and File Size

MPEG-4 recording format is used because it produces files that are routinely 75 to 90% smaller than the equivalent MJPEG formatted files that were produced by other recording tools.

The system **MPEG4 Quality** settings for MPEG-4 range from 1 to 10. The values represent the nominal bit rate divided by 500,000. The default value of 4 is suitable for most experiments and we recommend using that value unless there is a specific reason to modify it.



Note: If you increase the **MPEG4 Quality** setting, for example, increasing it from 4 to 10, the size of the AVI file increases significantly (assuming the same recording duration), but it does not dramatically increase the quality of the image. Conversely, if you reduce the **Quality** setting, for example, from 4 to 2, the size of the AVI file is reduced (for the same recording duration), however, the quality of the image degrades significantly.

5.9.3 MPEG Recording Processes

While it records, CineLyzer 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 CineLyzer 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.

Note: Windows Media Player and other video players might render files slower than real time if the files were recorded at 40 frames per second or greater. This can be confusing because the slider moves faster than the video plays such that the slider reaches the end of its travel before the video finishes playing.

The MPEG/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. CineLyzer software 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 this time stamp in visible numerals on the video image itself; see Section 5.7, “Configuring the Source Parameters” on page 77.

5.10 Where to Go Next

If you would like to configure tracking parameters, go to Chapter 6, Configuring the Tracking Parameters.

Plexon Inc

Chapter 6
Configuring the Tracking Parameters

6.1 Before You Start

Verify that you have completed the procedures in Chapter 5, Configuring the Recording Parameters.

6.2 Introduction to the CineLyzer Tracking Feature

This chapter describes the CineLyzer® 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 provide the detailed procedures.

1. Position the camera(s) 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**

Note: In LED or Color Markers tracking—After you set the tracking mode and configure the tracked objects for an experiment, you cannot change them. This ensures that the same tracking mode and tracked objects are used for all sessions in the experiment. (You can change some of the display options for the tracked objects, but not the objects themselves.)

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 4, Calibrating the Arena Dimensions)
8. Define the shape of the arena for each 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 7, Configuring the Behavioral Events Parameters
11. (If your experiment includes photometry) Set the parameters for photometry as described in Chapter 8, Configuring the Photometry Parameters.
12. Start recording
13. Stop recording

The CineLyzer System default window layout is suitable for most instances. However, to load, change or save a window layout, see Section 2.4, “Navigating the CineLyzer User Interface” on page 22. Additional information is available in Appendix B-Navigating the Plexon User Interface.

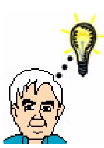
6.3 Setting Up Cameras and the User Interface

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 procedures for setting up cameras and the user interface are described in Section 2.3, “Setting Up Cameras and the User Interface” on page 19.

6.4 Navigating the User Interface

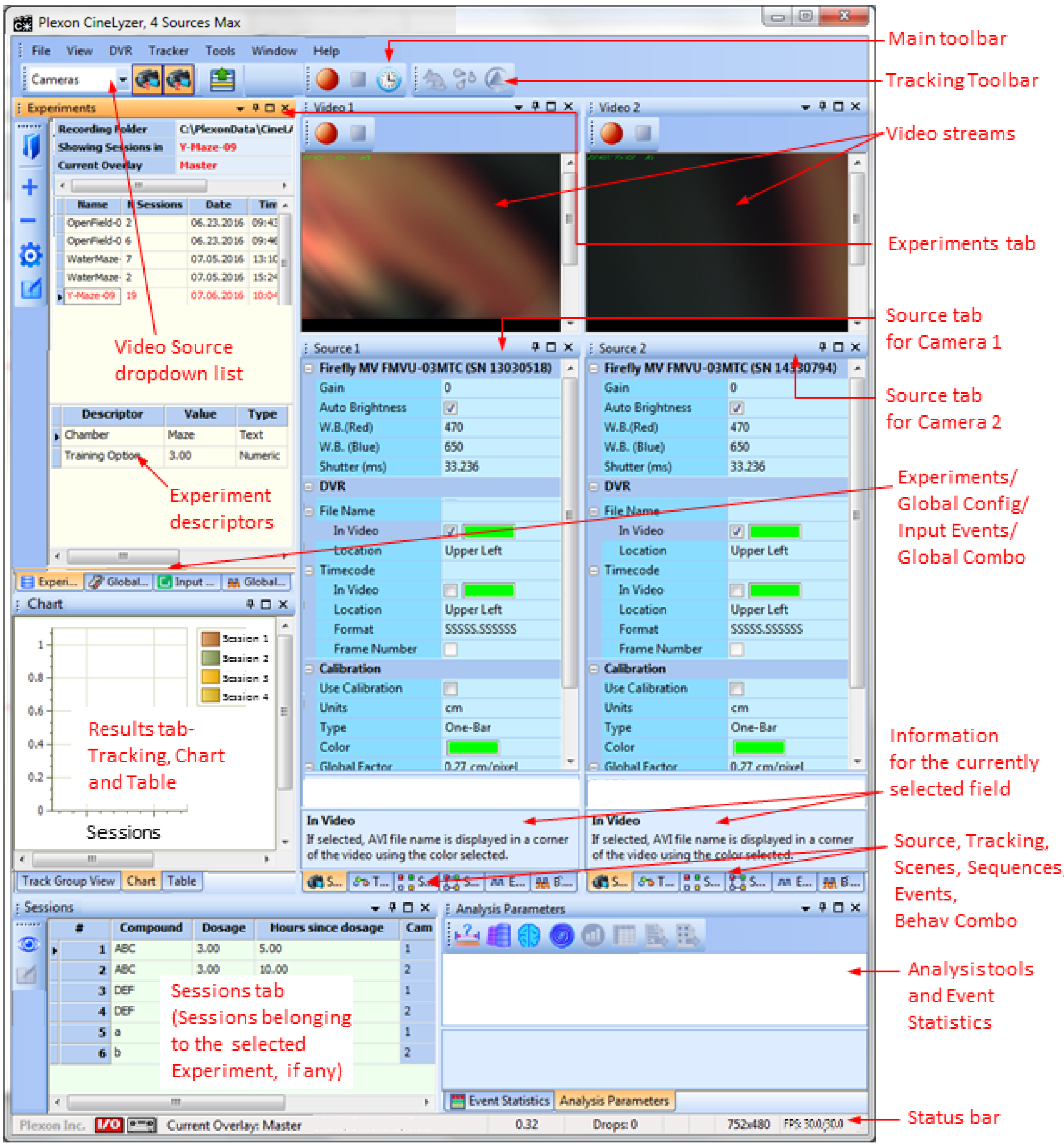
The image below shows the location of the windows in the default layout of the CineLyzer user interface. In this example, there are two cameras connected; up to four cameras can be licensed and connected (optional).

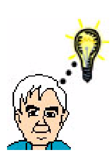


TIP

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**.

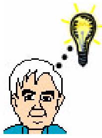




TIP

Verify "Cameras" Is Selected

Camera settings do not display in the user interface unless **Cameras** is set in the main toolbar.



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 list in the main menu.

Additional information is available in Appendix B-Navigating the Plexon User Interface.

There are a number of parameters that need to be configured to enable and manage tracking functions. These are configured in Section 6.6, “Selecting the Tracking Mode and Tracking Settings” on page 89.

6.5 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 32
- Section 3.2, “Planning the Database” on page 32
- Section 3.3, “Setting Parameters for an Experiment with Multiple Sessions” on page 33
- Section 3.4, “Setting the Recording Folder Location” on page 36
- Section 3.5, “Creating a New Experiment” on page 37
- Section 3.6, “Selecting a Previously Saved Experiment” on page 45
- Section 3.7, “Editing the Experiment Name, Descriptor and Variable Values” on page 46

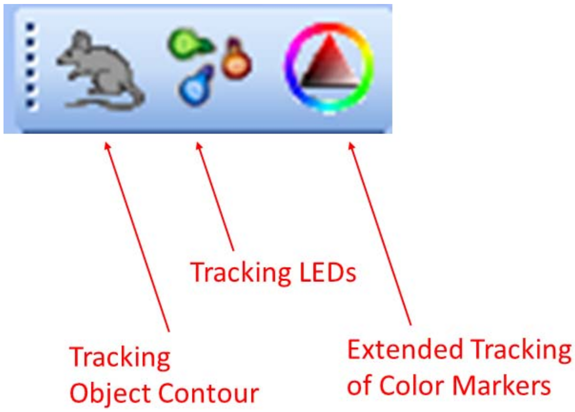
6.6 Selecting the Tracking Mode and Tracking Settings

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.

- Section 6.6.1, “Understanding the Tracking Mode Options” on page 90
- Section 6.6.2, “Guidelines for Selecting the Tracking Mode” on page 91
- Section 6.6.3, “Additional Guidelines for Tracking” on page 91
- Section 6.6.4, “Understanding the Threshold Setting” on page 92

6.6.1 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, you cannot change it. This ensures that the same tracking mode is used for all sessions in the Experiment.

- **Tracking Object Contour**: 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.

6.6.2 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 6.13, "Object Contour Tracking" on page 106)
LEDs	Yes	LED (See Section 6.14, "LEDs In Darkness with LED Tracking" on page 118) or Color Markers (See Section 6.15, "LEDs In Darkness or Light with Color Markers Tracking" on page 125 and Section 6.16, "Color Markers Tracking" on page 126)
LEDs	No	Color Markers (See Section 6.15, "LEDs In Darkness or Light with Color Markers Tracking" on page 125 and Section 6.16, "Color Markers Tracking" on page 126)
Colored markers	Not Applicable	Color Markers (See Section 6.16, "Color Markers Tracking" on page 126)

6.6.3 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.

6.6.4 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 6.13.2, "Setting the Threshold" on page 108. 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.

6.7 Setting Parameters In the Global Config Tab

Setting the general parameters in the Global Config tab

Details on setting the general parameters are provided in Section 5.5, "Configuring the Basic Global Configuration Parameters" on page 73.

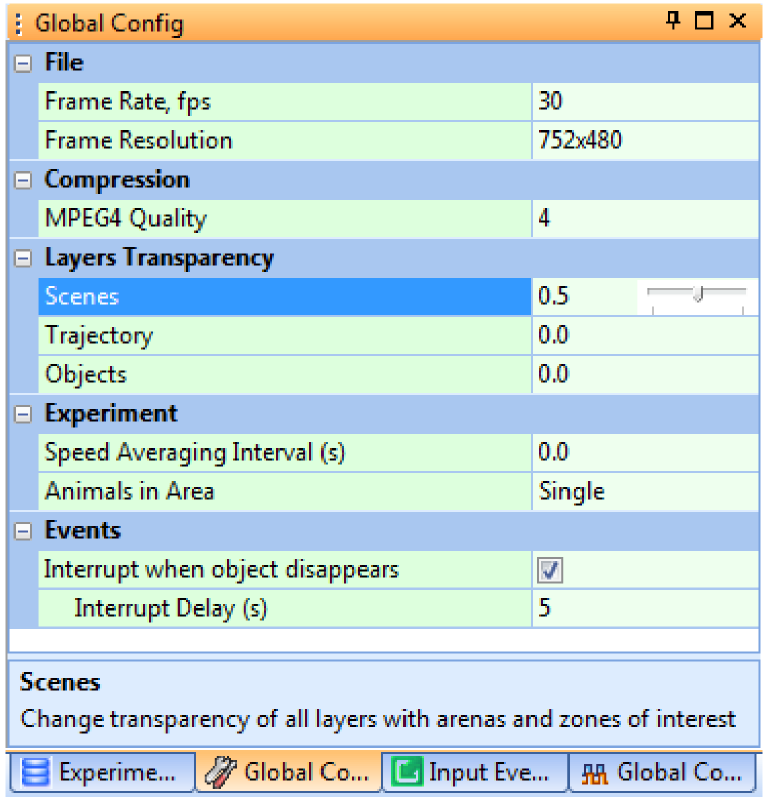
Setting the Tracking and Events parameters in the Global Config tab

If you have selected one of the Tracking modes, you will see additional parameters displayed in the **Global Config** tab. 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**—Transparency for user-specified shapes, such as arena and zones, as described previously.

    Note: When Photometry is present, the title will be as follows: **Scenes/Fiber Boundaries**—Transparency for user-specified shapes, such as arena and zones, as described previously, or fiber circle outlines.
  - **Trajectory**—Transparency for the tracked path of the animal during the session.
  - **Objects**— Transparency for all object layers (center of gravity, tracking window, contour and body).

    Note: When Photometry is present, the title of this row will be as follows: **Objects/Fiber Heat Maps**—Transparency for all object layers or photometry heat maps.



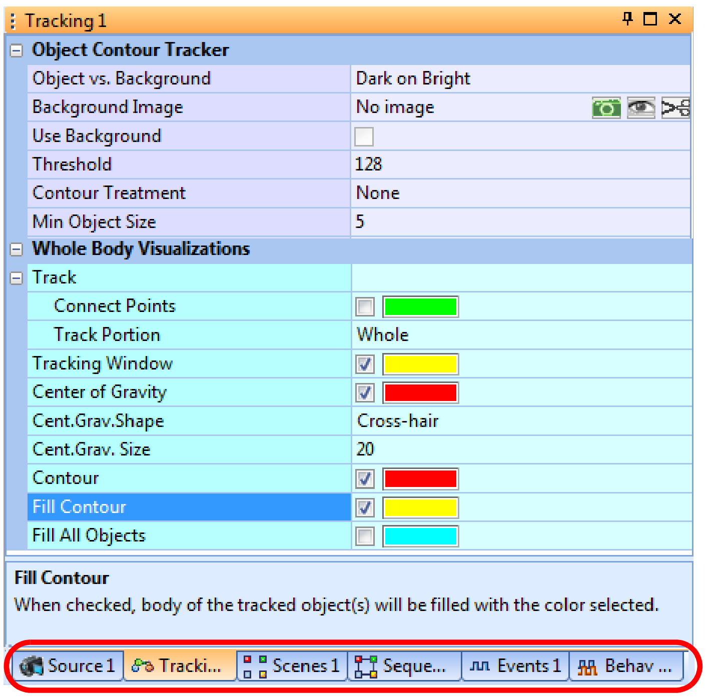
- **Speed Averaging interval (s)**. This setting specifies the size of the sliding time window, (0 to 1.0 seconds) that the system uses to average the speed values. If this parameter is set to 0 (the default value), the system reports the instantaneous speed value for each video frame.
- **Animals in 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.

6.8 Calibrating the Arena Dimensions

In most experiments, it is useful to calibrate the video images from the cameras so that 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 4, 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.

6.9 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 cameras or from existing files. There will be multiple Source tabs if there are multiple cameras on the system. This tab contains parameters that relate to optical settings for each 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 7, Configuring the Behavioral Events Parameters

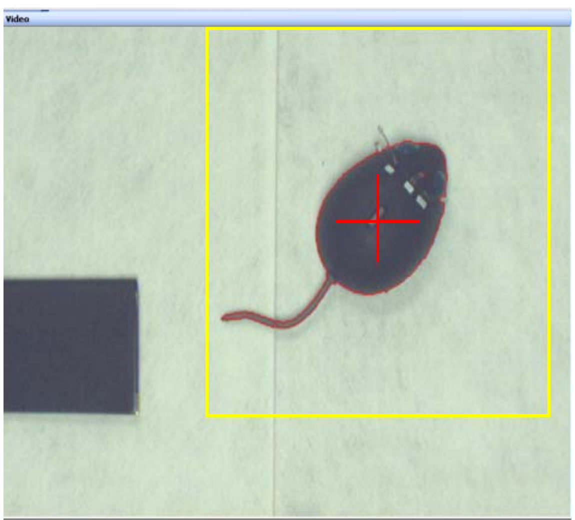
6.10 Understanding Tracking Windows and Arenas

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

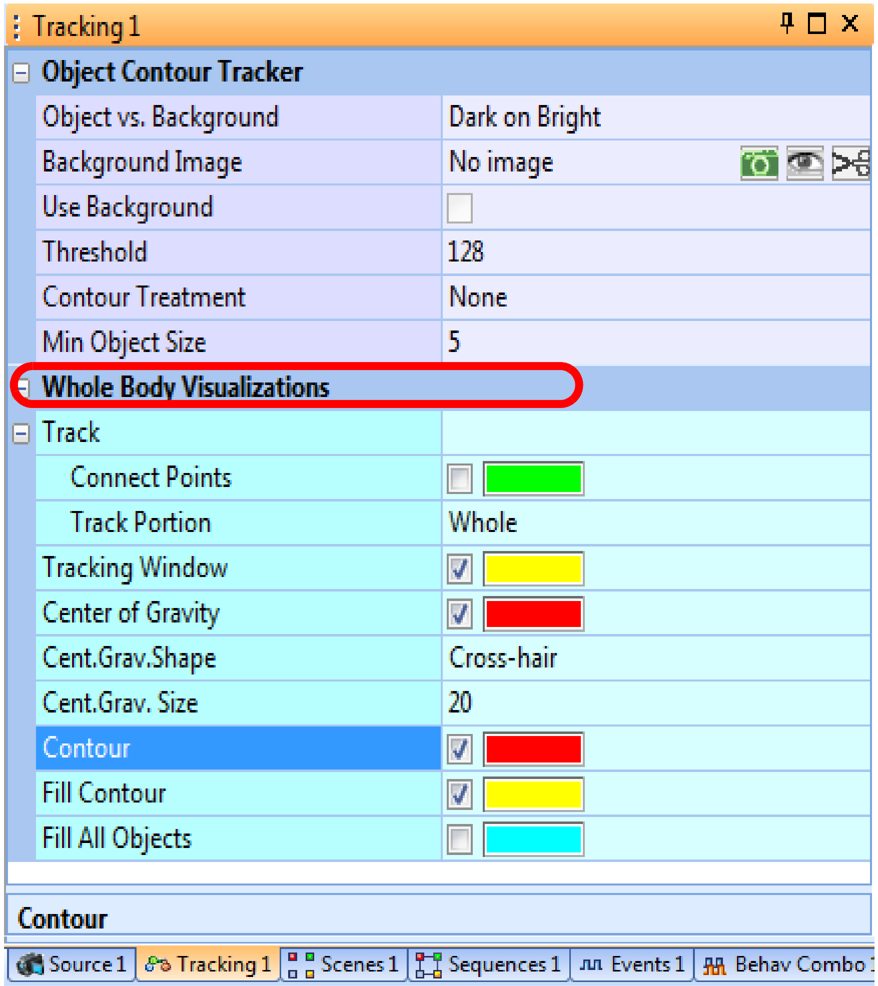
6.10.1 System-generated Tracking Windows

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).

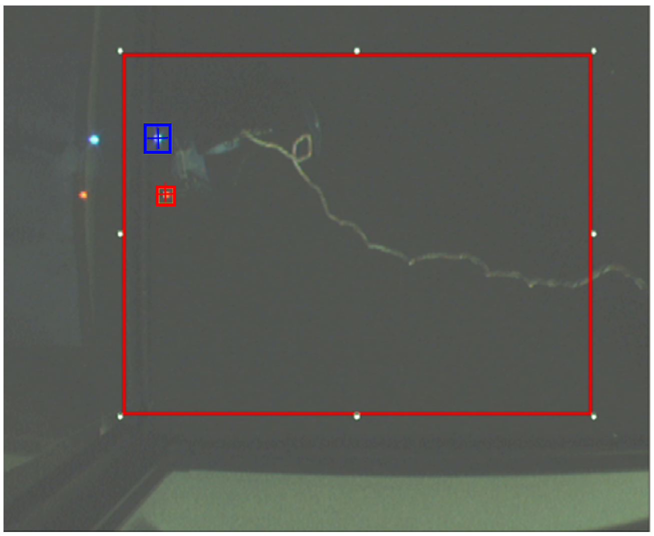
6.10.2 User-defined Arenas

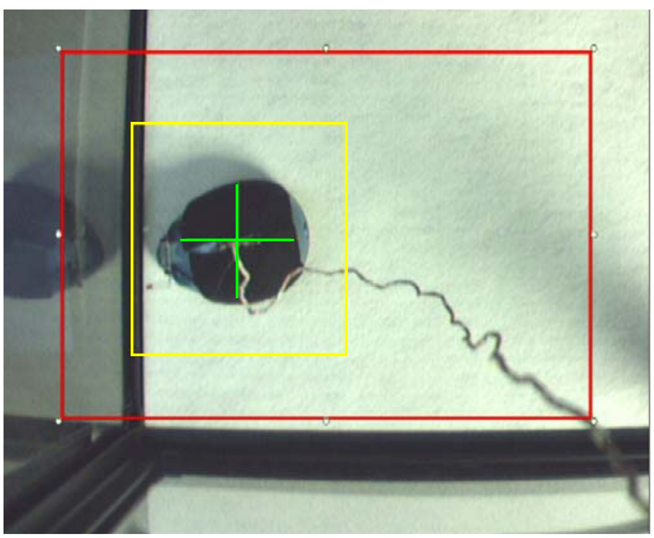
You can define an arena (or area of interest) for each camera image. Arenas reduce problems with unwanted reflections and parasite objects outside of the working area.

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.

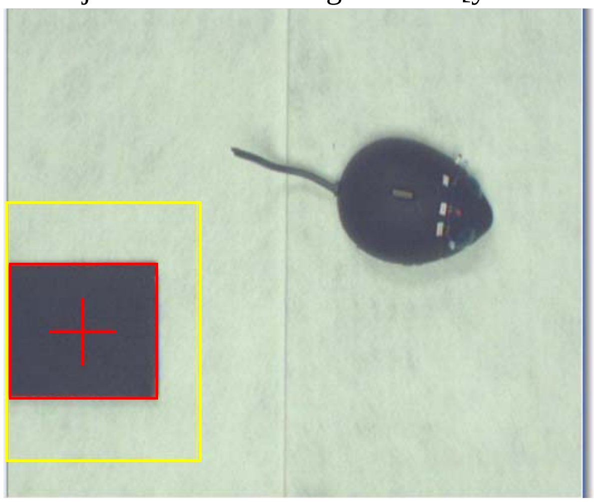




Example - Using Arenas to Exclude Background Objects or Colors

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.

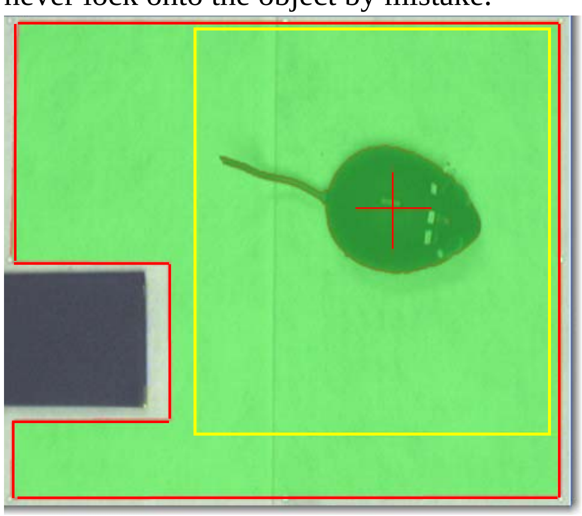
This image shows an animal to be tracked along with a background object of approximately the same color. The background object is larger than the animal and the system is tracking the background object (the red cross hair is centered on the object and the tracking window [yellow rectangle] is surrounding 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 6.13.5, “Background Subtraction” on page 113)

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 CineLyzer® software is installed, that file can be retracked with new or changed arenas.

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

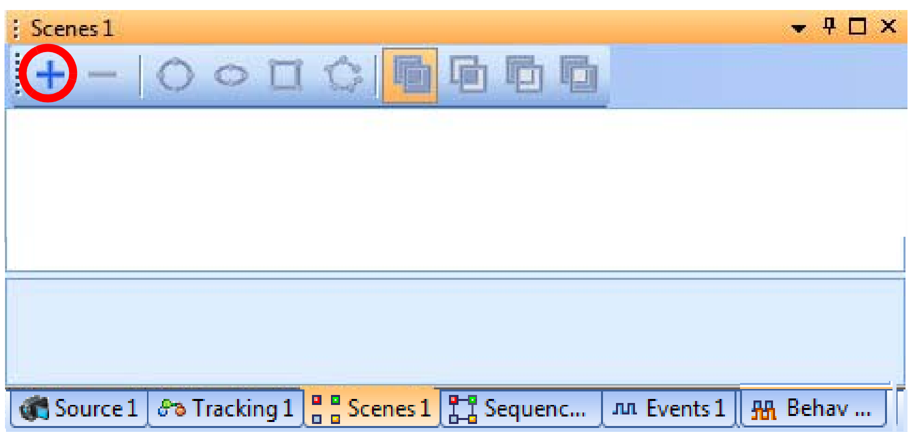
However, it is also important to determine that the computer on which the installation will be done meets the minimum system requirements. The requirements are summarized in Section 1.8, “Using a Standalone Computer for Data Analysis” on page 12. Requirements that are more current are available from Plexon Support (support@plexon.com).

6.11 Defining the Arenas

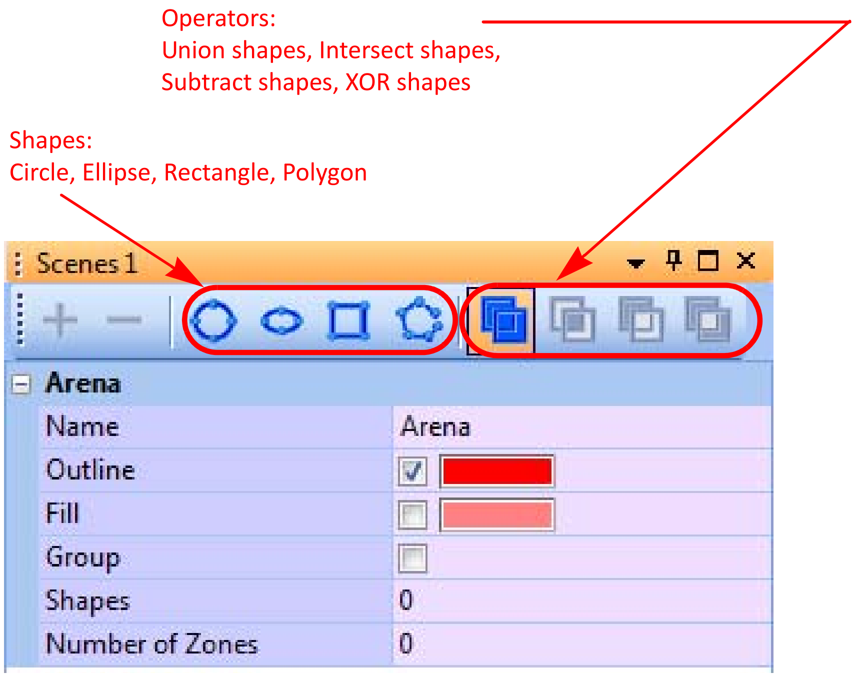
This section explains how to define an arena for each camera, beginning with Camera 1. One arena is allowed per camera.

Adding the Arena for Camera 1

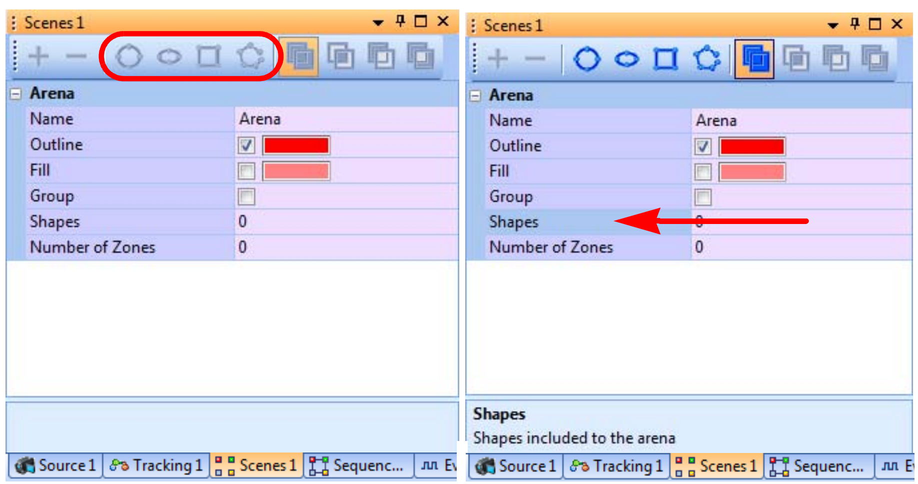
1 In the Scenes tab for Camera 1, click the **Add arena (+)** icon.



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.



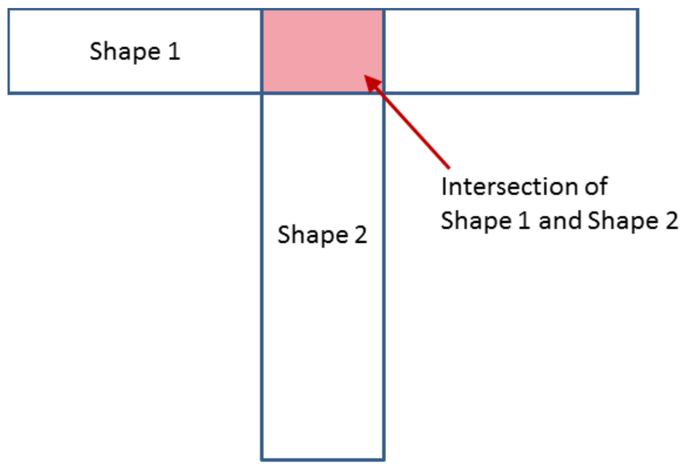
3 One or more shapes can be added to the arena. To draw a shape, click the desired shape on the toolbar (**Circle**, **Ellipse**, **Rectangle**, or **Polygon**). For a circle, ellipse or rectangle, left-click and hold the mouse on a desired point on the image and move the mouse to size the arena.

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:

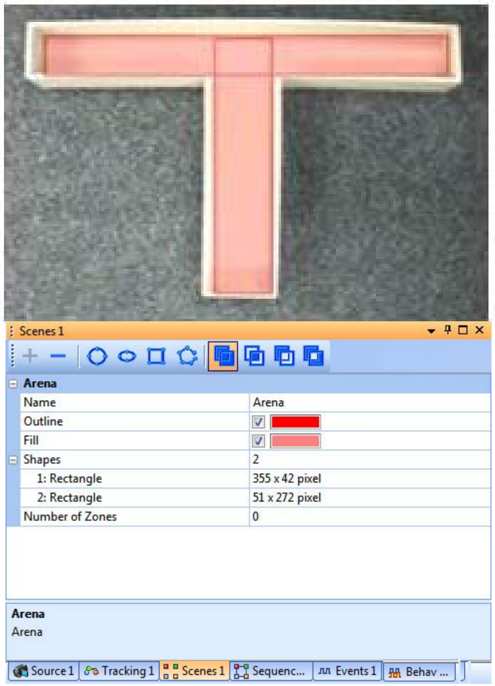
- Click on the operator that will combine the first and second shape.
- Then click on the icon for the second shape and draw the second shape.
- To view the combined shape more clearly, click on the **Fill** checkbox to select it.

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.

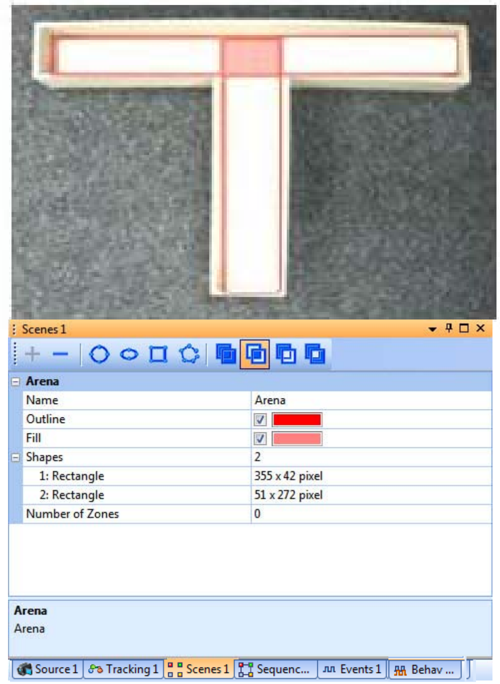


The images below show examples of combined arena shapes. Tracking will only occur in the shaded regions.

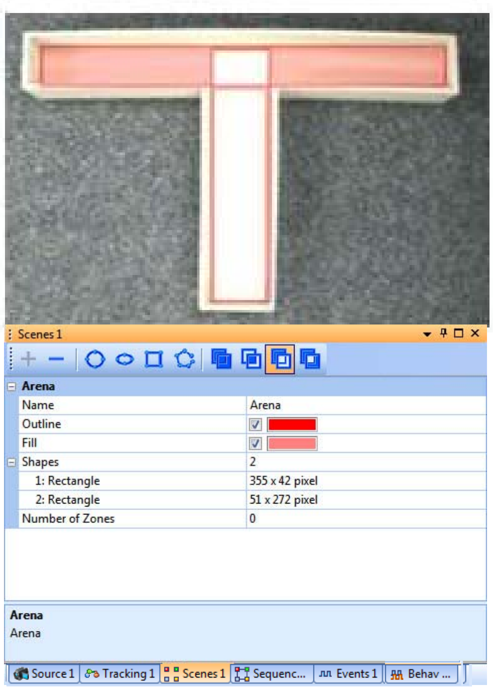
Union shapes



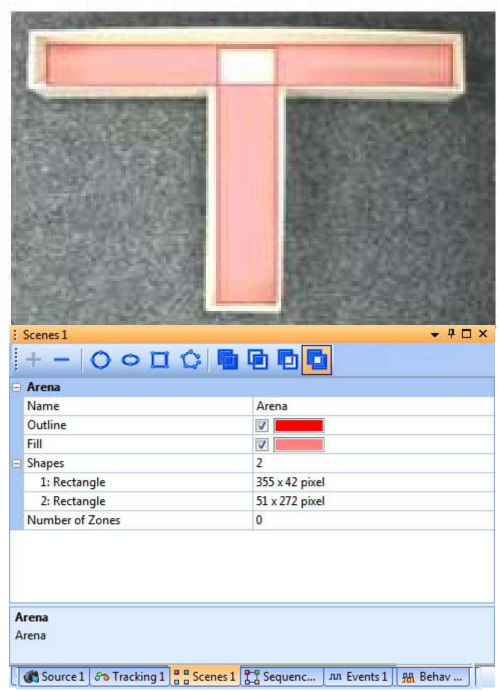
Intersect shapes



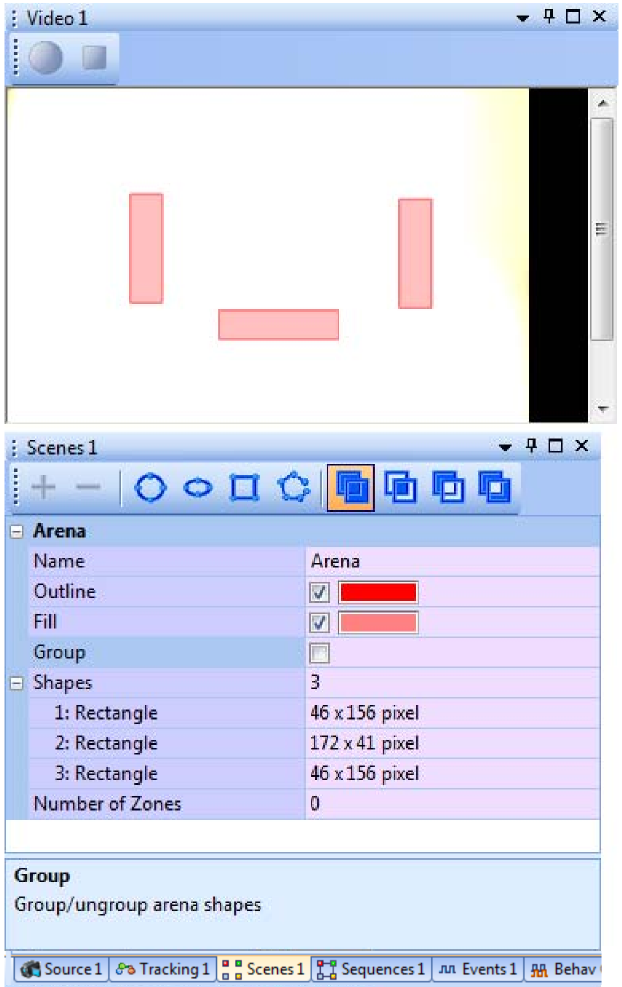
Subtract shapes



XOR shapes



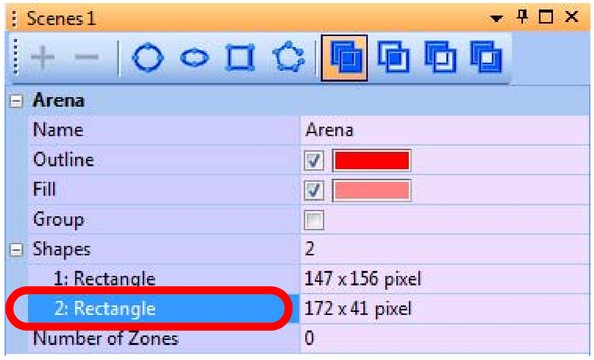
Arenas drawn from several shapes do not need to be contiguous (as was the case in the examples above). The image below shows an arena that contains noncontiguous shapes. Tracking will only occur in the shaded regions.



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.



Adding Arenas for Additional Camera(s)

8 Repeat Step 1 through Step 7 for all additional camera(s) to be used in the experiment.

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 10.14, “Using the Overlay Feature during Analysis” on page 305
— Appendix E, Modifying Arenas and Zones

6.12 Tracking Parameters - List of Configuration Procedures

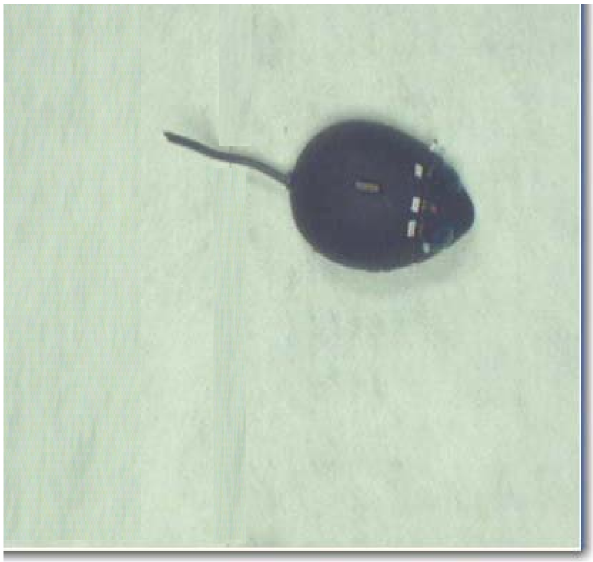
The following sections contain procedures that configure the tracking parameters for specific experimental applications. Select the appropriate procedure for your experiment:

- Section 6.13, “Object Contour Tracking” on page 106
- Section 6.14, “LEDs In Darkness with LED Tracking” on page 118
- Section 6.15, “LEDs In Darkness or Light with Color Markers Tracking” on page 125
- Section 6.16, “Color Markers Tracking” on page 126

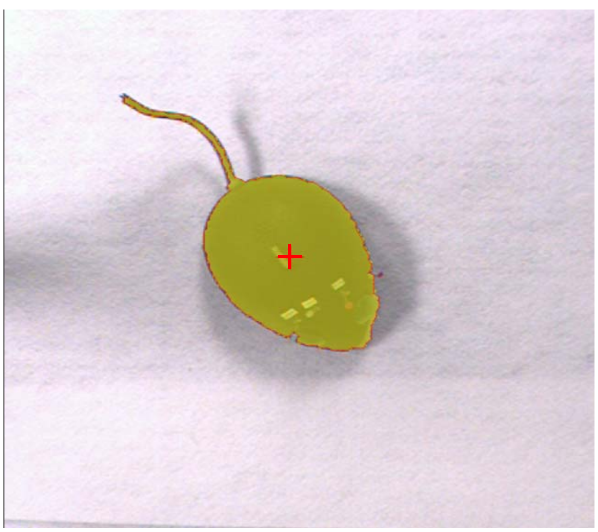
6.13 Object Contour Tracking

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.



6.13.1 Configuring Parameters for Object Contour Tracking

Perform these steps to select and configure Object Contour tracking.

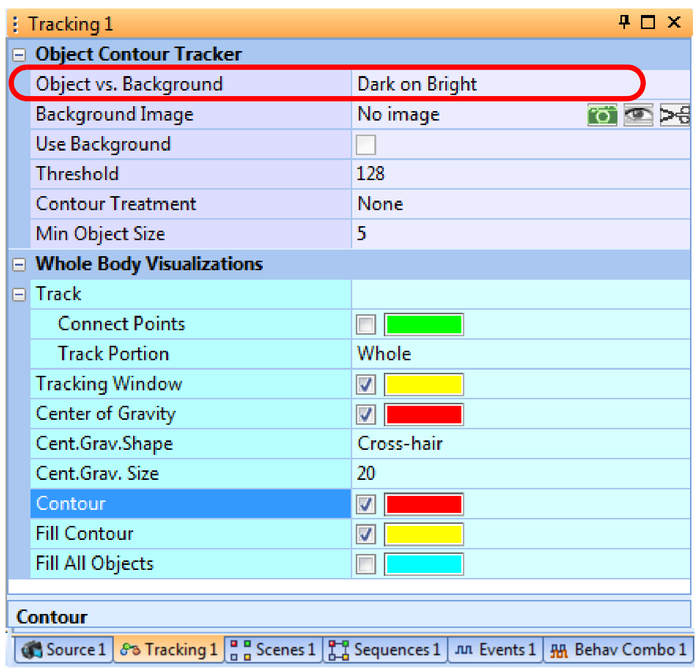
1 Place the animal in the camera field of view.

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 Section 2.3, “Setting Up Cameras and the User Interface” on page 19.

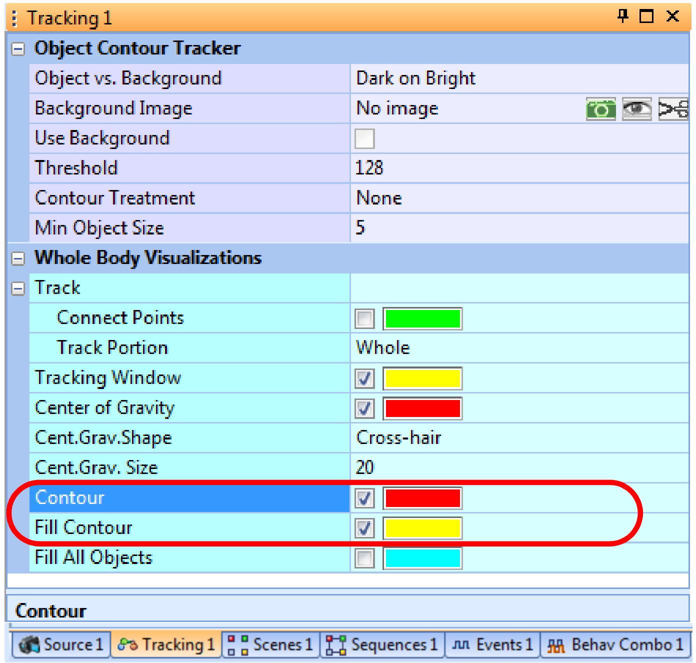
3 To select the Object Contour tracking mode, click the appropriate icon on the Tracking toolbar.

4 Click the Tracking tab to display the tracking parameters.

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:

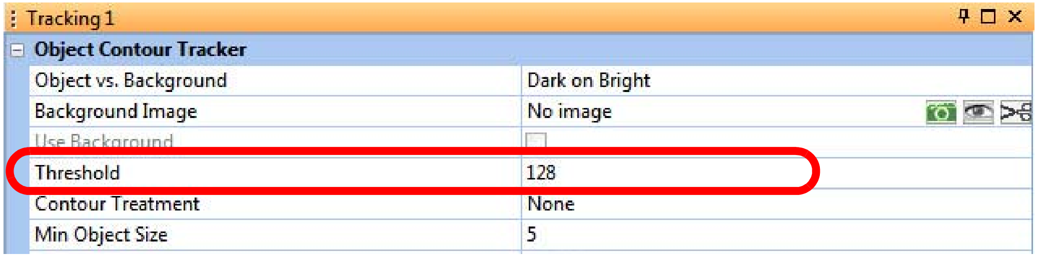
- Section 6.13.2, “Setting the Threshold” on page 108
- Section 6.13.3, “Object Contour Mode Advanced Functions—Overview” on page 110

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

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

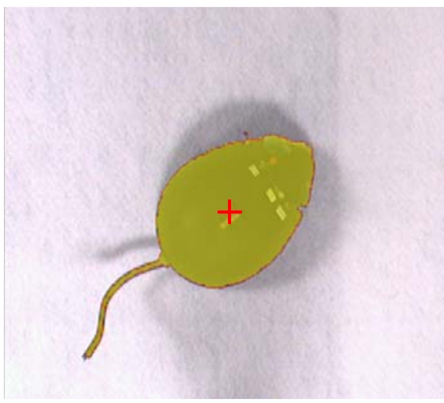
6.13.2 Setting the Threshold

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.



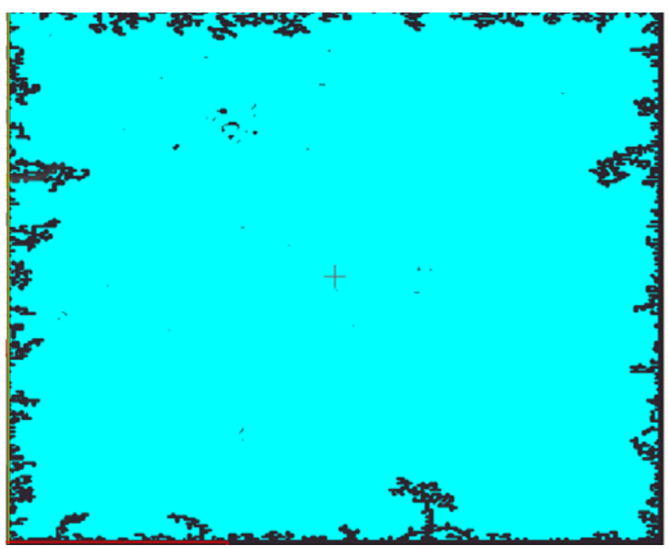
Note: If the background is not uniform or does not have good contrast with the animal, setting the **Threshold** parameter might not be sufficient for tracking. If this condition exists, perform the procedure in Section 6.13.5, "Background Subtraction" on page 113 to remove the background from the video.

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

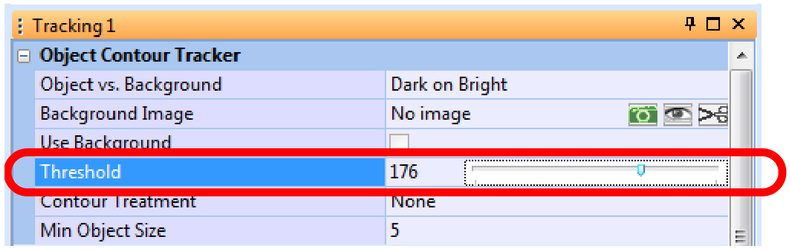


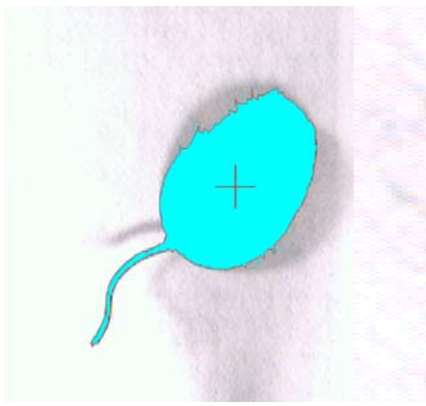
Use the guidelines below to adjust the **Threshold** setting.

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 any of the enabled cameras 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 9, Recording and Monitoring Video.

6.13.3 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:

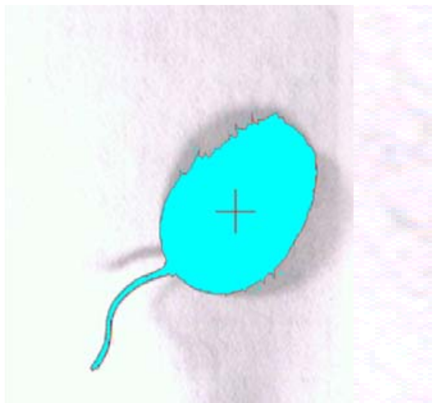
- Section 6.13.4, “Detail Filtering Adjustment” on page 111
- Section 6.13.5, “Background Subtraction” on page 113
- Section 6.13.6, “Close Contour Option” on page 117

6.13.4 Detail Filtering Adjustment

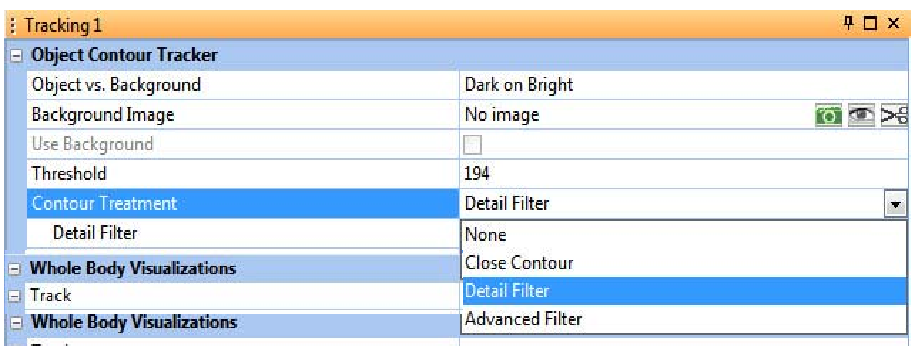
Use Detail Filtering to remove small features of the image that distort the tracking results. For example, a long tail on a target animal can skew the centroid calculation; cables attached to the target can also skew the results. The Detail Filtering adjustment parameter, which has a range of 1 - 10,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

To set the Detail Filtering Adjustment value

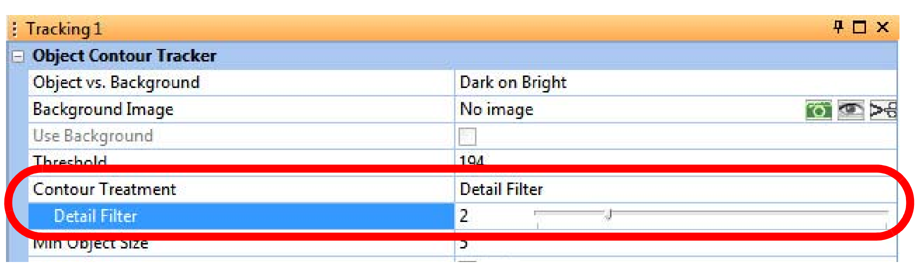
1 In the Properties window **Object Contour Tracker** area, click **Show Contour**, **Fill Contour** and/or **Find All Objects** checkboxes, and view the video image.



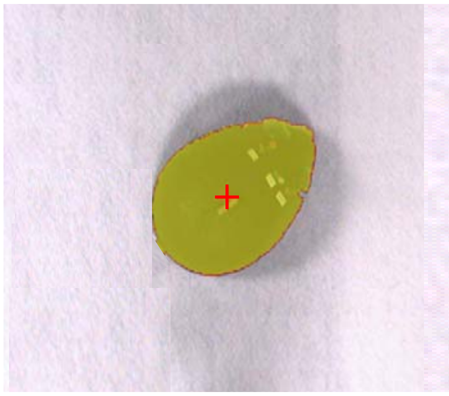
2 Select **Detail Filter** in the **Contour Treatment** dropdown list.



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.



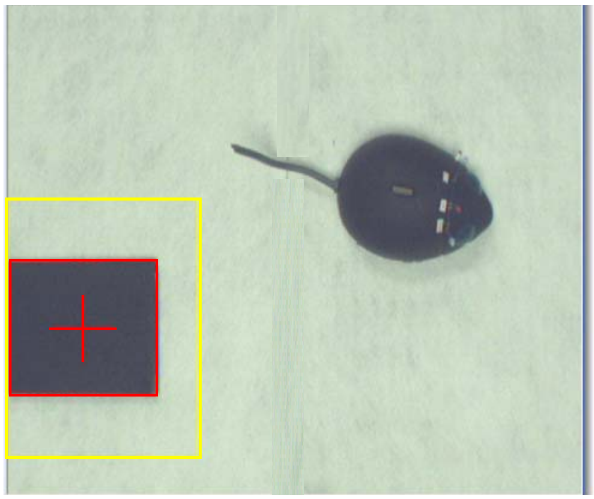
Note: The system automatically saves the parameter values you set.

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

6.13.5 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).

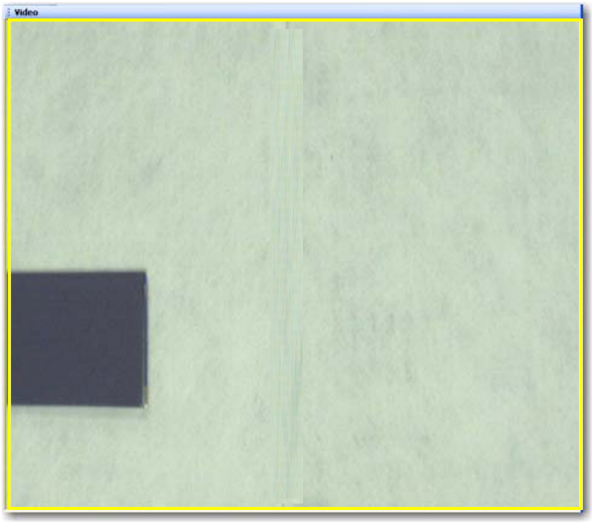


To avoid this situation, you can use one or both of the following tools:

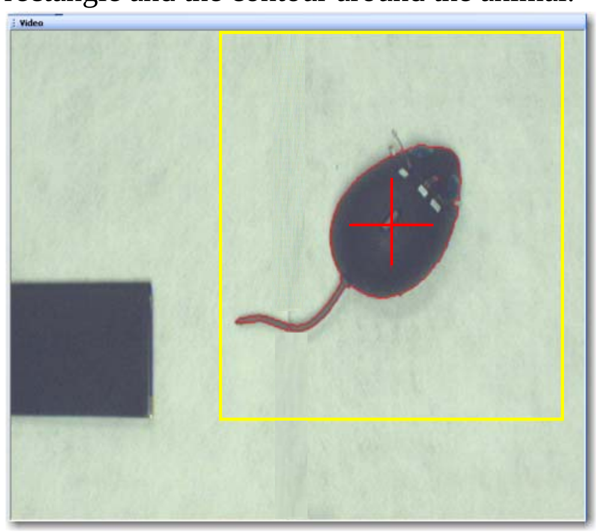
- Use background subtraction (discussed in this section)
- Define a specific arena shape in which to track (discussed in Section 6.10.2, "User-defined Arenas" on page 97)

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.

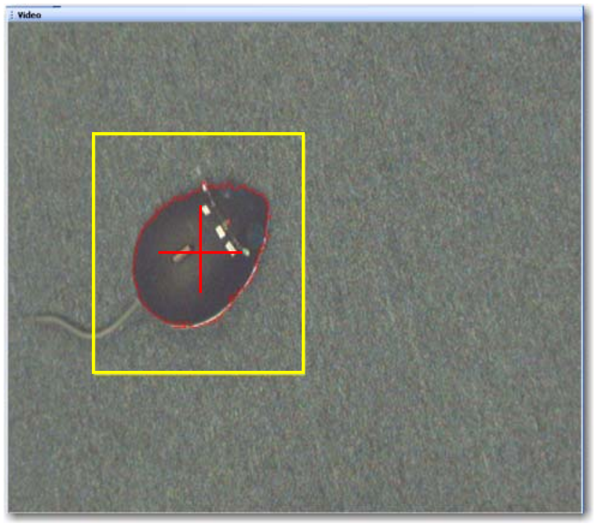


This image shows the background object and the animal together after the user has configured the background subtraction function. Note that the system is tracking the animal, as indicated by the positions of the red cross hair and yellow rectangle and the contour around the animal.



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

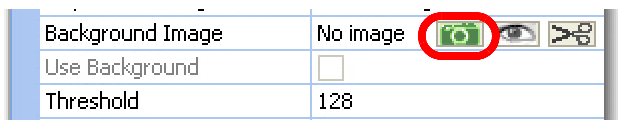
Shelf-lining paper can be used to alter background color

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.

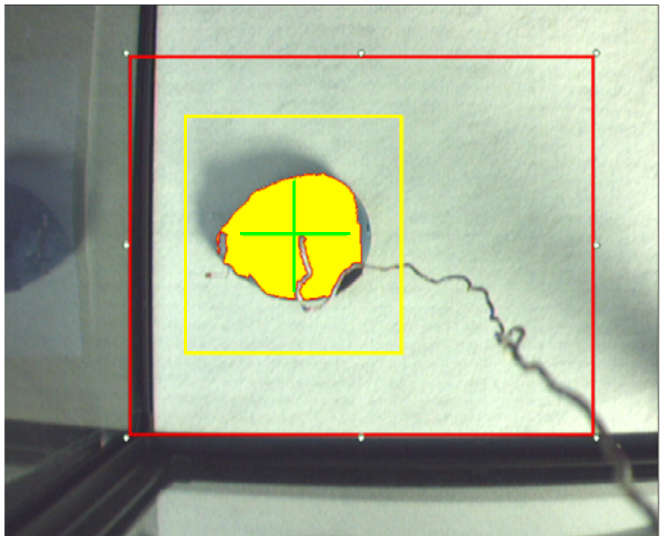
5. Replace the animal in the tracking area.
6. Adjust the Threshold (see diagram above) so the animal is detected and the animal's outline is filled in the Video window (see Section 6.13.2, "Setting the Threshold" on page 108).

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

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

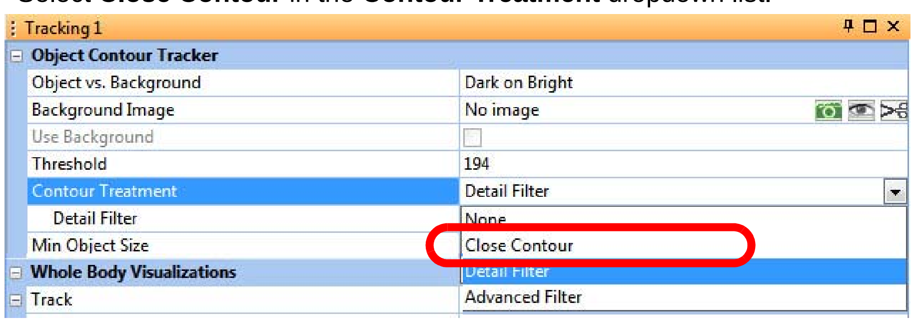
6.13.6 Close Contour Option

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.

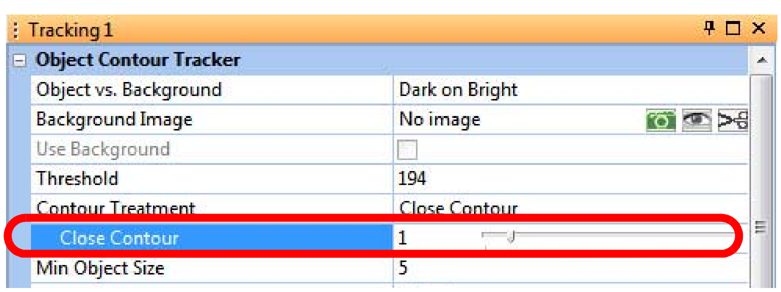


To apply the Close Contour option

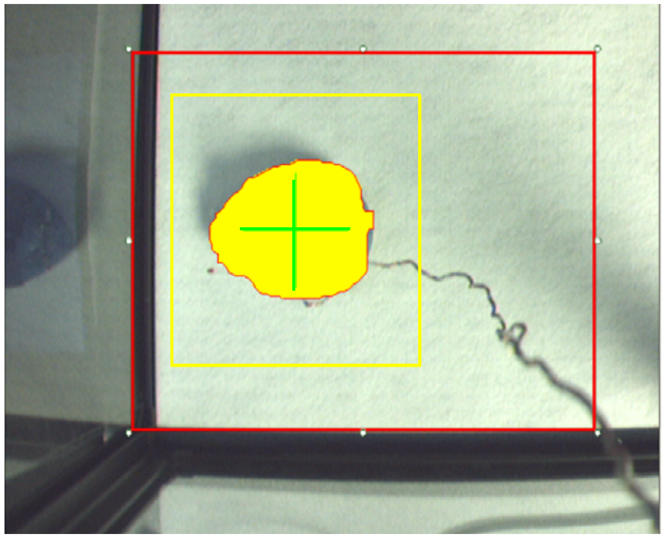
1 Select **Close Contour** in the **Contour Treatment** dropdown list.



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



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



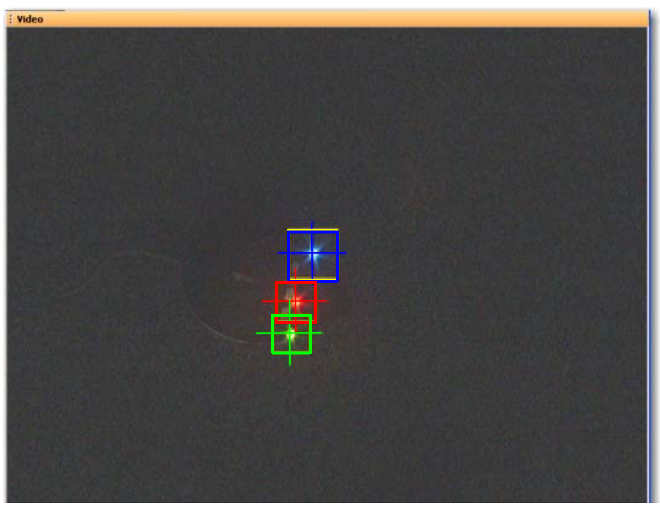
Note: The system automatically saves the parameter values you set.

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

6.14 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.

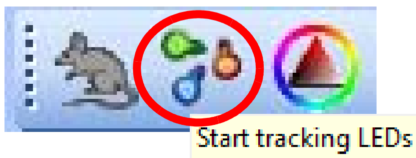
In darkness, the LEDs are the brightest spots in the window, therefore, the LED tracking mode is excellent for this condition. The image below shows three LEDs being tracked in darkness.

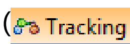


6.14.1 Configuring Parameters for LED Tracking

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 Section 2.3, “Setting Up Cameras and the User Interface” on page 19.
3. To select the LED tracking mode, click the appropriate icon on the Tracking toolbar.

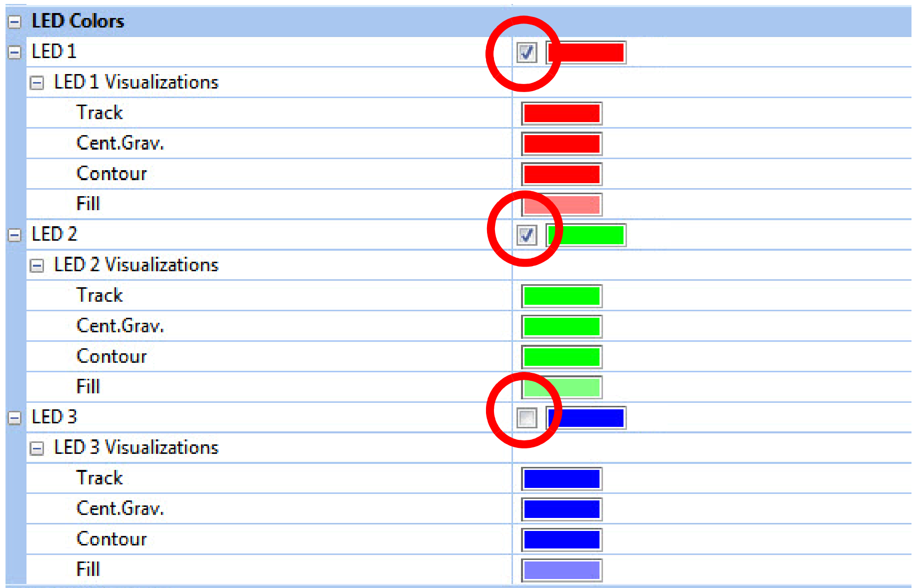


4. Click on the Tracking tab () to view the Tracking parameters.
5. In the **LED Visualization** group check **Contour** and **Fill Contour** so that the objects found by the system are visible. These selections are common to all LEDs used in the experiment.

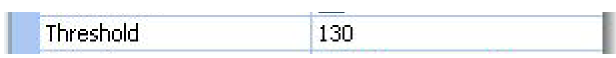
6. Check the **Pure Colors** checkbox in the LED Tracker area. Checking this box causes the standard red, green and blue LED colors to appear in the **LED Colors** area in the Tracking configuration tab.

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.



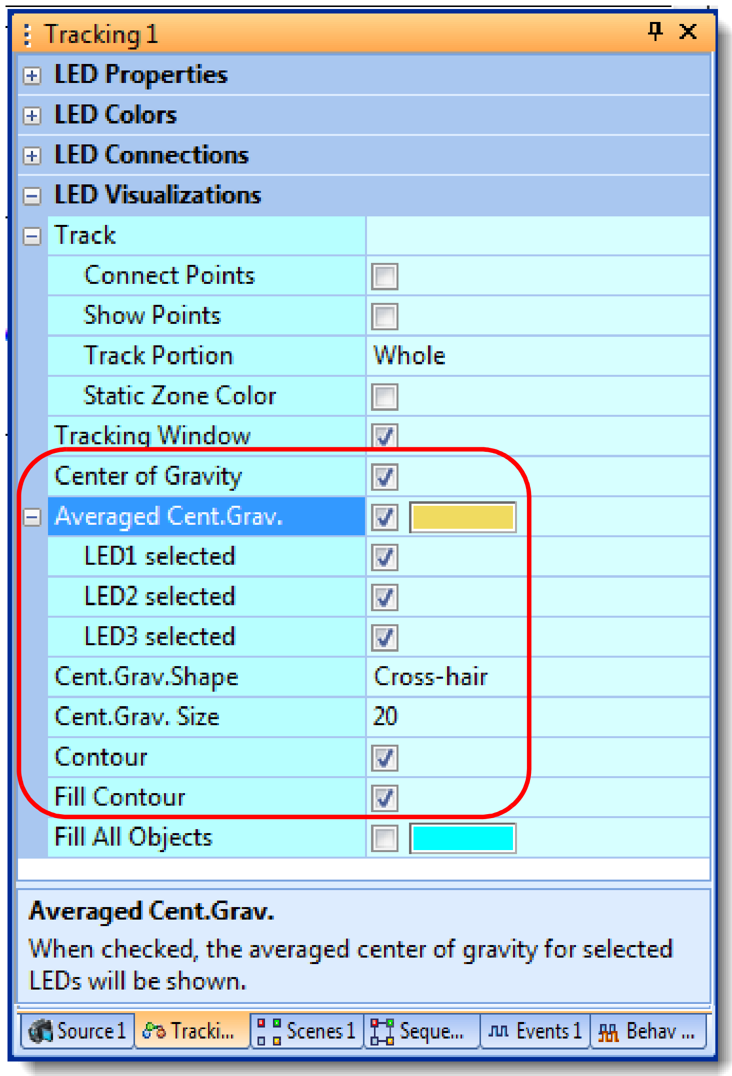
9 If the **Pure Colors** option does not give good results, use the pick-up tool to select the color from the video (see "Using the Color Pick-up Tool with LED Tracking" below). Adjust the **Threshold** as needed.

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 7, Configuring the Behavioral Events Parameters 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 7.13.5, “Events Based on Head Direction To Object” on page 170.



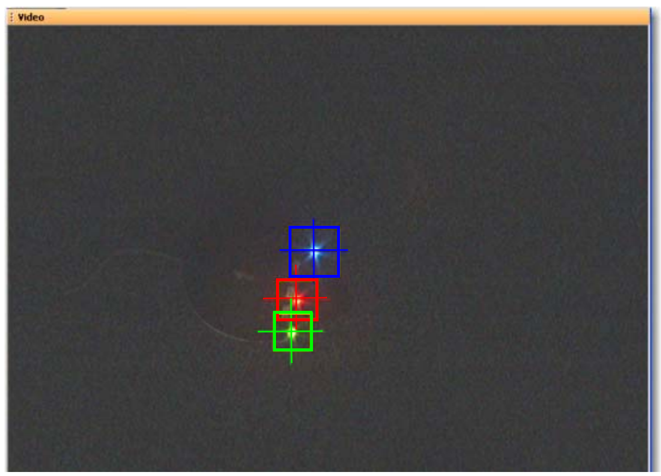
11 Now you may record sessions as explained in Chapter 9, Recording and Monitoring Video.

6.14.2 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.

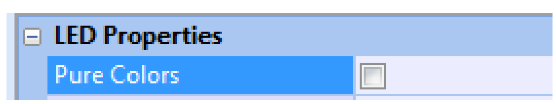
LED color shade selection is possible, but requires precision in the pixel selection process. This is because an LED image is a hot white center surrounded by pixels of varying shades of color. You must click on one of the pixels of the desired color, but not white.

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

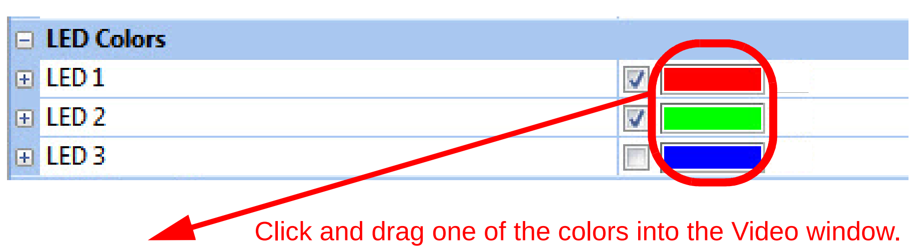


2 At the camera lens, adjust the zoom, focus, and iris settings for the best picture quality. For additional guidance on camera setup and video quality, see Section 2.3, “Setting Up Cameras and the User Interface” on page 19.

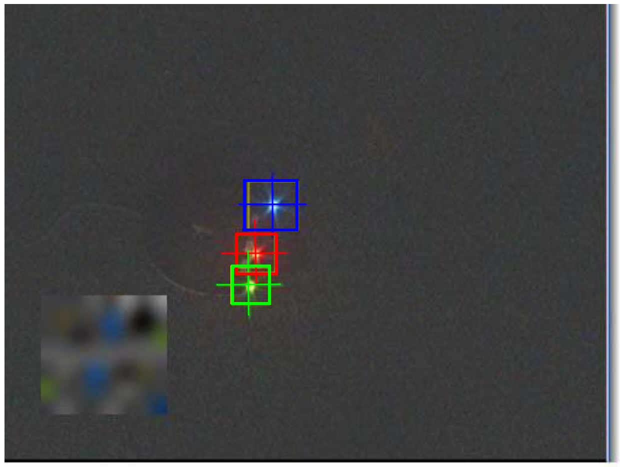
3 Deselect the **Pure Colors** option.



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.

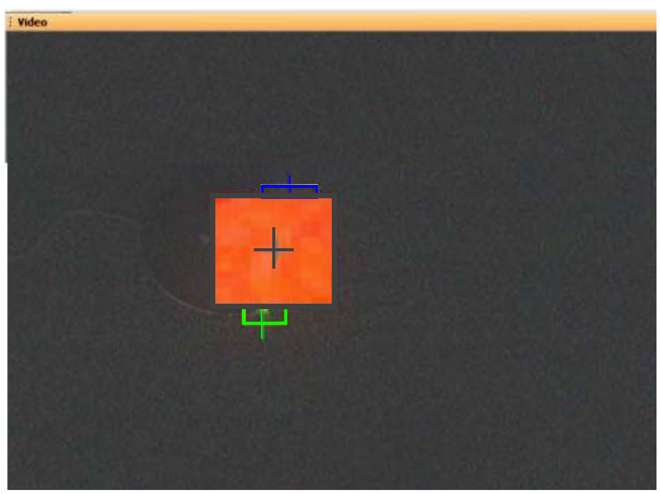


5 In the Video window a square area surrounding the cursor displays as a magnified window. Move the magnified window over the area to be shown in the **Color Selection** box. The image below shows the magnified cursor window in the lower left of the diagram - note the digitization of the area.



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.

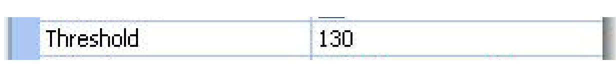
Note: Be sure that the pixel is the dominant color of the LED, and not the white center of the LED.



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 6.13.2, “Setting the Threshold” on page 108.

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 Section 2.3, “Setting Up Cameras and the User Interface” on page 19.

11 Repeat the steps in this section for any additional camera(s) to be used in the experiment.

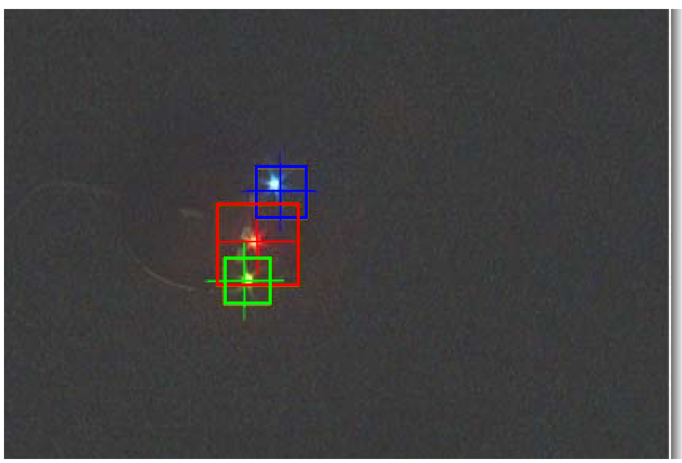
Note: The system automatically saves the parameter values you set.

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

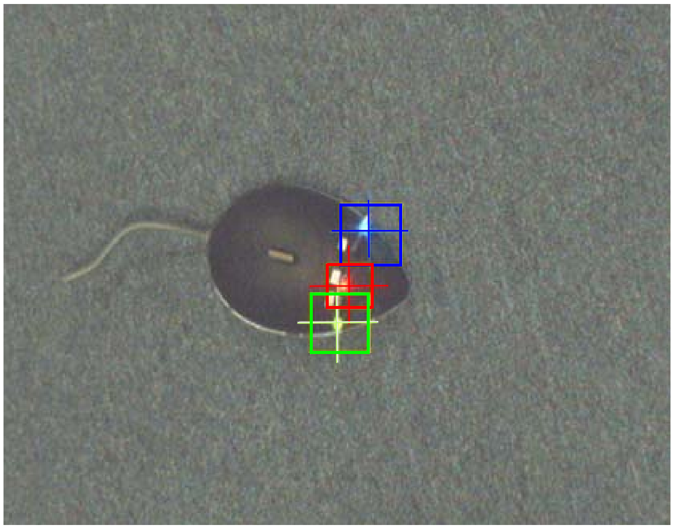
6.15 LEDs In Darkness or Light with Color Markers Tracking

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

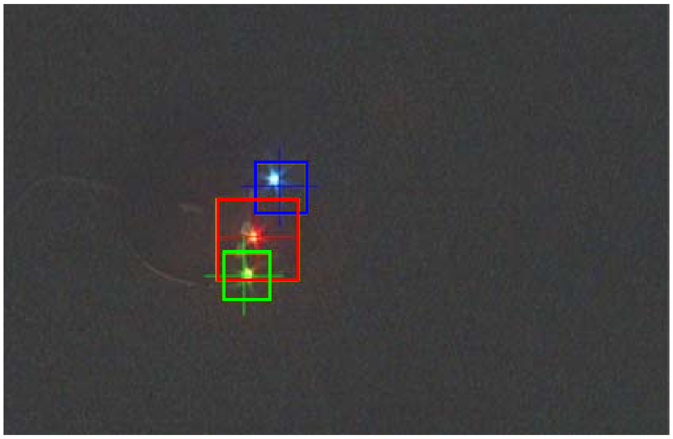
Follow the same procedure as with Color Markers Tracking (below).

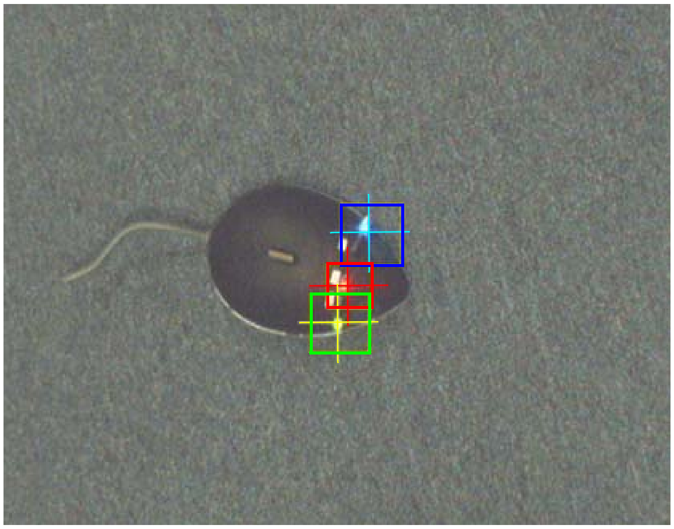
6.16 Color Markers Tracking

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

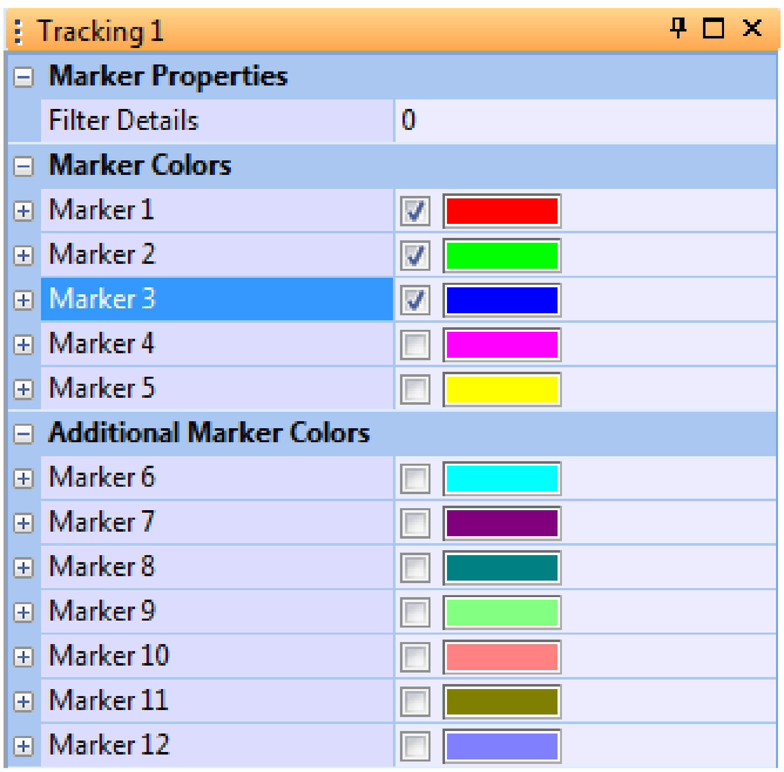

Color markers

6.16.1 Enabling Color Markers Tracking

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

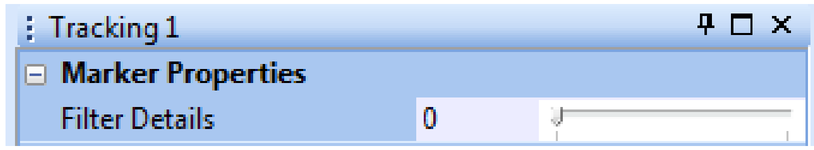
1. Place the colored targets in the camera field of view.
2. At the camera lens, adjust the zoom, focus, and iris settings for the best picture quality. For additional guidance on camera setup and video quality, see Section 2.3, “Setting Up Cameras and the User Interface” on page 19.
3. To select the **Color Markers** mode, click the appropriate icon on the Tracking toolbar.

4. Click on the Tracking tab (Tracking) for Camera 1 to view the Tracking parameters.



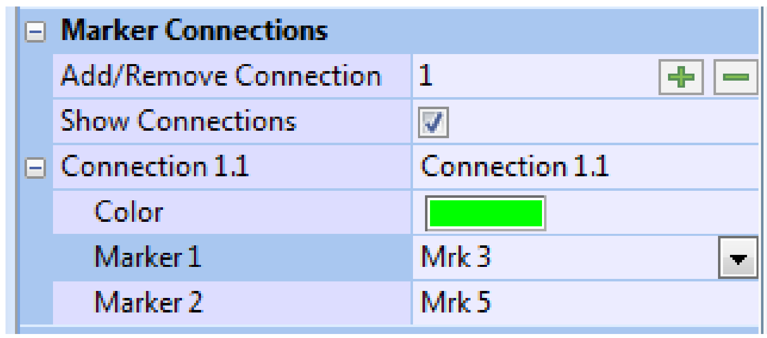
Note: The adjustment of the color for each Color Marker will be discussed in Section 6.16.2, “Using the Color Pick-up Tool with Color Marker Tracking” on page 131

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.

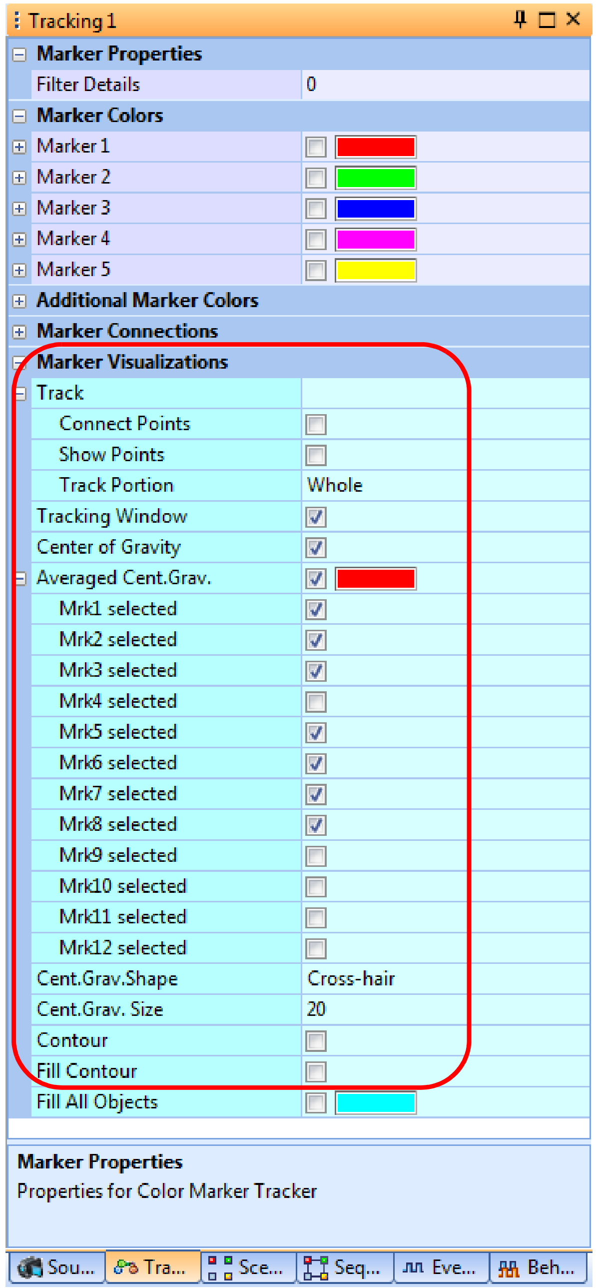
7 If desired, in the **Marker Connections** area, add connections between pairs of markers, and select the **Show Connections** checkbox if you want to display these connections.



The **Marker Visualizations** area (below) contains a number of parameters that allow you to customize the display of color markers in the 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 7, Configuring the Behavioral Events Parameters based on their typical usage.

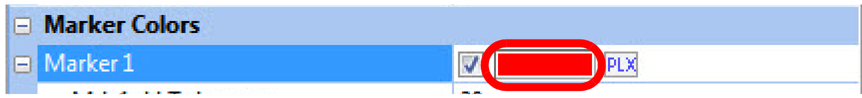
The **Averaged Cent.Grav.** is another useful parameter. You can select which LEDs will be included in the average. See the description in Section 7.13.5, “Events Based on Head Direction To Object” on page 170.


Tracking 1
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
Sou...
Tra...
Sce...
Seq...
Eve...
Beh...

6.16.2 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.

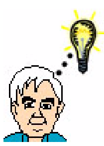


2 The image below shows a sample video window before clicking the **Color Selection** box.

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.

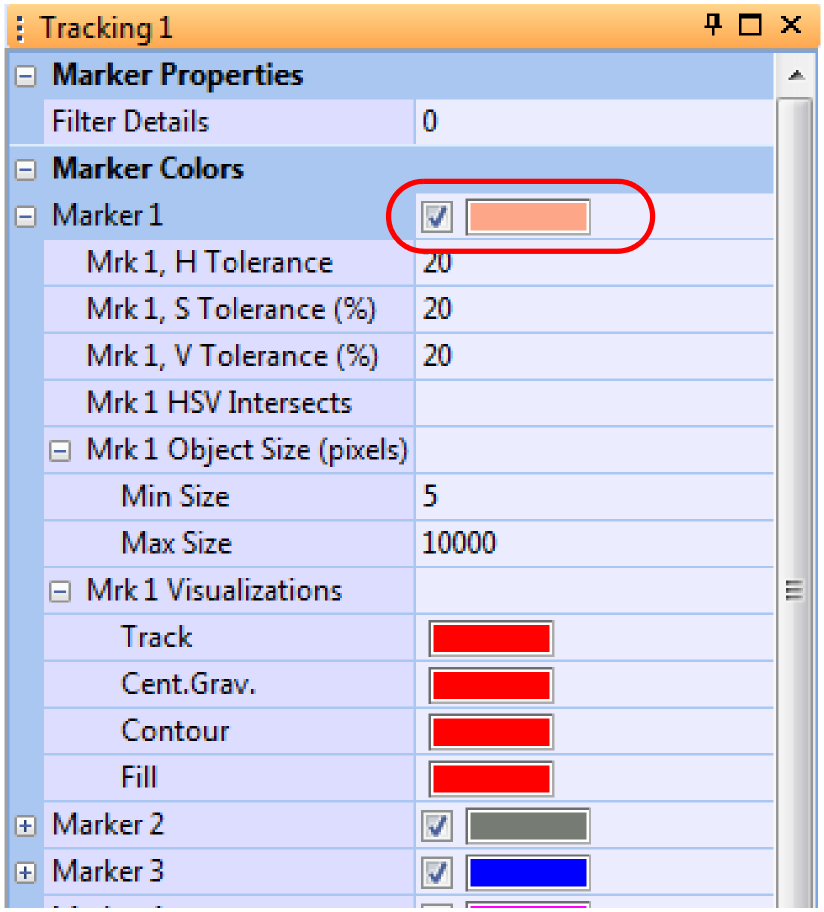


TIP

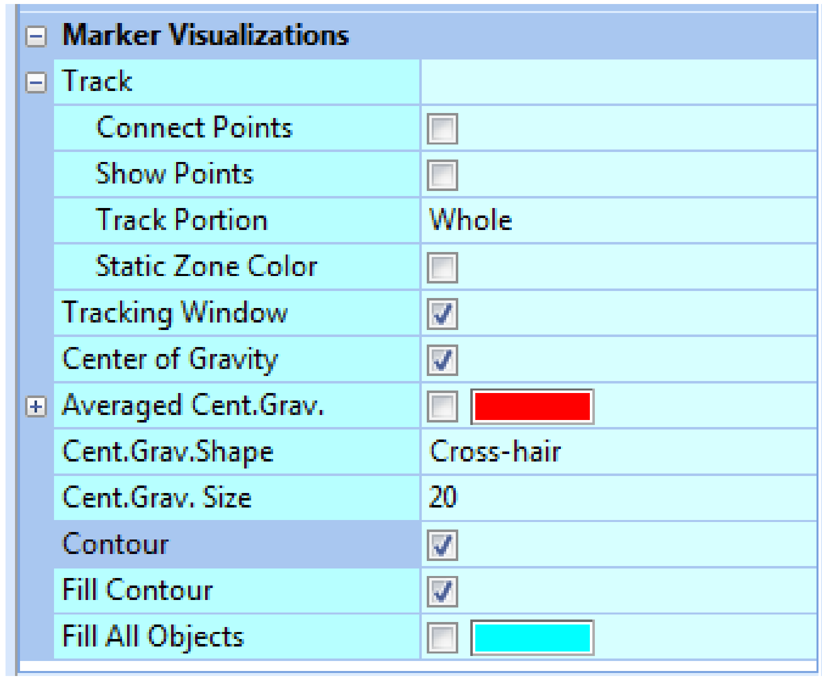
Select the pixel that gives the best tracking

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 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 Video window needs improvement, repeat the procedure in Section 2.3, “Setting Up Cameras and the User Interface” on page 19.

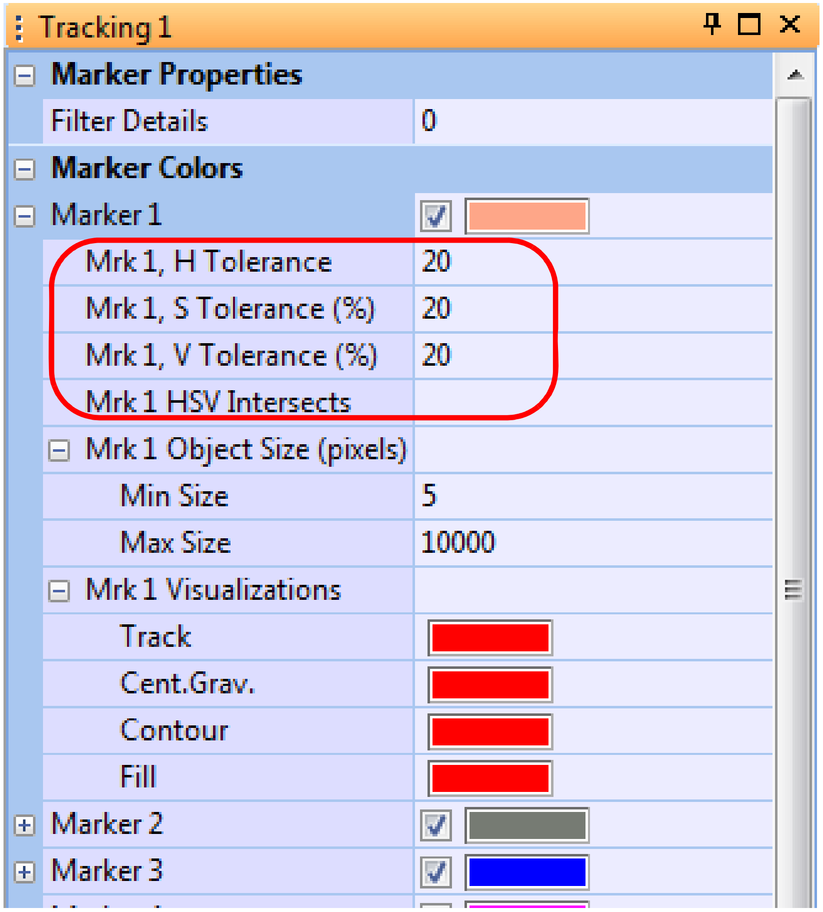
9 Repeat Step 1 through Step 8 for the other colors that will be tracked in the experiment.

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.

6.16.3 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 **Adjust 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.

6 Repeat the steps in this section for any additional camera(s) to be used in the experiment.

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

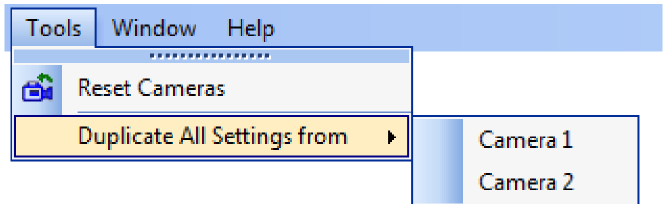
6.17 How to Duplicate Parameter Settings to All Cameras

Some characteristics of all video windows (assuming you are using two or more video streams) are duplicated automatically across all windows as you set them, regardless of the video window in which you configured the characteristic. These settings include, for example, the creation or deletion of arena and zone shapes. timecode display format, certain LED and Color Marker selections, the **Use Calibration** feature, and the calibration units (inches or cm). For these parameters, configuring a value in one video window causes the same value to be assigned automatically in all video windows.

In addition, some parameter settings can be copied from one camera to all the other cameras if you choose to do so. These include, for example, modifications to arena and zone dimensions and locations, the calibration **Global Factor** setting, and the display colors that appear in the video windows.

1 To duplicate settings, from the **Tools** menu, select **Duplicate All Settings from** and then choose one of the cameras in the dropdown list. The system

will immediately copy the applicable parameter settings from the camera you select to all the other cameras.



Note: It is highly likely that some of the duplicated settings will need to be modified for the other cameras.

2 Before you start recording any sessions, review all the values you have set for all cameras and ensure that they are set appropriately. It is recommended that calibration be individually adjusted for each camera. This is because it is unlikely that all cameras are positioned at the same distance, viewing angle and magnification from their respective arenas.

6.18 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 10.14, “Using the Overlay Feature during Analysis” on page 305
- Appendix E, Modifying Arenas and Zones

6.19 Where to Go Next

If you would like to configure basic behavior parameters, go to Chapter 7, Configuring the Behavioral Events Parameters.

Chapter 7
Configuring the Behavioral Events Parameters

7.1 Before You Start

Verify that you have completed the procedures in Chapter 6, Configuring the Tracking Parameters.

7.2 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.

7.2.1 Procedures for Behavioral Events and Data Analysis

Following are the procedures for behavioral events and analysis that the system provides in addition to the tracking features described in Chapter 6, 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 from one or multiple cameras to generate logical events and electrical signals
- 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 on a headstage.
- 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 (within each Video window and across multiple Video windows) according to user-specified variables associated with each session.
- 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.

Note: As an example, you can connect a cable from the Plexon® PlexBright® 4 Channel Controller (PlexBright Controller) to the CineLyzer® System DIO Interface and configure the PlexBright Controller to start playing a specified stimulation pattern when a digital output signal is generated by the CineLyzer System. For details, see "Connecting CineLyzer System to Optogenetic Stimulation Device" on page C-9. The DIO Interface is described in Appendix C, USB Digital Input/Output Interface." The cable and PlexBright Controller are sold separately.

7.2.2 Terminology

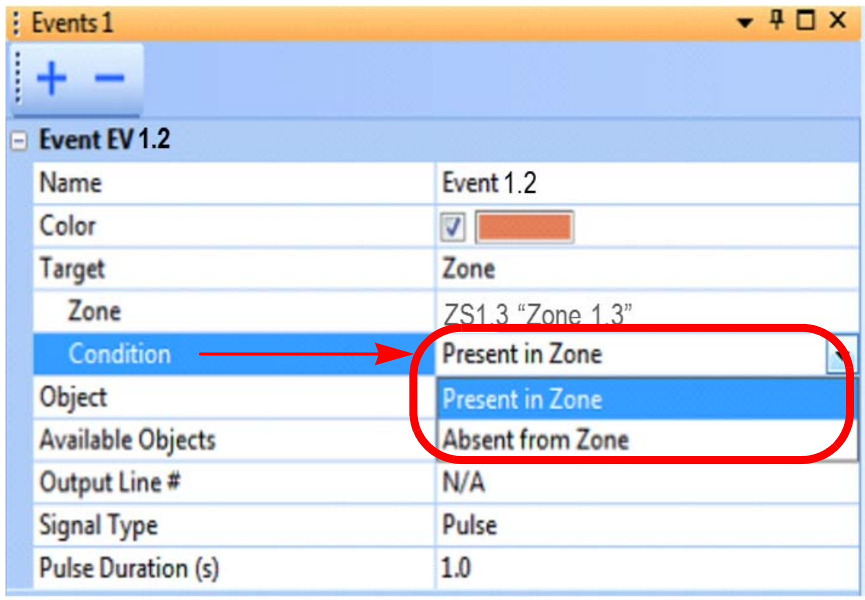
The following terminology is useful in discussing behavior recording, tracking and analysis:

- **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.

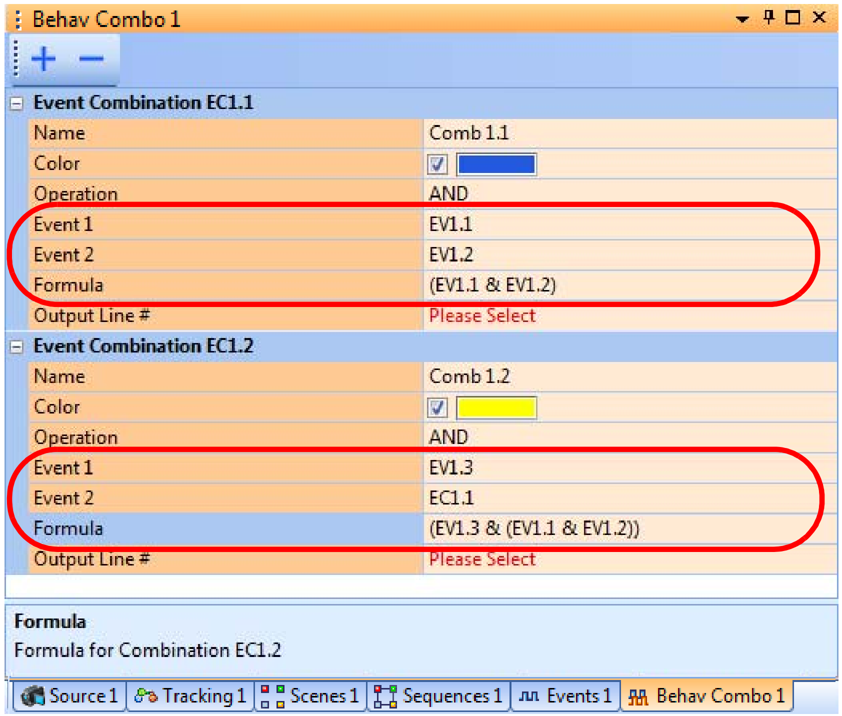
- **Static Zones**: Zones that do not move with respect to the image or the arena are referred to as static zones. Often they are used in object recognition, place preference or conditioning experiments. For example, a static zone could be an area the animal should be present in or avoid to receive a 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.

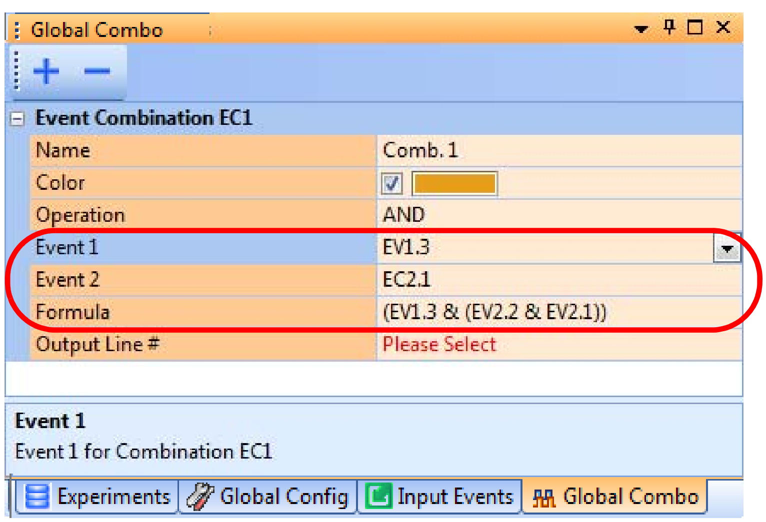


Note: The system can generate electrical pulses or levels (high or low) based on logical event states or state changes. This is particularly useful in some optogenetic protocols for triggering laser firing events based on the subject’s location.

- **Combination Events per video source (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.

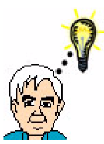


7.3 Setting Up Cameras and the User Interface

The procedures for setting up cameras and the user interface are described in Section 2.3, "Setting Up Cameras and the User Interface" on page 19.

7.4 Navigating the User Interface

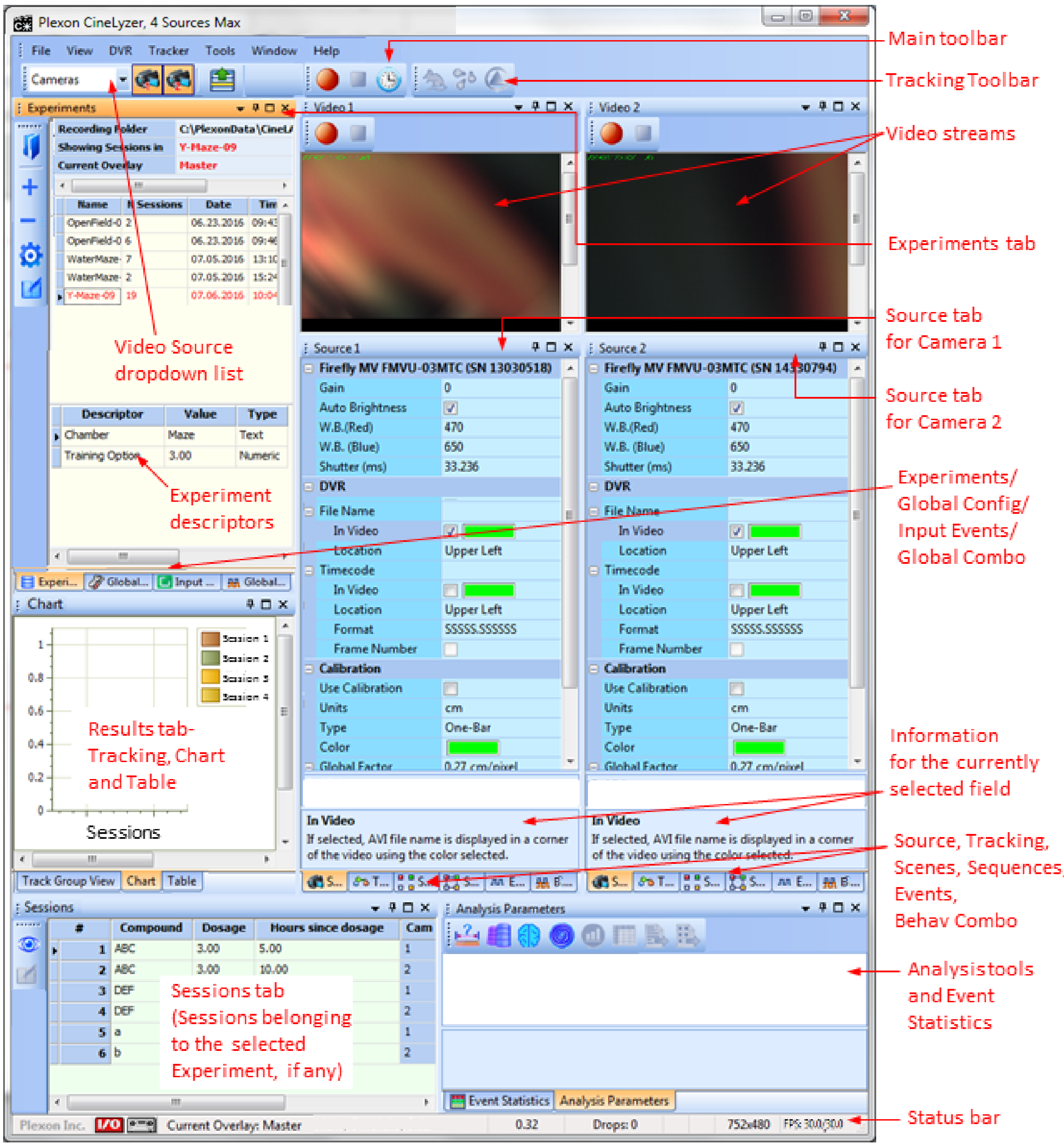
This image shows the location of the windows in the default layout of the CineLyzer user interface. In this example, there are two cameras connected; up to four cameras can be licensed and connected (optional).

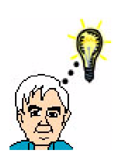


TIP

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**.

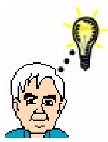




TIP

Verify "Cameras" Is Selected

Camera settings do not display in the user interface unless **Cameras** is set in the main toolbar.



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 list in the main menu.

Additional information is available in Appendix B-Navigating the Plexon User Interface.

There are additional parameters that need to be configured to enable and manage the basic behavior functions, and to analyze the data. These parameters are covered in this chapter.

7.5 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 32
- Section 3.2, “Planning the Database” on page 32
- Section 3.3, “Setting Parameters for an Experiment with Multiple Sessions” on page 33
- Section 3.4, “Setting the Recording Folder Location” on page 36
- Section 3.5, “Creating a New Experiment” on page 37
- Section 3.6, “Selecting a Previously Saved Experiment” on page 45
- Section 3.7, “Editing the Experiment Name, Descriptor and Variable Values” on page 46

7.6 Calibrating the Arena Dimensions

In many experiments, it is useful to calibrate the video image for each camera so that sizes, positions and velocities are reported in the desired units of measure instead of pixels. See Chapter 4, Calibrating the Arena Dimensions, for the procedure.

7.7 Digital Inputs and Outputs

The system can receive up to 12 digital inputs through the Plexon CineLyzer USB Digital Input/Output Interface (DIO Interface), which is included as a standard item in the CineLyzer System order. See the detailed procedure in Section 5.6, “Configuring the Input Events Parameters” on page 74.

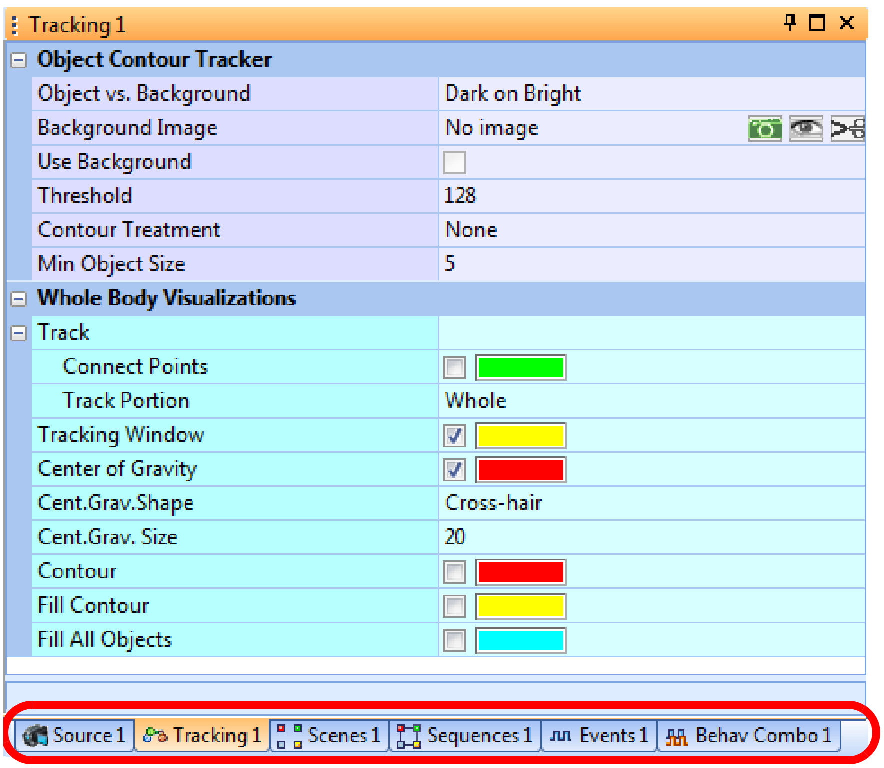
The DIO Interface is described in detail in Appendix C, USB Digital Input/ Output Interface.”

The system can generate up to 12 digital outputs when an individual event or a combination of events occurs. See the detailed procedure in Section 7.17, "Specifying Digital Outputs" on page 190.

One of the important applications for digital inputs and outputs is to connect the CineLyzer System with the Plexon PlexBright® 4 Channel Controller (PlexBright Controller) to provide coordination between an animal's behavior and optogenetic stimulation. The connection is made through the Opto Controller DIO Bare Wire cable (sold separately) as described in "Connecting CineLyzer System to Optogenetic Stimulation Device" on page C-9.

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

Each 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:

- **Source**—Used to set parameters for the video stream, either from cameras or from existing files. There will be multiple Source tabs if there are multiple cameras on the system. This tab contains parameters that relate to optical settings for each 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).
- **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.
- **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 7.14, "Defining Combination Events per Video Source" on page 179.

7.9 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.

7.9.1 Before You Start

The procedures in Chapter 6, Configuring the Tracking Parameters are required for configuring an arena for each camera. Arena and tracking parameters must be configured before zones and behavioral events can be created.

7.9.2 Overview of Procedure

The procedure consists of the following tasks:

- Section 7.10, "Adding Zones (Static and Dynamic)" on page 149
- Section 7.11, "Defining Sequences of Zones" on page 157
- Section 7.12, "Modifying Arenas, Zones and Calibration During an Experiment" on page 159
- Section 7.13, "Defining Logical Events" on page 160

7.10 Adding Zones (Static and Dynamic)

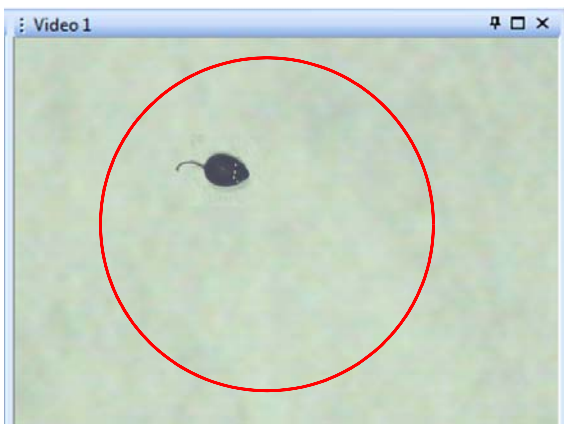
There are two types of zones, static and dynamic. Static zones are created by clicking the **Static Zone** icon and defining the shape, size and location of the zone in the video image. Dynamic zones are created by clicking the **Dynamic Zone** icon and selecting the LED or color marker around which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

Note: See See “Terminology” on page 141 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.

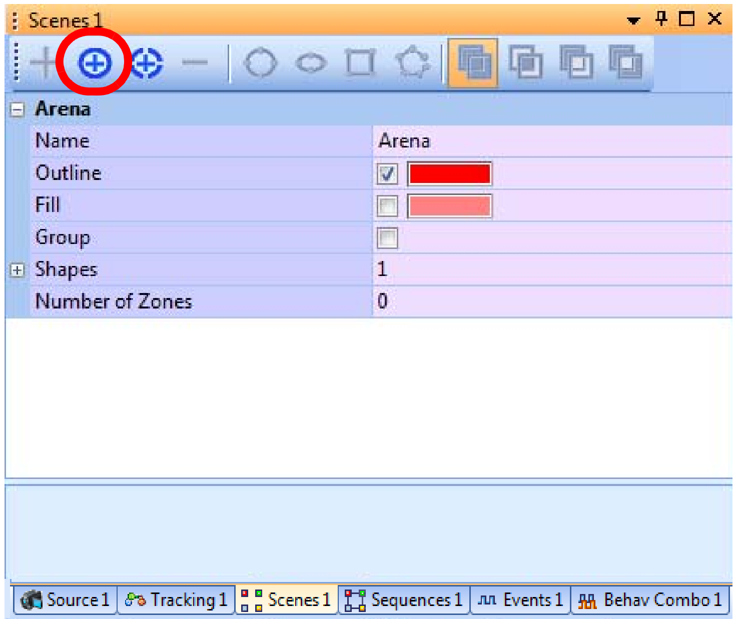
7.10.1 Adding a Static Zone

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

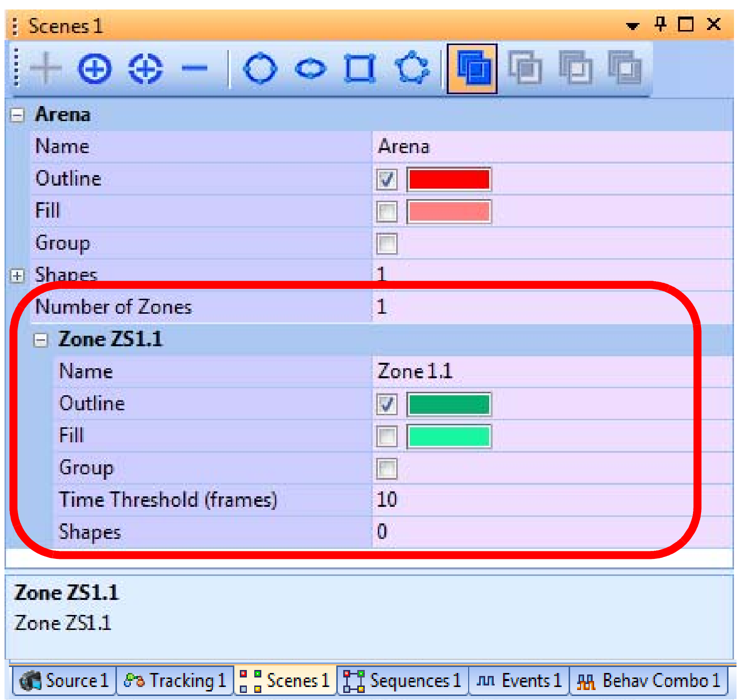
In this example, we use a red circle for the arena.



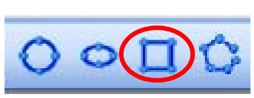
1 On the **Scenes** tab click the **Add static zone to current arena** icon.



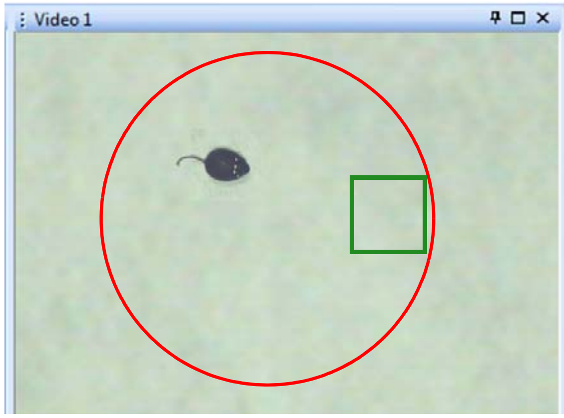
The parameters are displayed for the new Static Zone.



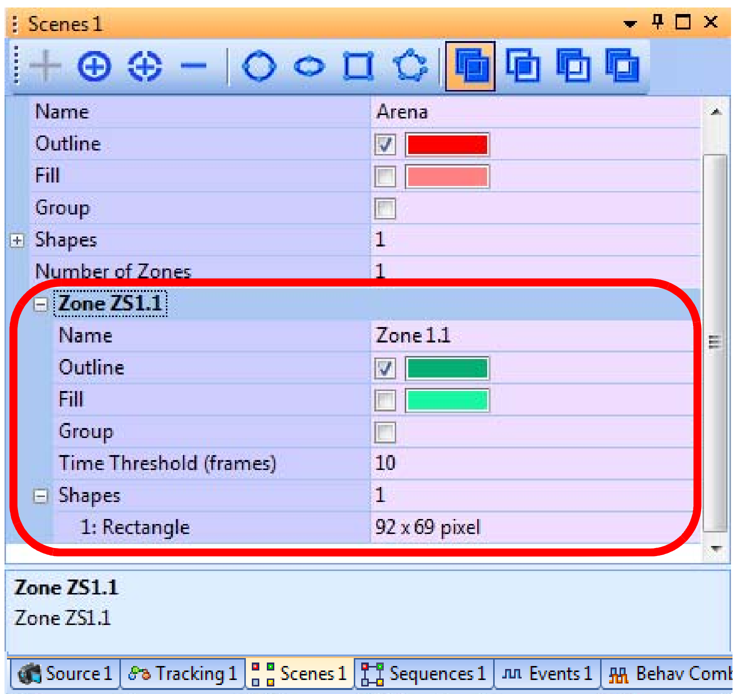
2 Select the drawing tool that makes the most sense for the zone that needs to be created. In the example that follows, the rectangle shape will be selected.



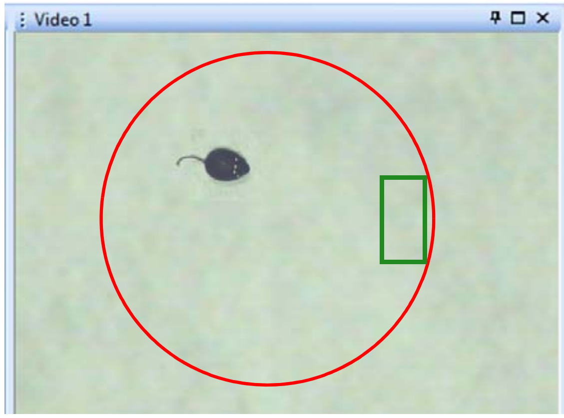
3 Draw the zone outline over the image corresponding to the first physical zone of interest. For the image below, the existing arena is a red circle, and a green rectangle has been added as the zone. This rectangle might represent, for example, a novel object the subject can view.



The Scenes tab shows the parameters for the zone shape.

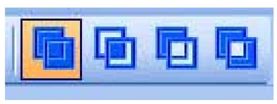


4 If desired, adjust size and shape of the zone shape. For the image below, the rectangle was adjusted to better fit the actual object of interest.



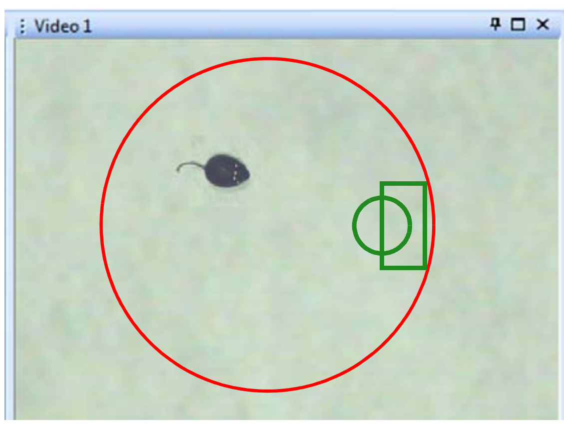
5 If a complex zone is desired, select a different drawing tool if needed, and the operator to be applied.

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:

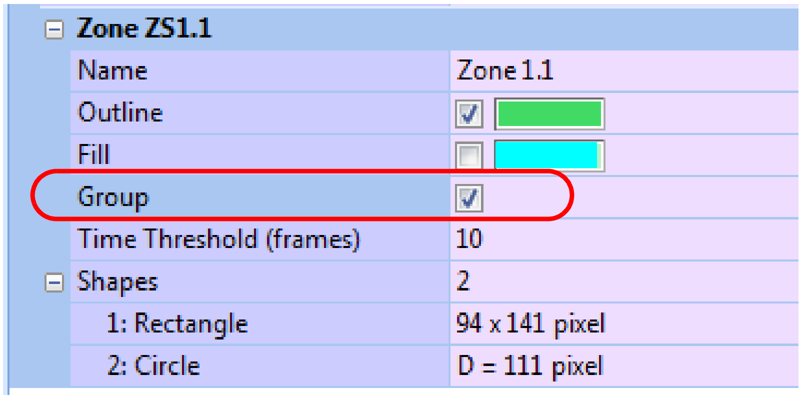


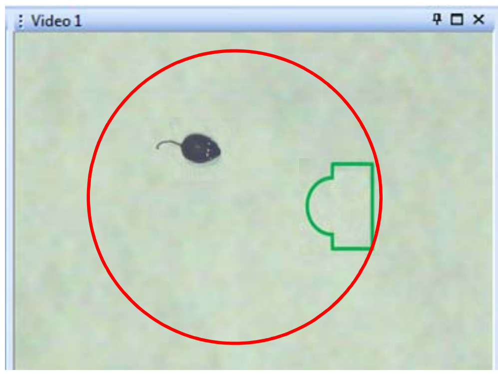
For a detailed description of the operators, see Section 6.11, “Defining the Arenas” on page 100.

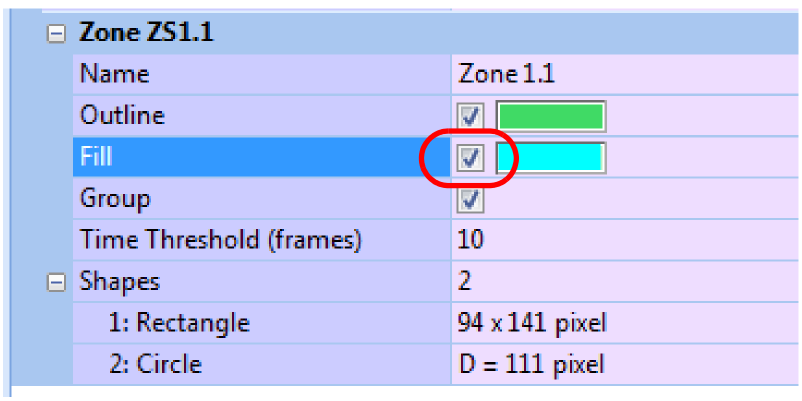
The image below shows an example of a complex static zone obtained using the **Union shapes** operator on round and rectangular shapes.

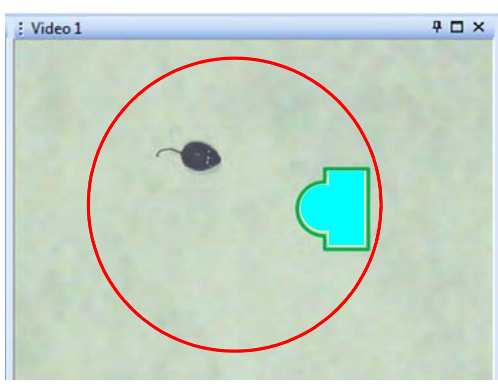


6 If desired you can group the shapes, so the system treats the combined shape as a single entity. You can also fill the shape with a color. See the examples below.







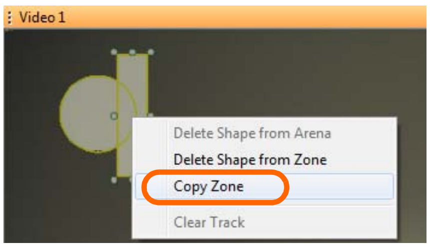


Note: If you have created a group shape (a combined zone object) you will be able to move the entire combined zone object as a unit.

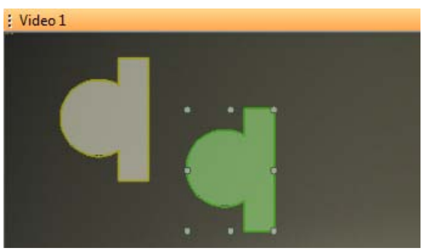
TIP

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.

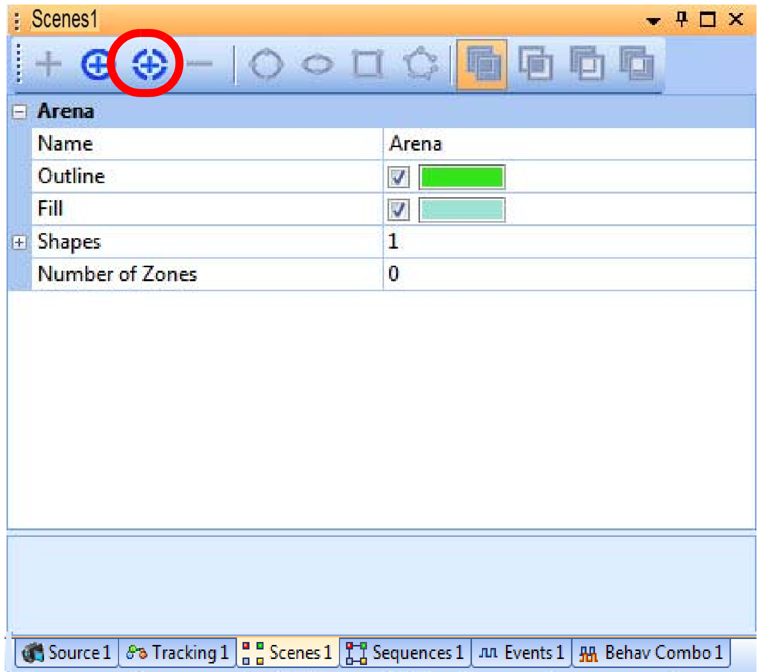
7 If necessary, adjust the value for the **Time Threshold (frames)** parameter. This parameter specifies the number of frames that an object must be detected in the zone before it is considered “present.” Whether an object is “present” or not can be used to trigger a zone-related event. You can adjust this value at any time before you start recording. For a further description of this parameter, see Section 7.13.1, “Events Based on a Zone” on page 7-161.

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

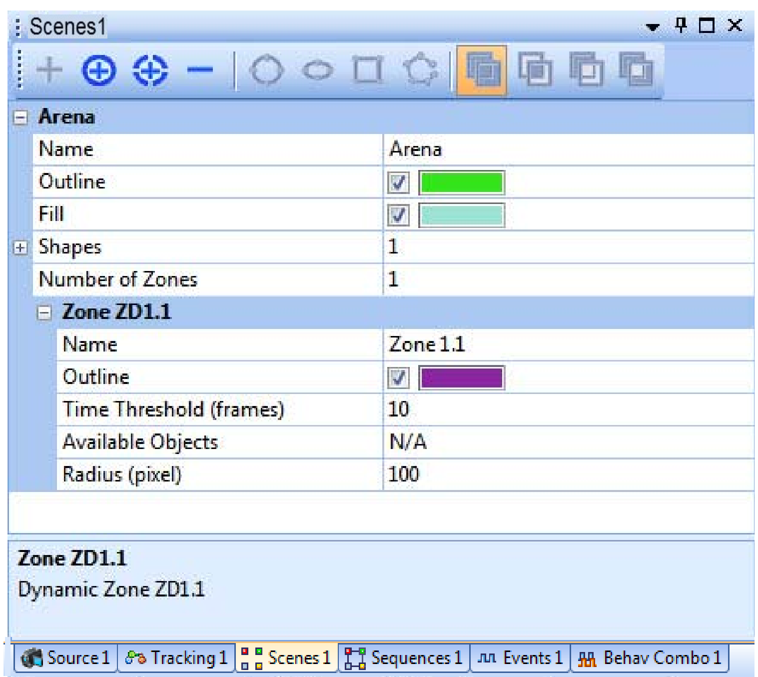
7.10.2 Adding a Dynamic Zone

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

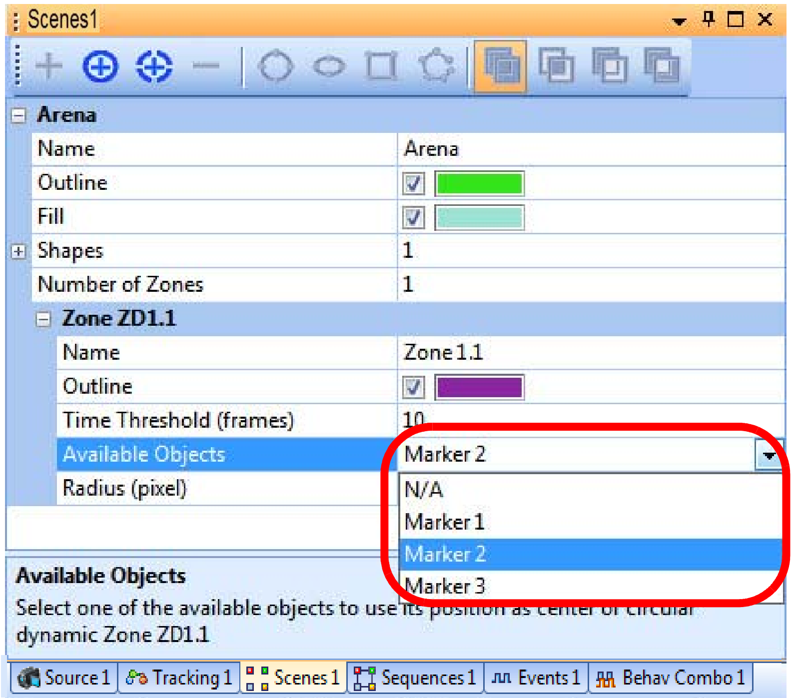
1 On the Scenes tab click the **Add dynamic zone to current arena** icon.



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.

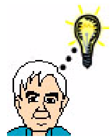
The **Time Threshold (frames)** parameter specifies the number of frames that an object must be detected in the zone before it is considered “present.” Whether an object is “present” or not can be used to trigger a zone-related event. You can adjust this value at any time before you start recording. For a further description of this parameter, see Section 7.13.1, “Events Based on a Zone” on page 7-161.

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.

7.11 Defining Sequences of Zones

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

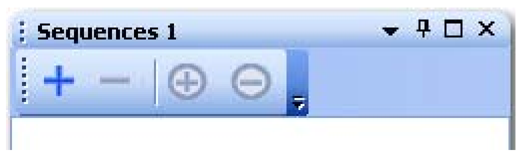


TIP

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

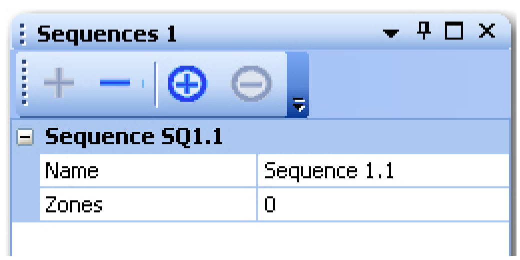
If some of the windows are not visible in the user interface, it may be helpful to restore the default screen layout. From the **Window** dropdown list, select **Layout**, then **Reset to Default Layout**.

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

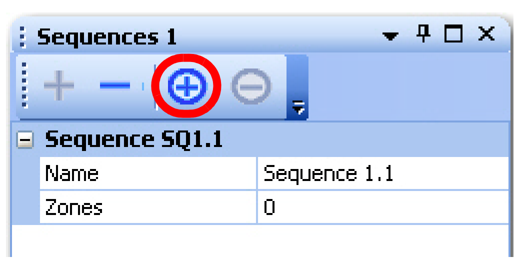


2. Click the **Add new sequence** icon to add a sequence.

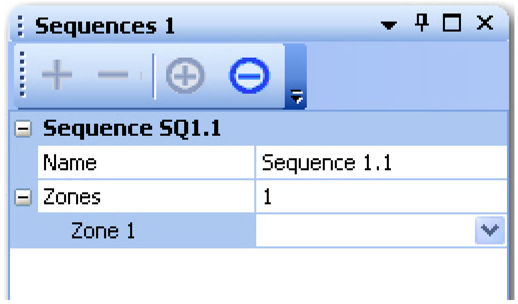
A new sequence is added to the tab.



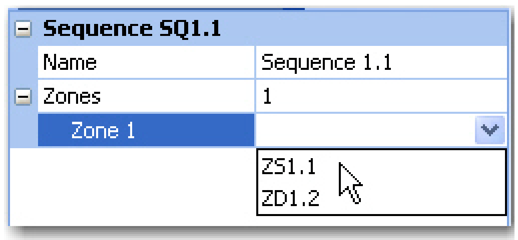
3. Click the **Add zone to selected sequence** icon to add a zone to the sequence.



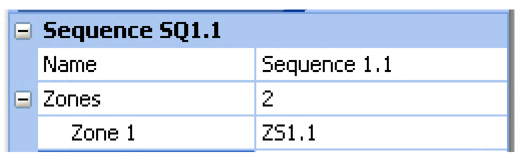
A zone is added to the sequence.



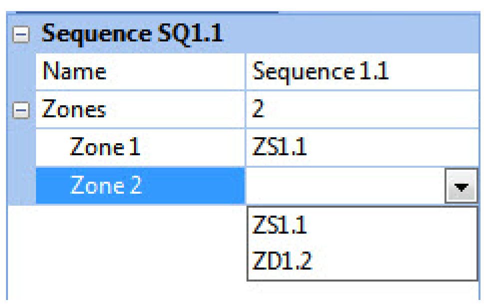
4 Select available zones from the drop down list for the new sequence.



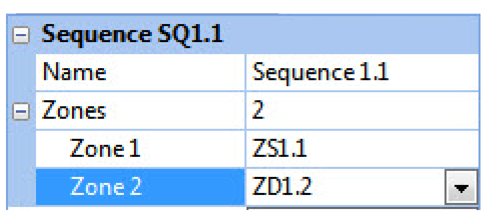
The selected zone is displayed in the **Zone 1** setting.



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 7.13.2, “Events Based on a Sequence of Zones” on page 163.

7.12 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:

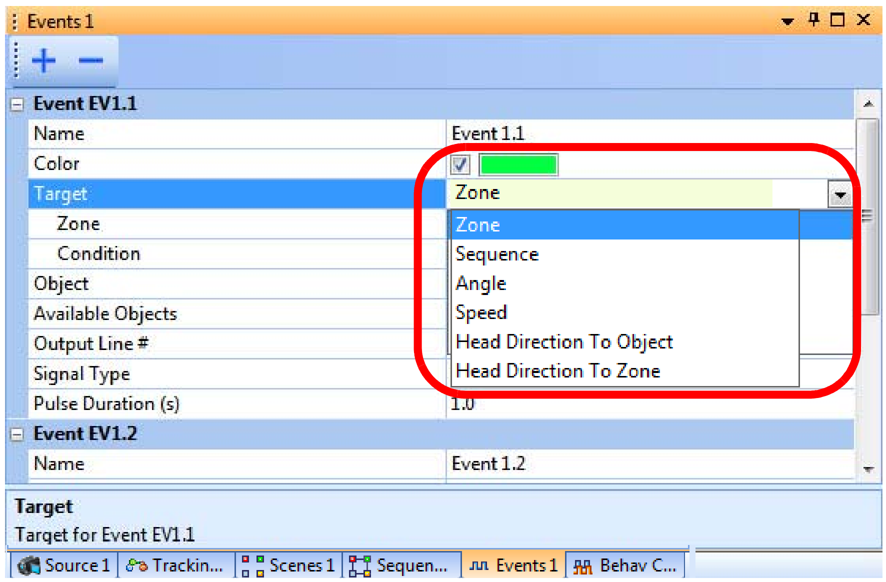
- Section 10.14, “Using the Overlay Feature during Analysis” on page 305
- Appendix E, Modifying Arenas and Zones

7.13 Defining Logical Events

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:

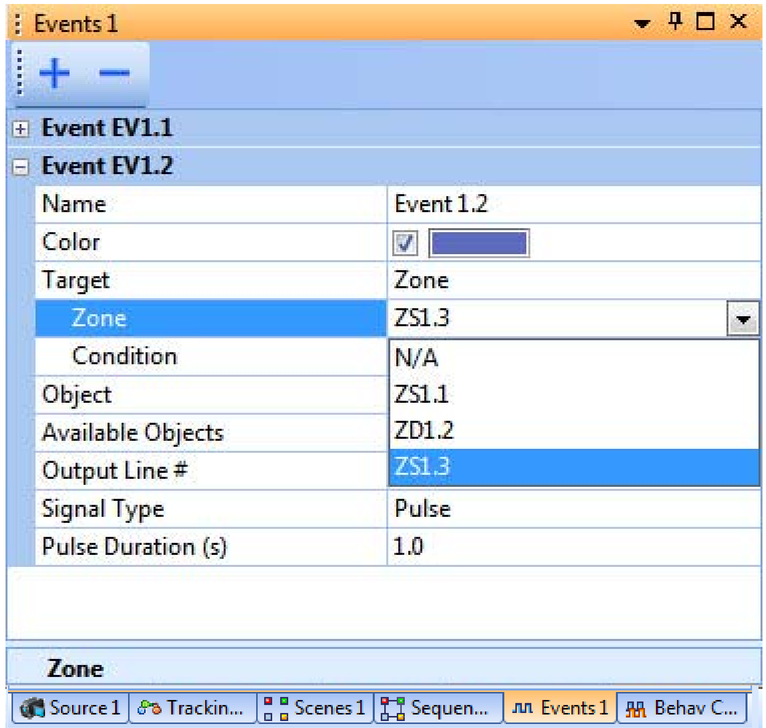
- For Object Contour tracking, the targets listed in the dropdown are **Zone**, **Sequence** and **Speed**.
- For LEDs and Color Markers, the targets listed in the dropdown are **Zone**, **Sequence**, **Angle**, **Speed**, **Head Direction To Object**, and **Head Direction To Zone**.

See the specific procedure for each type of target:

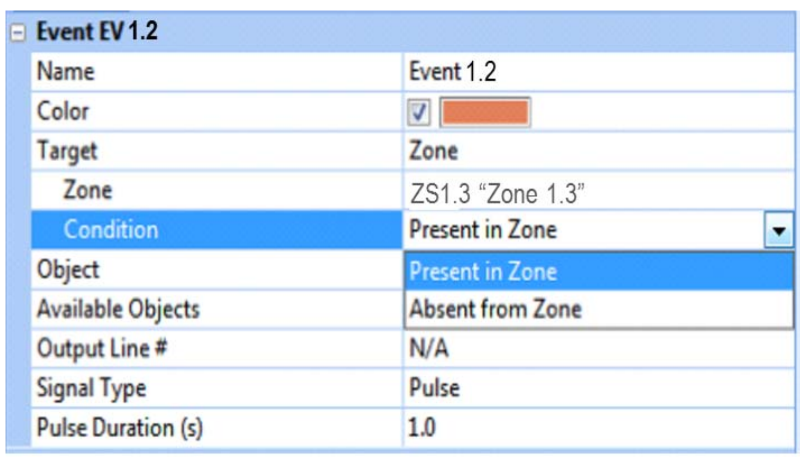
- Section 7.13.1, "Events Based on a Zone" on page 161
- Section 7.13.2, "Events Based on a Sequence of Zones" on page 163
- Section 7.13.3, "Events Based on a Connection Angle" on page 165
- Section 7.13.4, "Events Based on Speed" on page 168
- Section 7.13.5, "Events Based on Head Direction To Object" on page 170
- Section 7.13.6, "Events Based on Head Direction To Zone" on page 176

7.13.1 Events Based on a Zone

1 Select a **Zone**.



2 Define the **Condition**.



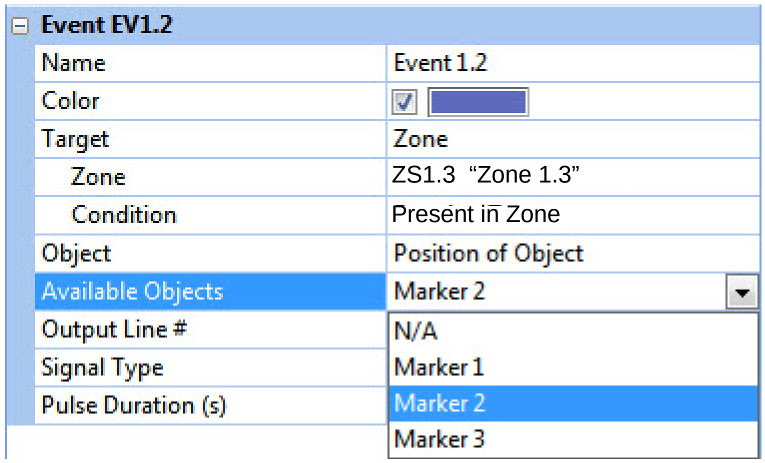
The **Present in Zone** condition is dependent on the **Time Threshold (frames)** parameter that was set in the Scenes tab (see Section 7.10.1, "Adding a Static Zone" on page 149 or Section 7.10.2, "Adding a Dynamic Zone" on page 155 as applicable).Specifically:

- 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. Events are triggered upon 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.

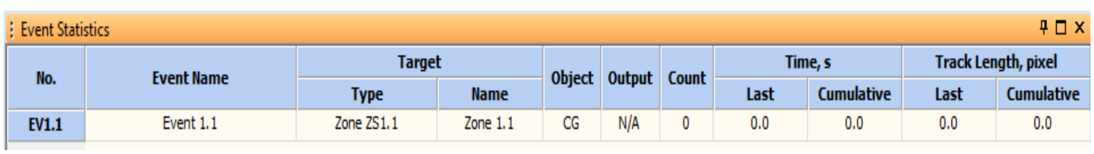
Note: (The meaning of “object” is explained in the **Available Objects** discussion in the next step.)

3 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.

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

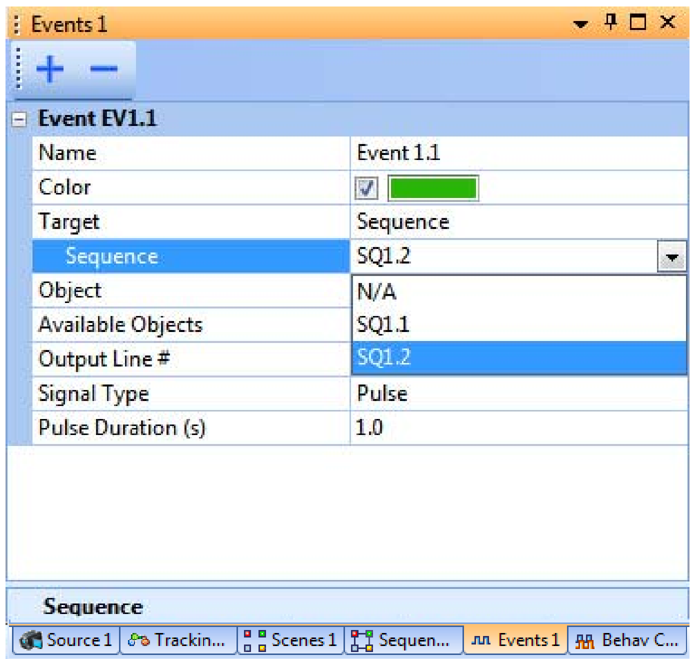


7.13.2 Events Based on a Sequence of Zones

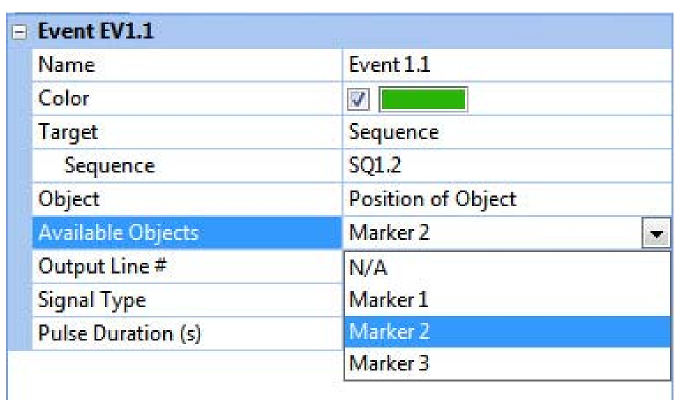
This section explains how to configure events based on a sequence of zones and how the system detects and reports these events. It is recommended that you have a thorough understanding of the information in this section when you are defining sequences (as described in Section 7.11, “Defining Sequences of Zones” on page 157) and when you are creating events based on those sequences (as discussed below).

Procedure

1 Select a **Sequence**.

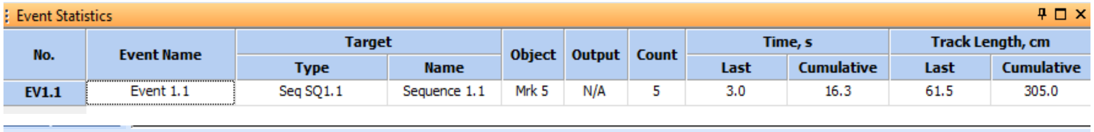


2 In the **Available Objects** row, select a tracked object to traverse the specified sequence of zones. In the example below, the tracked object is Marker 2.



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.

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



Event Statistics

No.	Event Name	Target		Object	Output	Count	Time, s		Track Length, cm	
		Type	Name				Last	Cumulative	Last	Cumulative
EV1.1	Event 1.1	Seq SQ1.1	Sequence 1.1	Mrk 5	N/A	5	3.0	16.3	61.5	305.0

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

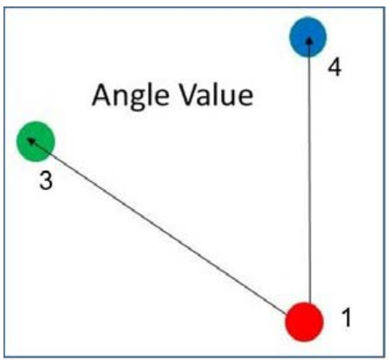
If a sequence restarts, it means that the event has not occurred. In that case, the system does not increment any counters and it resets the timer.

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.

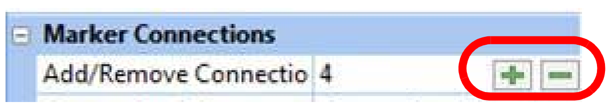
7.13.3 Events Based on a Connection Angle

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.

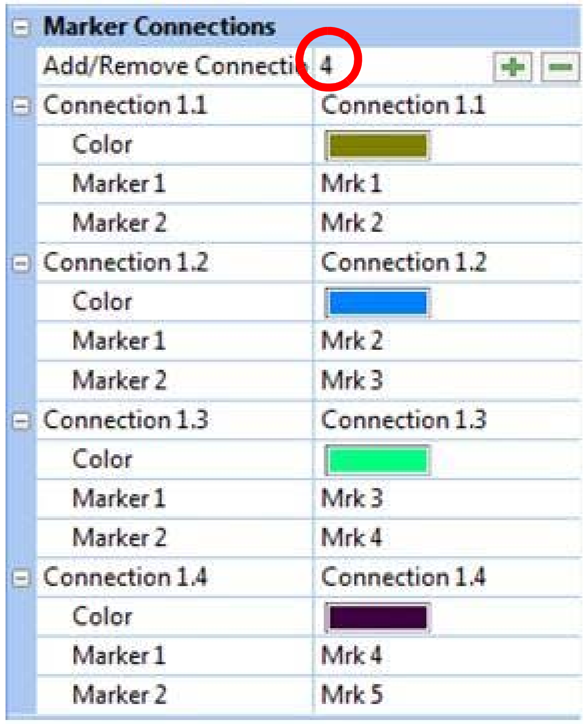
The procedure for creating an angle-based event involves defining *connections* between pairs of markers (or LEDs) and then defining an *angle* between a pair of connections. In the example shown below, one connection is defined from the red marker to the green marker, and a second connection is defined 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.



2 Define two or more connections. In the example below, the user is defining four connections.

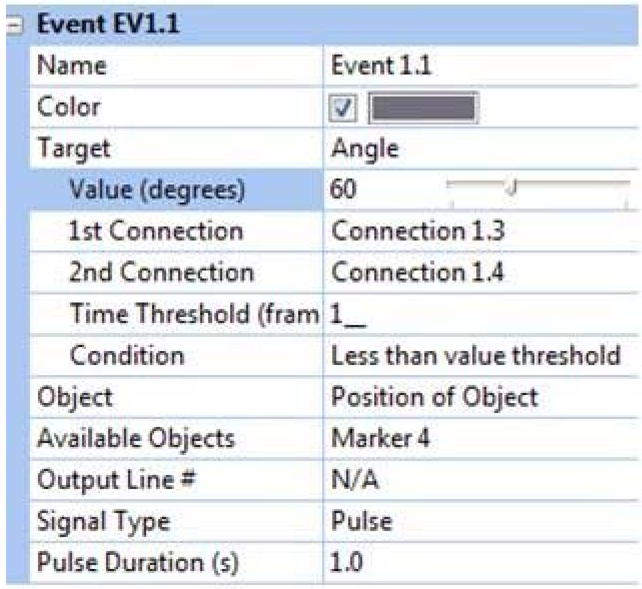


3 Specify the threshold angle **Value (degrees)** and the additional parameters in the **Event** group. In the example below, if the **Angle** is less than 60 degrees for at least one frame (the **Time Threshold**), the event becomes TRUE.

Note: **Time Threshold (frames)** specifies the number of frames for which the **Condition** must be met for the event to be triggered, 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.

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

7.13.4 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 triggering 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

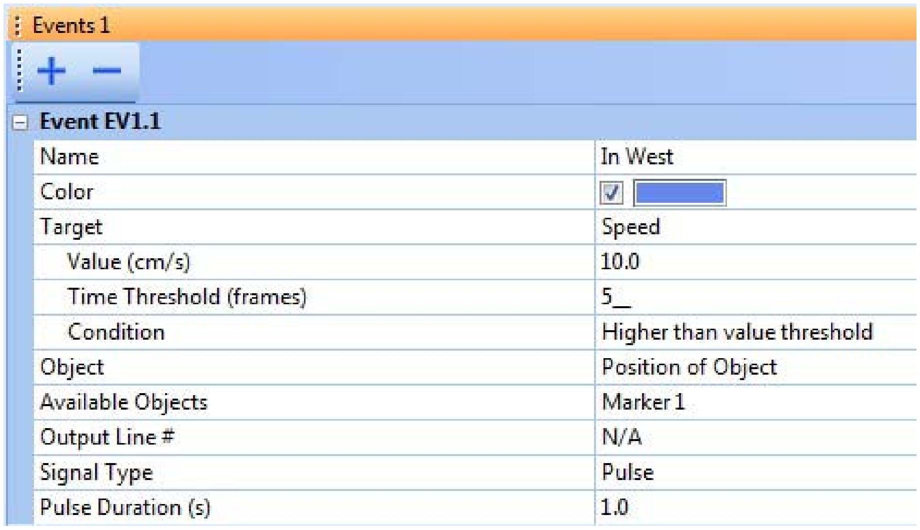
1. Ensure that the **Speed Averaging Interval (s)** setting is configured in the Global Config tab according to the needs of your experiment.
2. In the Events tab, specify the **Target** as **Speed**.
3. Specify the speed (**Value**), **Time Threshold** and **Condition**, plus any additional parameters 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 triggered, 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.

7.13.5 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. For example, an event could be generated when the animal's head faces toward a colored marker attached to a lever (within a certain number of degrees).

Head direction with respect to X-axis of the arena coordinate system

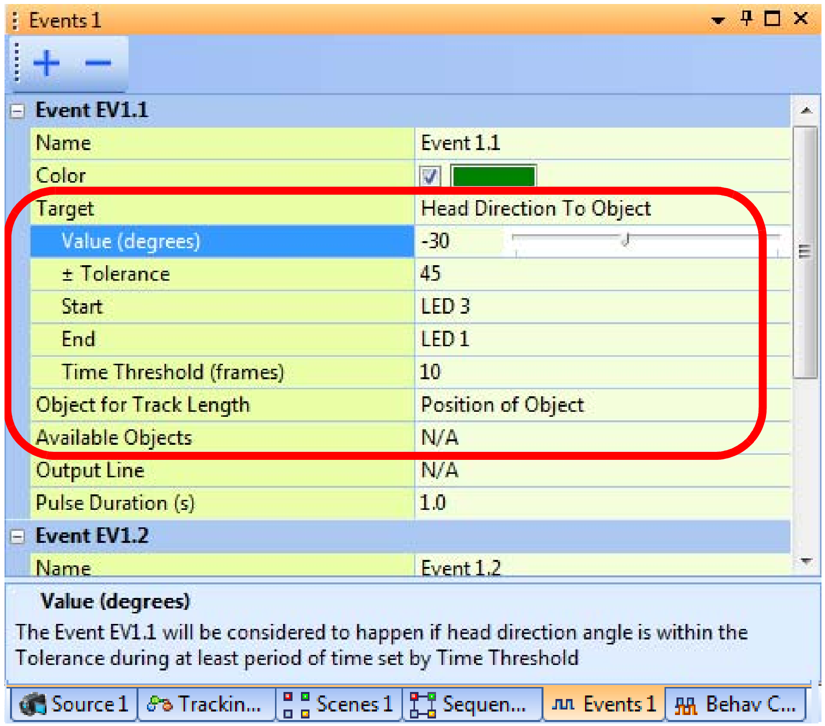
You can specify the head direction with respect to the X-axis of the arena or a vector rotated from the X-axis by a user specified number of degrees.

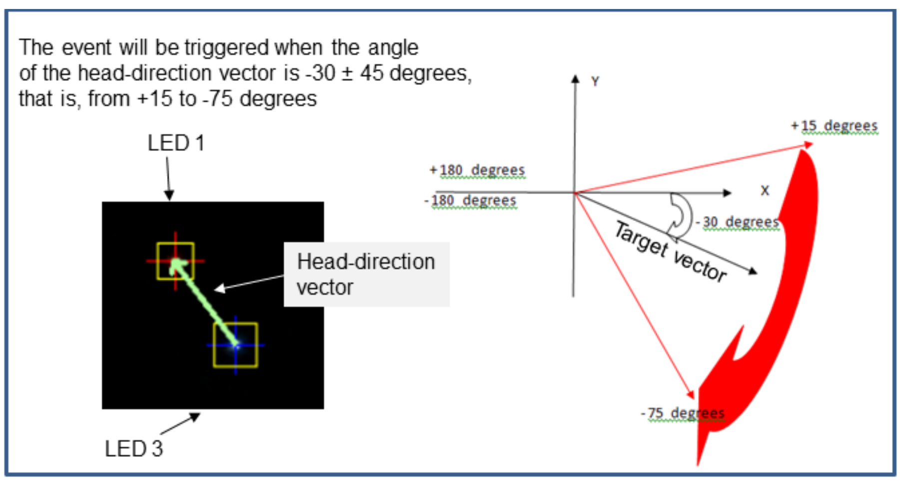
The system determines two vectors:

- The head-direction vector, which points from the **Start** LED or marker to the **End** LED or marker
- The target vector, which is a vector rotated with respect to the X-axis of the arena by a user specified number of degrees.

When the head-direction vector points toward the target vector within a customer specified tolerance and duration, the event is triggered.

1. In the Events tab, specify the **Target** as **Head Direction To Object** and specify the **Object for Track Length** as **Position of Object**.
2. In the **Available Objects** row, select **N/A** from the dropdown list. Note that this causes the **Value** line to appear.
3. In the **Value** line, use the slider to specify a value from –180 to +180 degrees with respect to the X-axis of the arena.
4. 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 triggered.





5 (Optional) If you want to send a trigger to an external device, follow the procedure in Section 7.17, “Specifying Digital Outputs” on page 190.

Head direction with respect to another object in the arena

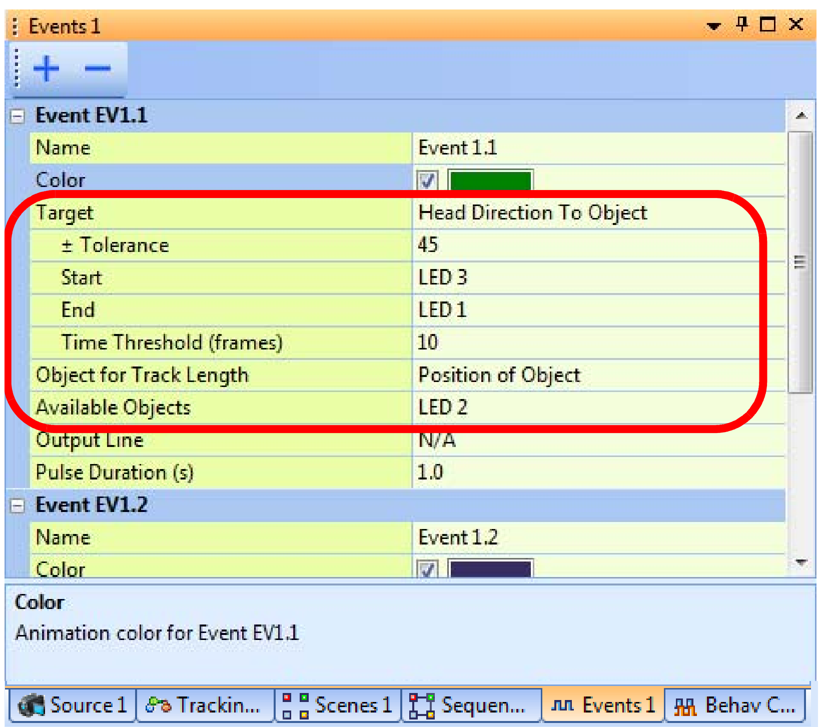
You can specify the head direction with respect to another object in the arena.

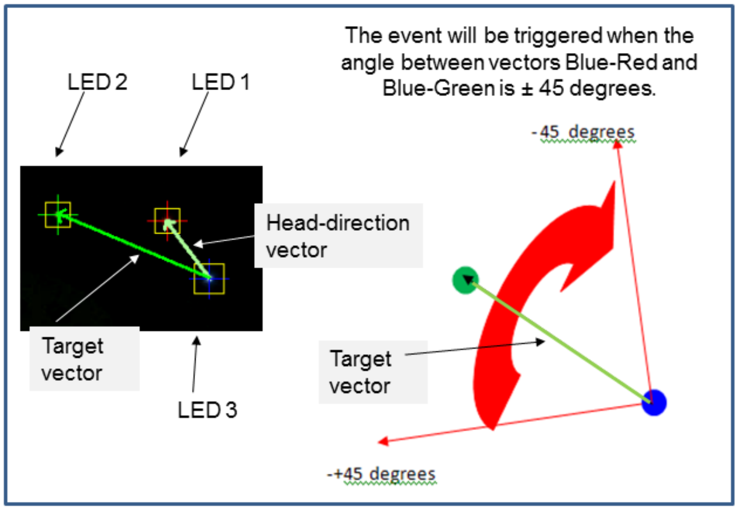
The system determines two vectors:

- 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 another LED or marker in the arena (**Available Objects**).

When the head-direction vector points toward the target vector within a customer specified tolerance and duration, the event is triggered.

1. In the Events tab, specify the **Target** as **Head Direction To Object** and specify the **Object for Track Length** as **Position of Object**. 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 triggered.
2. For **Available Objects**, select the LED or marker of interest. The system will generate a target vector that starts at the **Start** LED or marker and points toward the LED or marker of interest.





3 (Optional) If you want to send a trigger to an external device, follow the procedure in Section 7.17, “Specifying Digital Outputs” on page 190.

Head direction with respect to average center of gravity

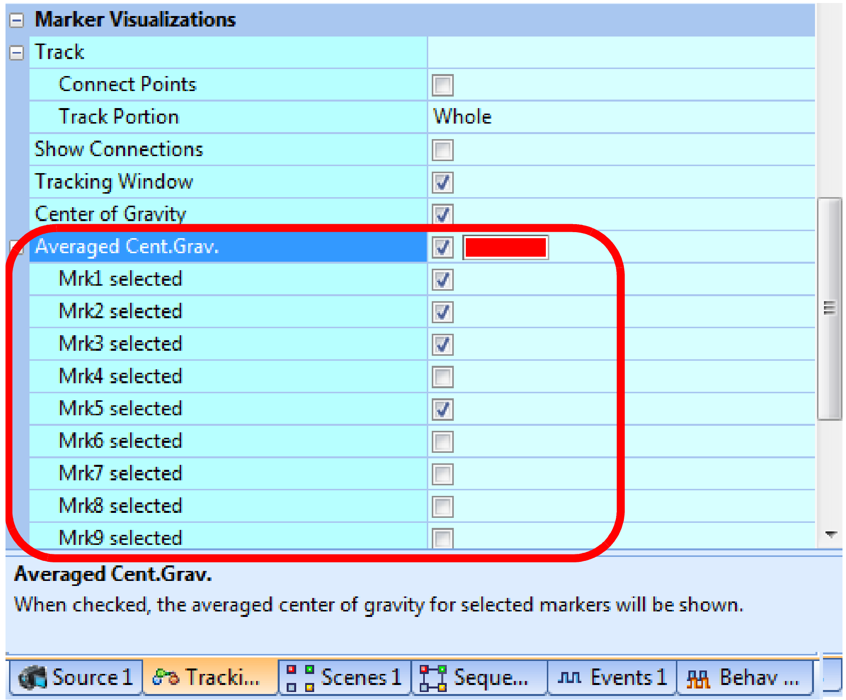
You can specify the head direction with respect to the averaged center of gravity of a group of LEDs or markers. The specific LEDs or markers to be included in the averaged center of gravity determination are set in the **Marker Visualizations** or **LED Visualizations** section of the Tracking tab.

The system determines two vectors:

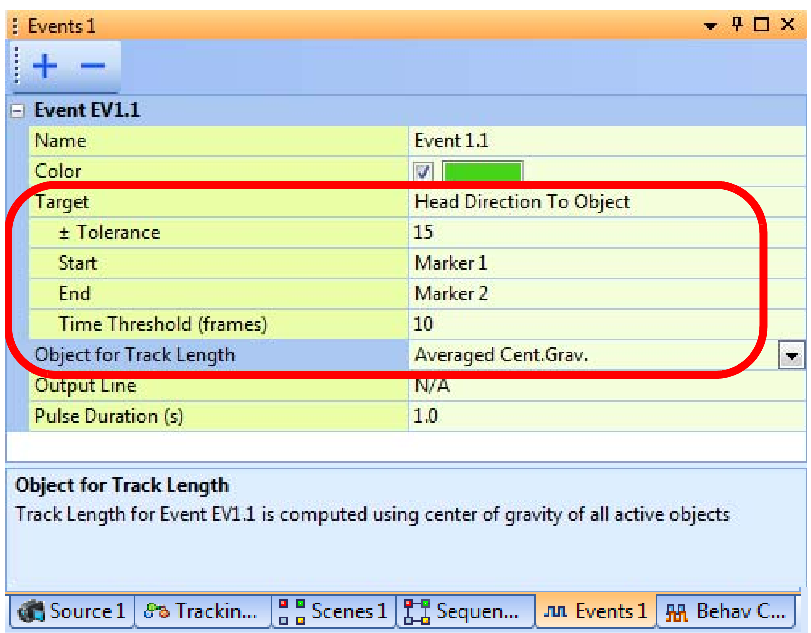
- The head-direction vector, which points from the **Start** LED or marker to the **End** LED or marker
- The target vector, which points from the **Start** LED or marker to the averaged center of gravity (**Averaged Cent.Grav.**) of all specified LEDs or markers

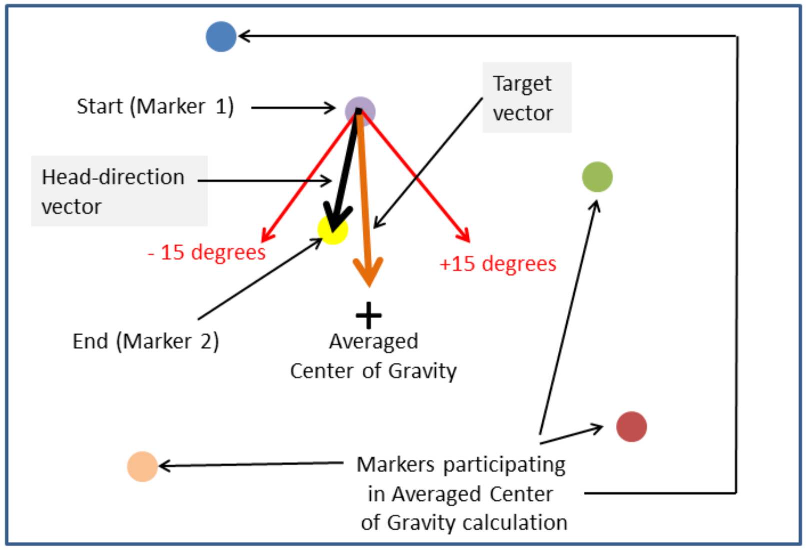
When the head-direction vector points toward the target vector within a customer specified tolerance and duration, the event is triggered.

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 triggered.



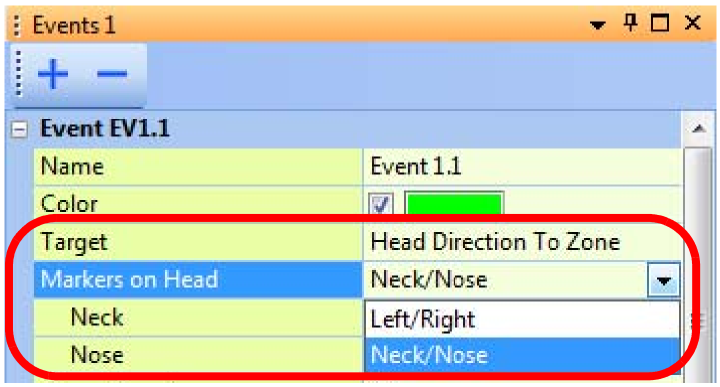


3 (Optional) If you want to send a trigger to an external device, follow the procedure in Section 7.17, "Specifying Digital Outputs" on page 190.

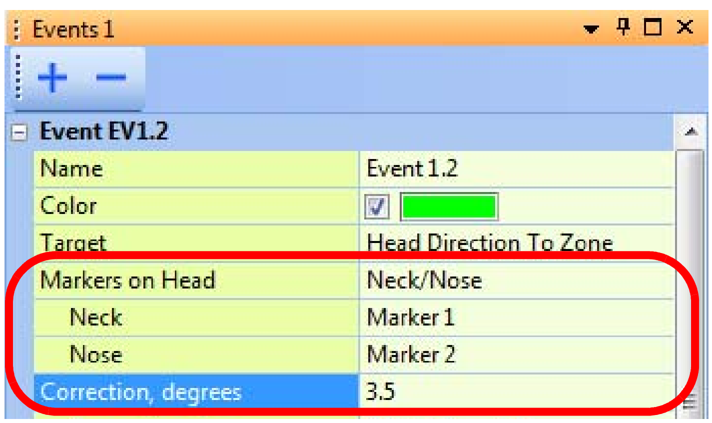
7.13.6 Events Based on Head Direction To Zone

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.

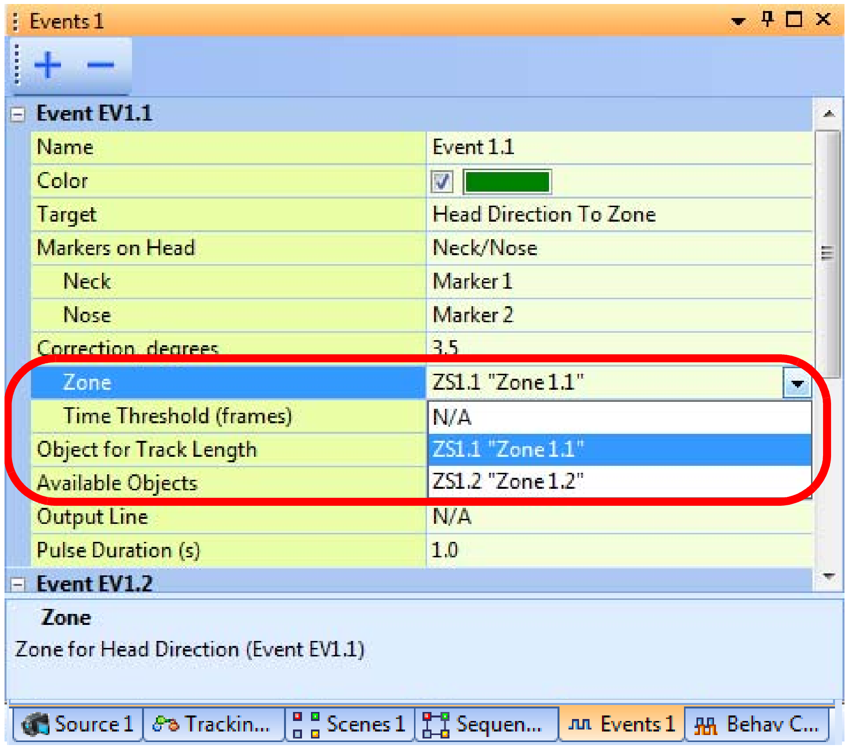
1 In the Event tab, specify the **Target** (**Head Direction To Zone**) and the location of LEDs or markers on the animal's head (either **Left/Right** or **Neck/ Nose**).



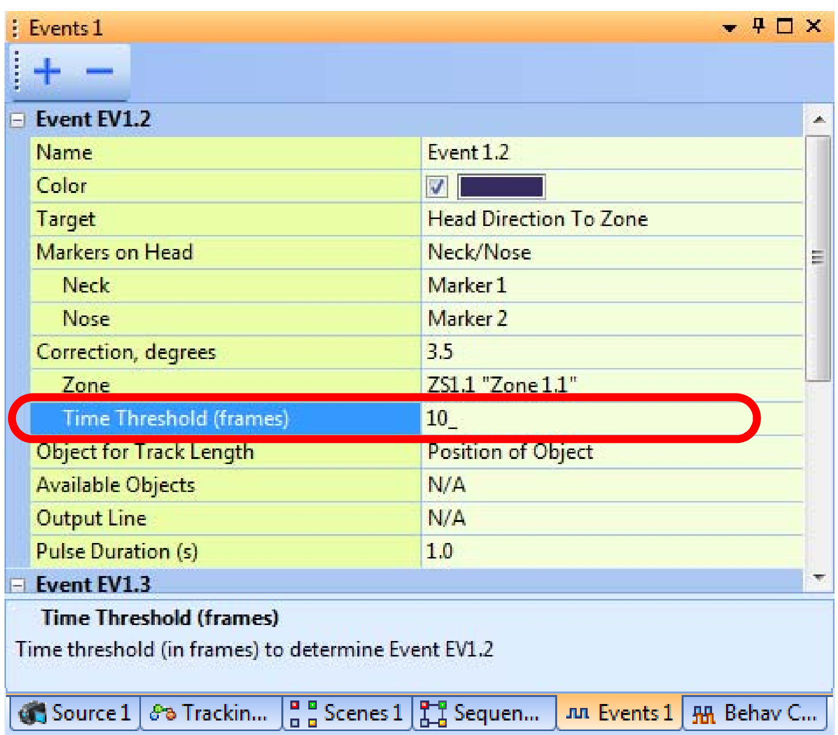
2 Specify which LEDs or markers are being tracked and a correction value (if any) to compensate for the possible offset of the LEDs or markers from their intended mounting positions on the animal's head.



3 In the **Zone** row, specify the zone of interest.



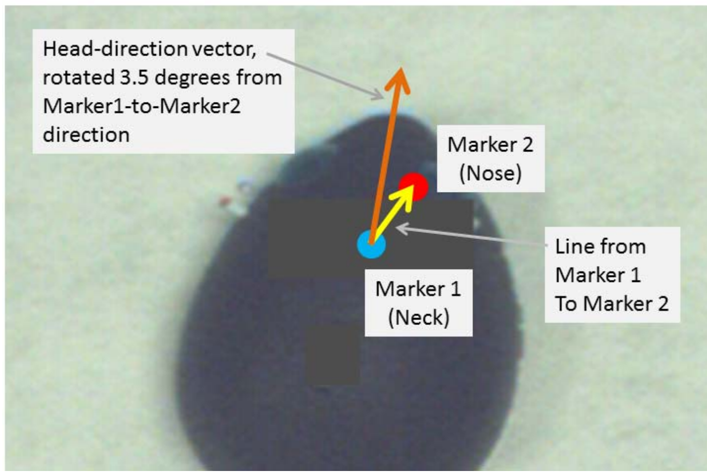
4 Specify the value of **Time Threshold (frames)**, which is the minimum number of frames the condition must be true to cause an event to be triggered.



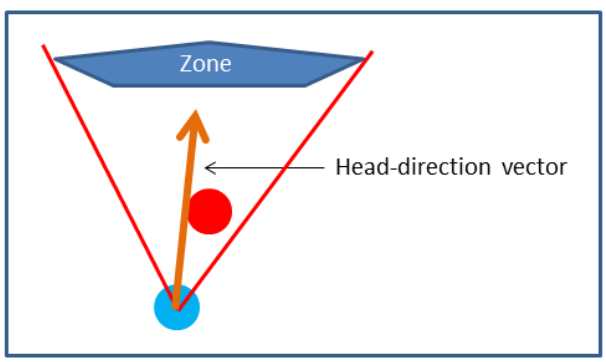
5 Specify the **Object for Track Length** as either **Position of Object** or **Averaged Cent.Grav.**

- 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.

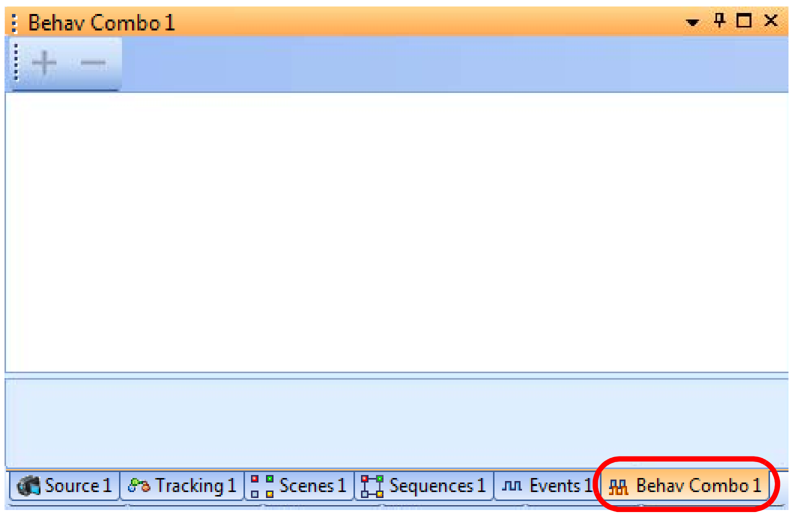


7.14 Defining Combination Events per Video Source

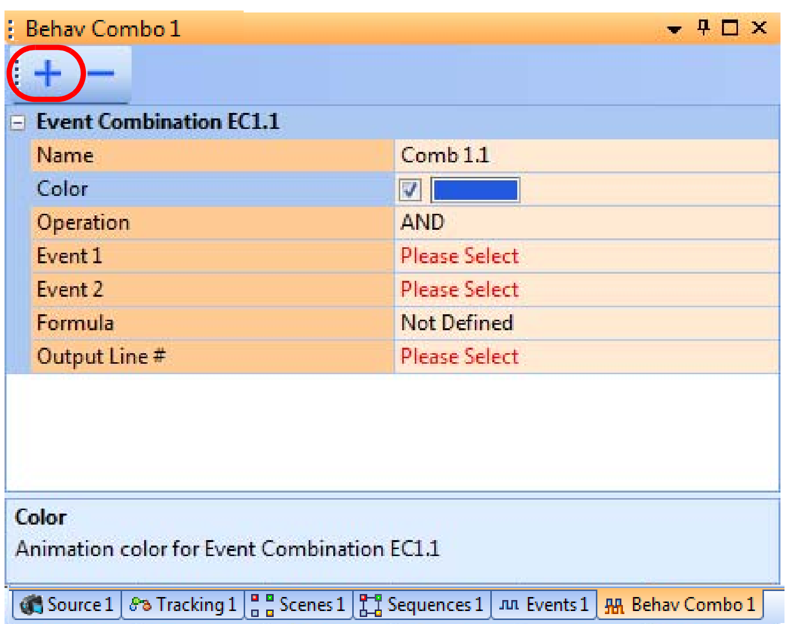
This section explains how to configure combination events within a single video source (video stream). After you create individual events, you have the option of creating combination events for each video source. For example you might want to trigger an event in the Video 1 arena when a subject [1] enters a particular zone AND [2] turns its head toward a certain object.

You can create combination events for each of the video sources in your system—Video 1 ... Video 4—up to the number of cameras licensed.

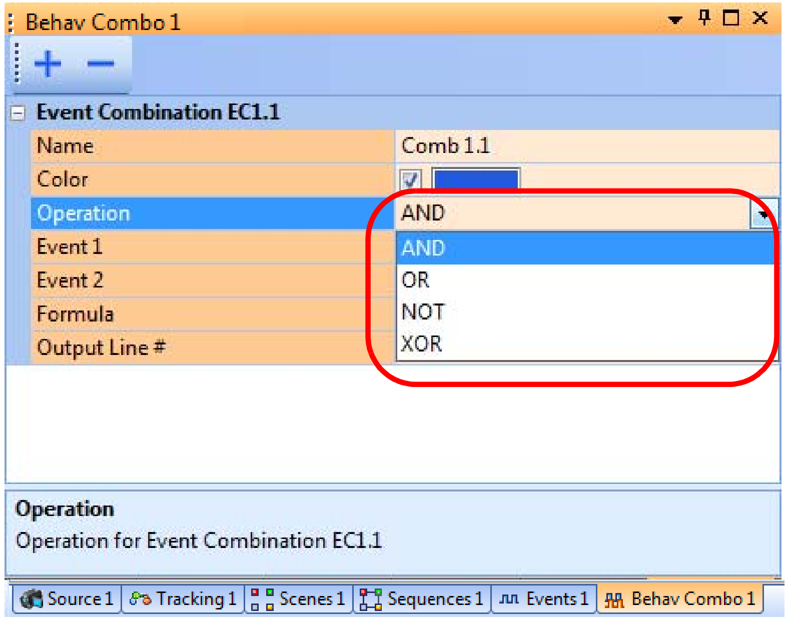
1 Click the **Combination Events** tab.



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.

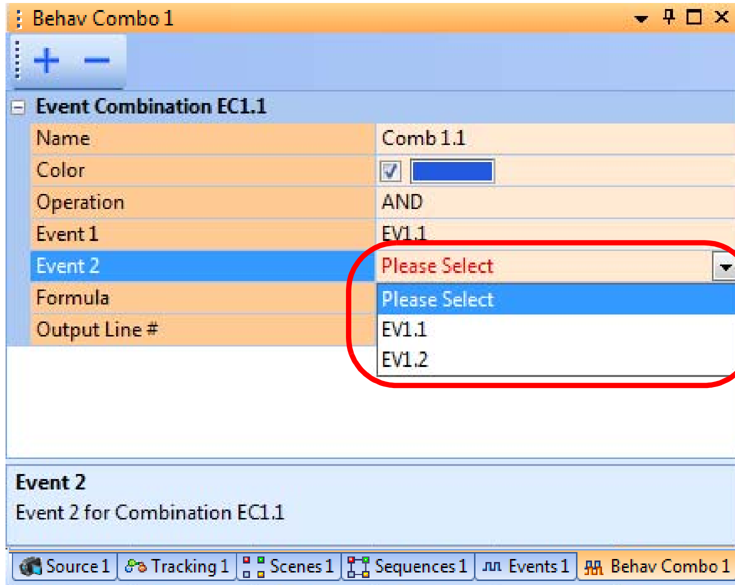
For the combination of two events, the **AND**, **OR** and **XOR** operators are available. The resulting combination event is considered true under the following conditions:

AND—Both of the individual events are true

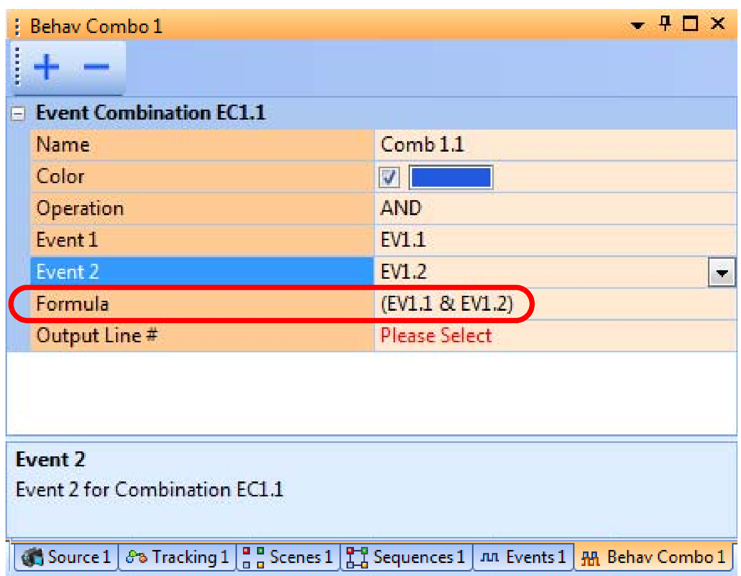
OR—Either one of the individual events is true, or both of the individual events are true

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

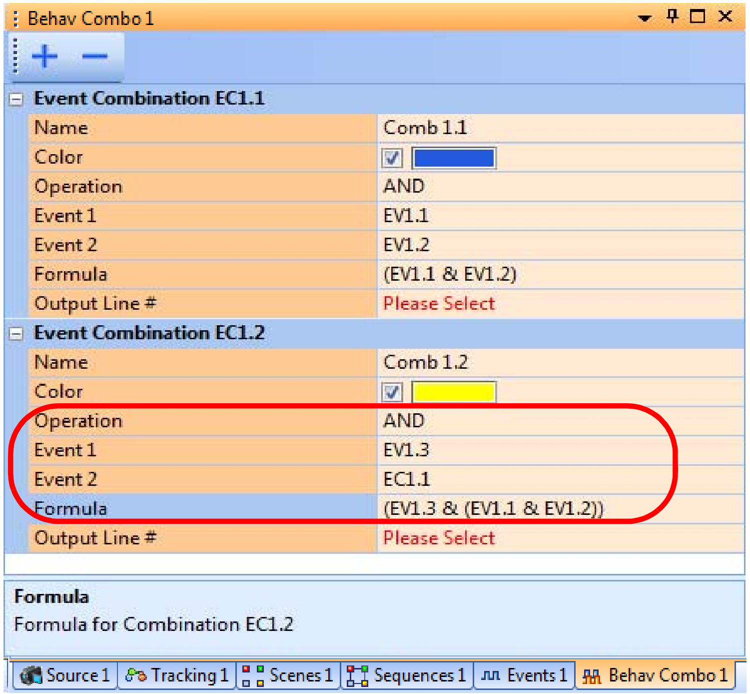
4 Select each event in the combination from the drop down list of available events.



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.



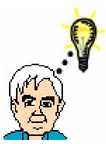
Note: You can also create *global combination events* across multiple video sources. For that procedure, see Section 7.15, "Defining Global Combination Events" on page 7-183.

7.15 Defining Global Combination Events

This section explains how to configure *global* combination events.

Procedure

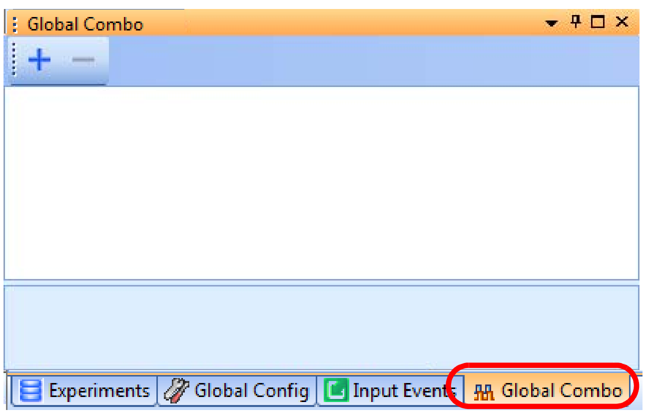
After you create events in the individual video sources (Video 1 ... Video 4), you can create one or more global combination event(s) that combine events from multiple video sources. For example you might want to trigger an event when a subject [1] enters a particular zone in the Video 1 stream *and* [2] turns its head toward a target object in the Video 2 stream.



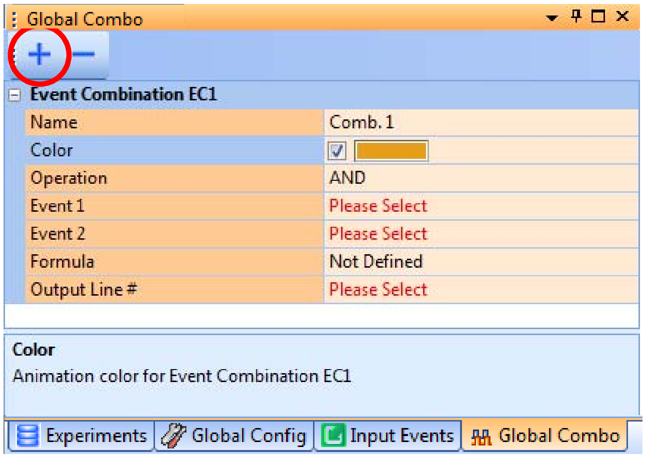
TIP
Configuring combination events for single and multiple video sources

It is usually more convenient to configure combination events in the individual video sources whenever possible (as described in Section 7.14, "Defining Combination Events per Video Source" on page 179), and create a global combination event only when it is necessary to combine events occurring across multiple video sources.

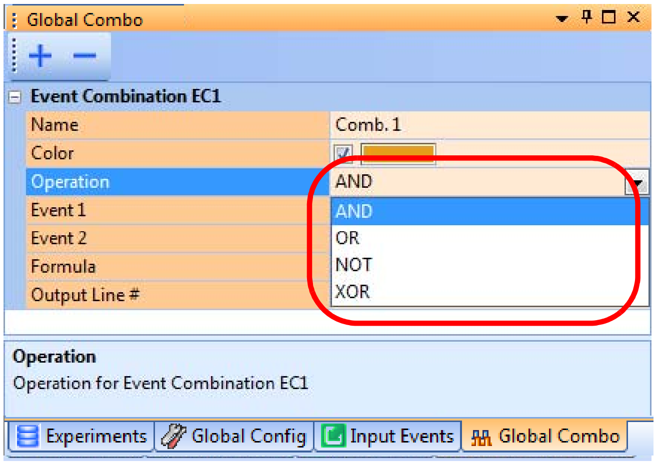
1 Click the **Global Combo** tab in the global configuration window.



2 Click the **Add new combination of tracking events** icon to view the parameters.

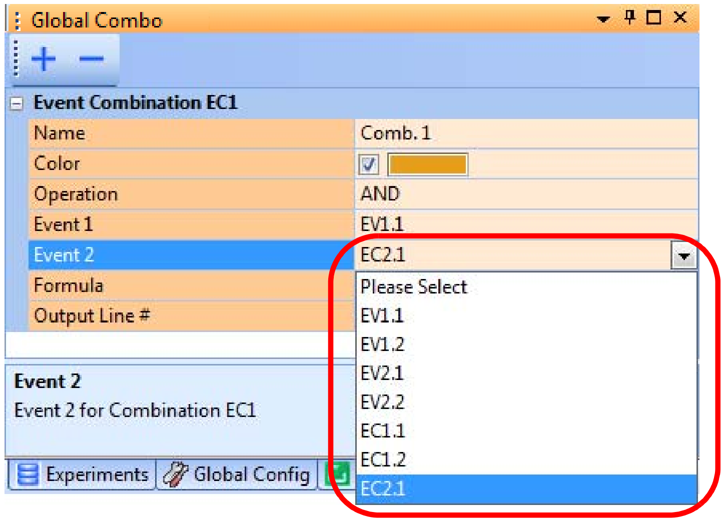


3 Select the logical operation to use.

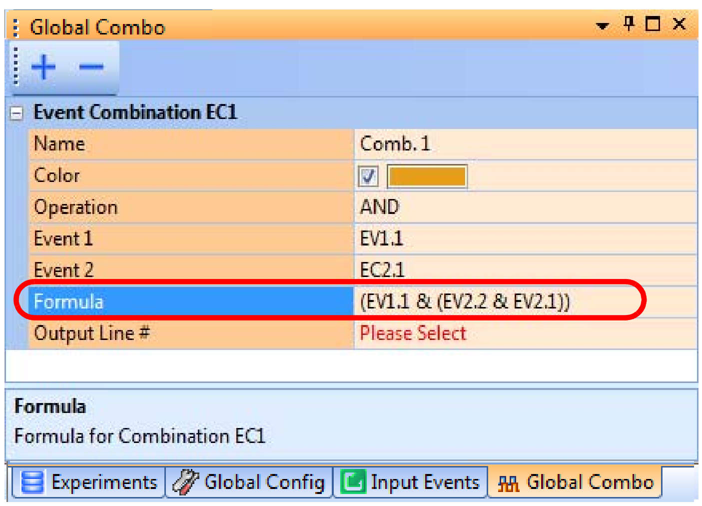


The four logical operators are **NOT**, **AND**, **OR** and **XOR**. See Section 7.14, “Defining Combination Events per Video Source” on page 179 for definitions of these operators.

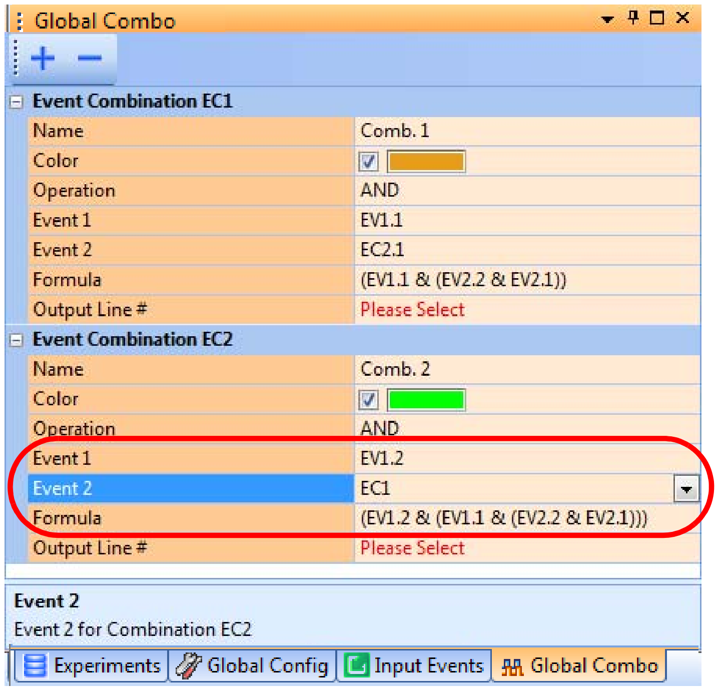
4 Select each event in the combination from the drop down list of available events.



The system generates an entry in the **Formula** line based on the Operation and Event(s) you selected in the above steps.



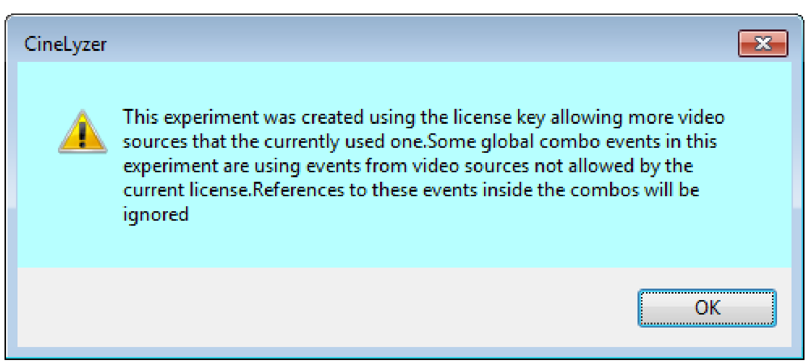
5 If desired, a global combination event can contain other combination events, as shown in the following example. Events of arbitrary complexity can be created by combining already combined events with combined or non-combined (single) events.



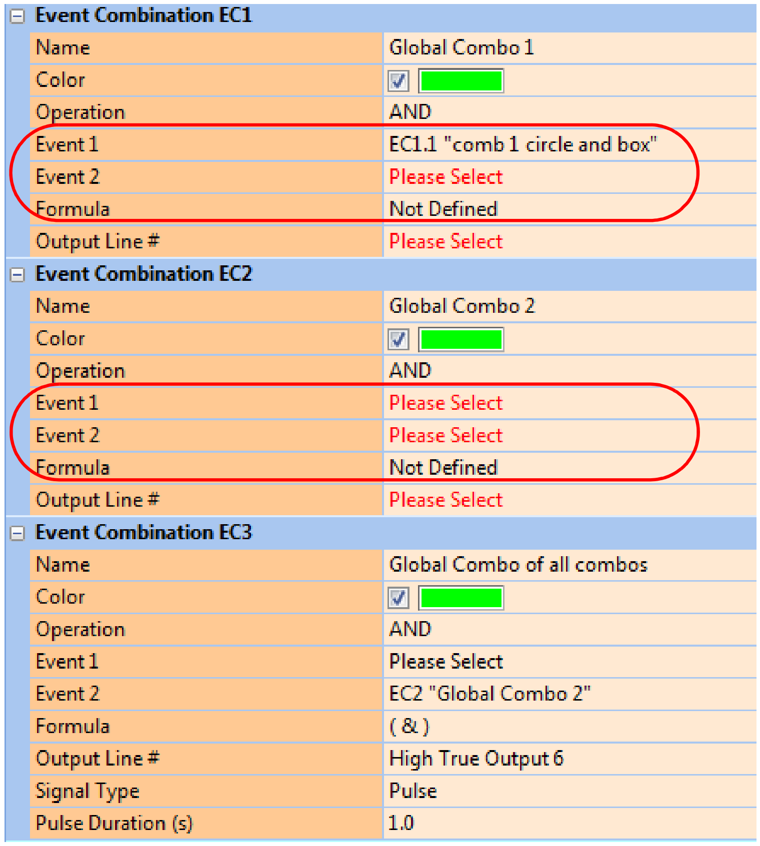
Global Combination Events—Licensing considerations

If you create sessions with a multicamera CineLyzer license, then attempt to create additional sessions (in the same experiment) with a different CineLyzer license key that is valid for fewer cameras, the system will preserve your global combination events data, but you will not be able to access certain data that was created with the original license.

For example, if you have a four-camera license key and create a global combination event involving two or more cameras, then switch to a single-camera license key, you will not be able to use that multiple-camera combination event. You will see this dialog:



The **Global Combo** tab will look similar to this example:



If you do not make any changes to these global combination events (the items that say “Please Select” in the above image), but simply switch back to the original four-camera license key, these global combination events will be restored.

7.16 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 triggered 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 triggered.

In the Tracking toolbar:

1. Select **LED** or **Color Markers** tracking mode. In this example, we will select markers.

In the Tracking tab:

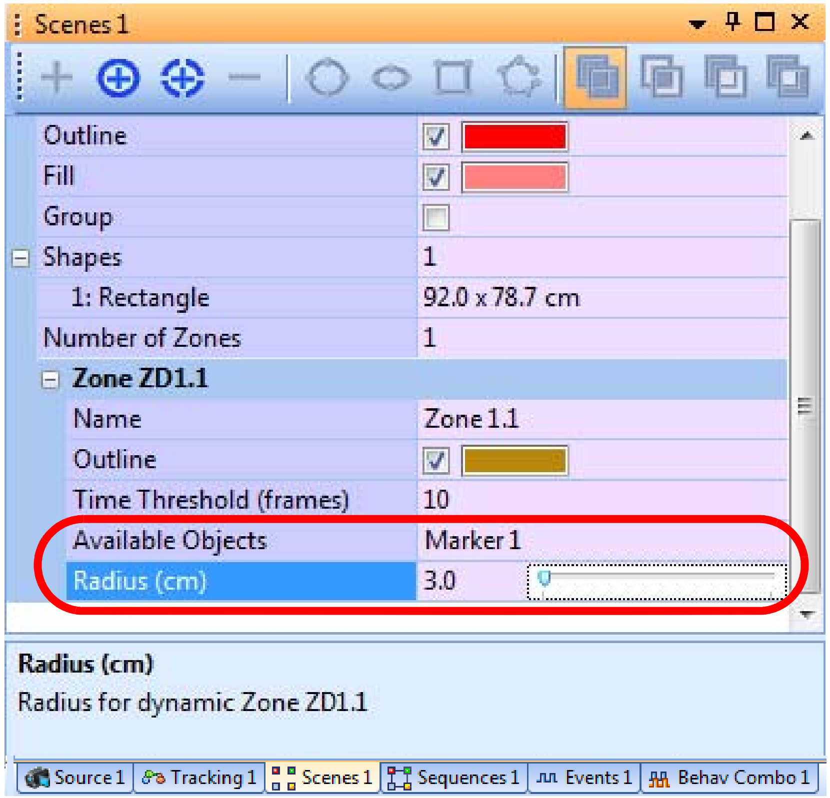
2. Select the checkboxes for Marker 1 (the marker on the head of the first animal) and Marker 2 (the marker on the head of the second animal).

In the Sources tab:

3. Calibrate the arena in cm.
4. Select the **Use Calibration** checkbox.

In the Scenes tab:

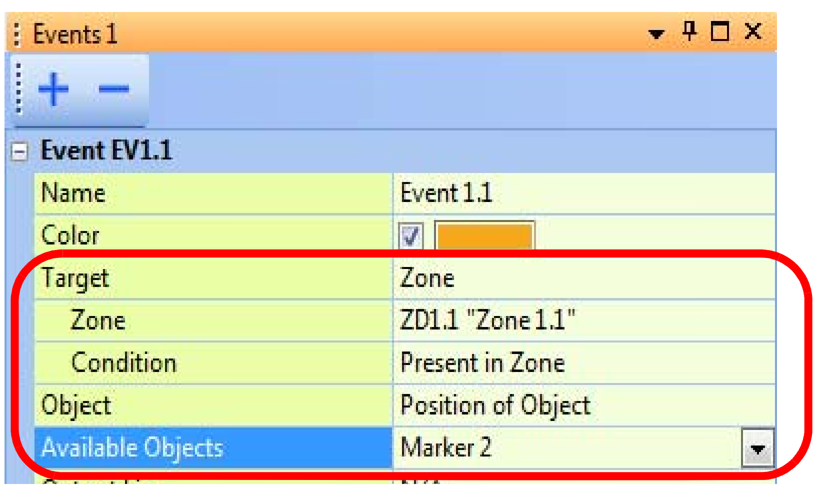
5. Draw the arena.
6. Define a dynamic zone around Marker 1.
7. Set **Available Objects** to Marker 1 and **Radius (cm)** to 3.0cm.



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) is triggered.

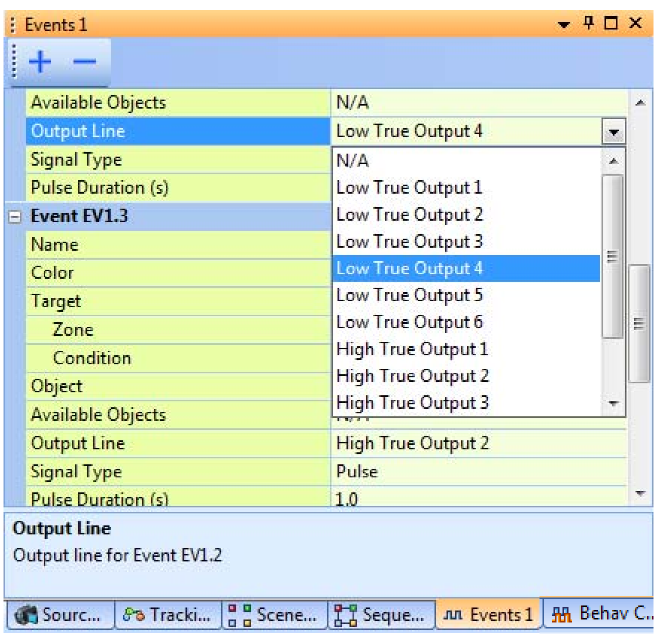
7.17 Specifying Digital Outputs

The system can output a digital signal when an individual event or a combination event becomes true. Up to 12 different digital outputs can be specified (up to six low true and six high true). The outputs can be based on a level or pulsed for a user-specified duration.

As an example of using digital outputs, you can connect a cable from the Plexon PlexBright® 4 Channel Controller (PlexBright Controller) to the CineLyzer System DIO Interface and configure the PlexBright Controller to start playing a specified stimulation pattern when a digital output signal is generated by the CineLyzer System. For details, see Section 11.3, “Sending CineLyzer Output Signals to the PlexBright Controller” on page 312 and “Connecting CineLyzer System to Optogenetic Stimulation Device” on page C-9. The cable and PlexBright Controller are sold separately.

Outputs can be generated at the camera frame rate, 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.

Note: Digital outputs can be transmitted to external devices only if the Plexon CineLyzer USB Digital Input/Output Interface (DIO Interface) is installed. The DIO unit connects to a USB port on the host PC. (Be sure to restart the CineLyzer software after connecting the USB cable.) The **Output Line** in the Events tab and the DIO Interface are shown in the example below.

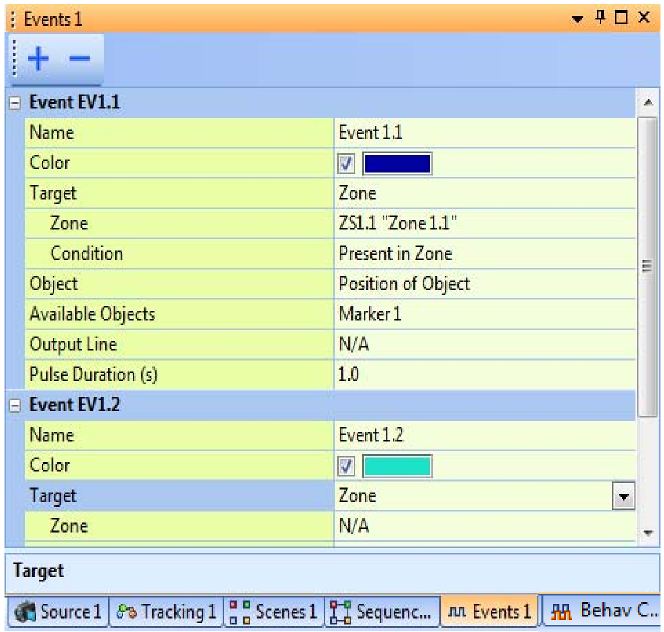


Note: Each digital output line is restricted to one event at a time.

For additional information about the DIO Interface and associated accessories, see Appendix C, USB Digital Input/Output Interface.”

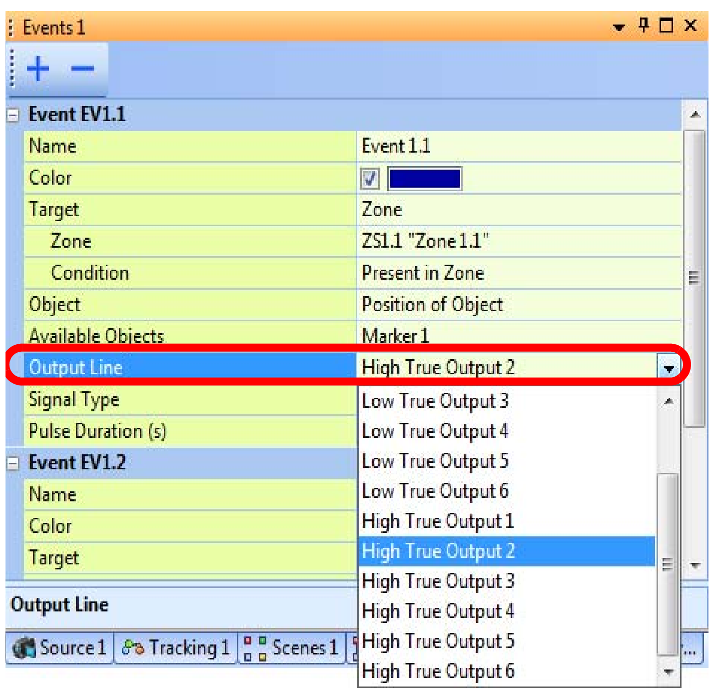
1 Ensure that the CineLyzer DIO Interface is connected, as described above.

2 For events, open the Events tab.

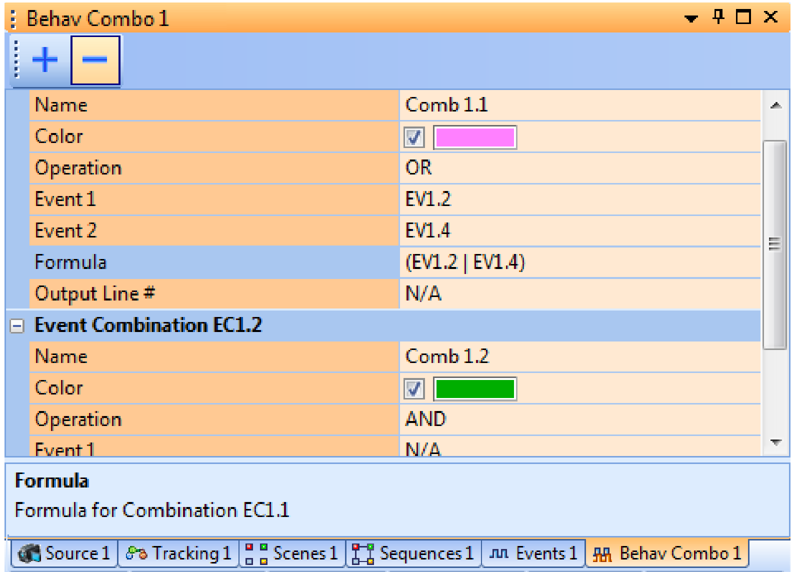


3 To specify that a digital output is generated when an event occurs (becomes TRUE), select the desired **Output Line** from the drop down list associated with the event. The output line numbers correspond to the line numbers shown in the side view of the DO unit (see the photograph of the DO unit, above).

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

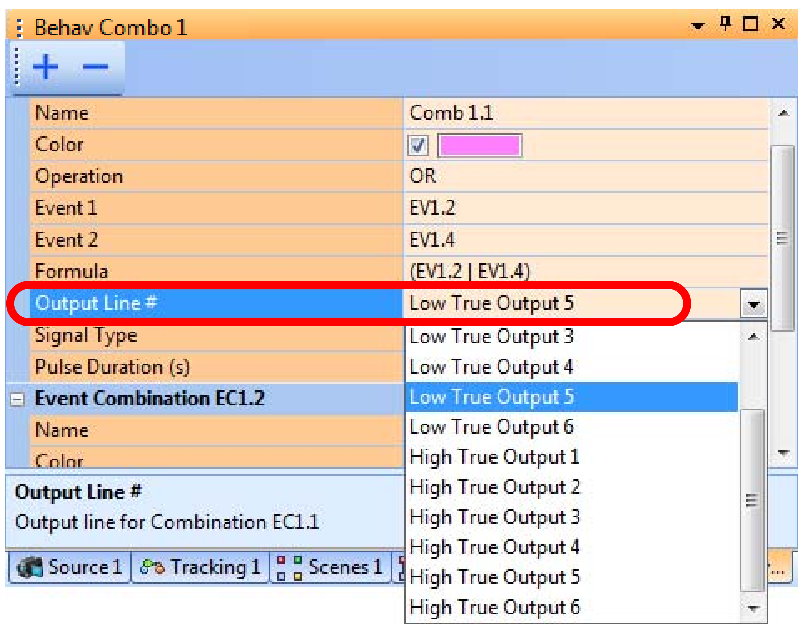


4 For combination events, click on the Combination Events tab.

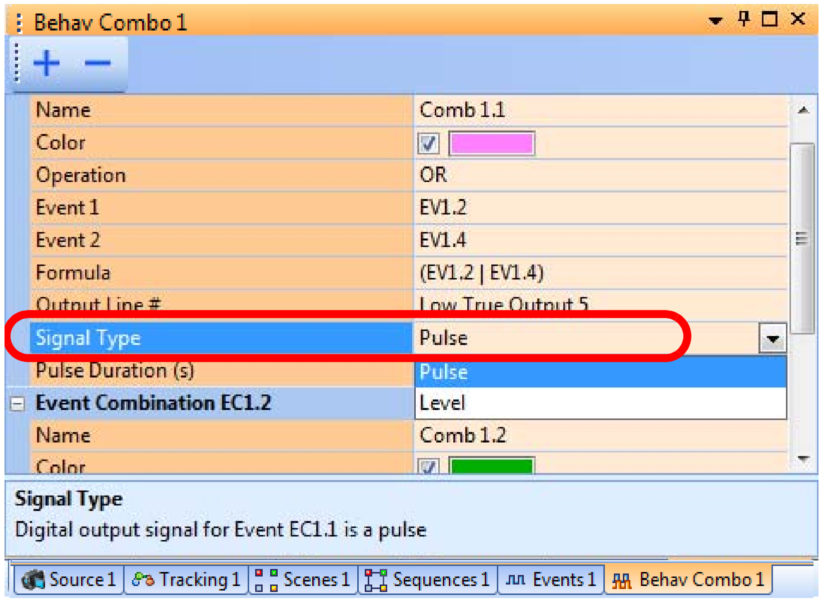


5 To specify that a digital output is generated when a combination event occurs (becomes TRUE), select the desired **Output Line** from the drop down list associated with the combination event.

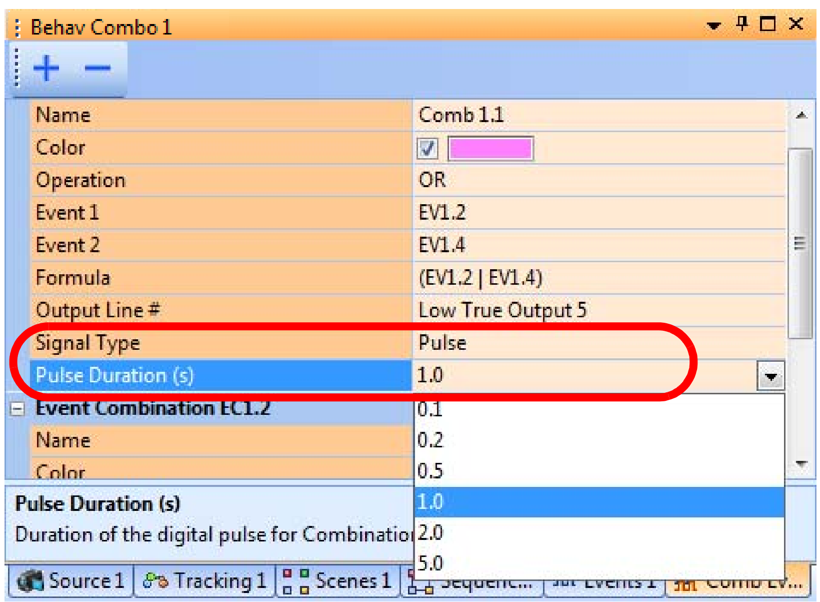
Note that when you select an output line, a new row appears—**Signal Type**.



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

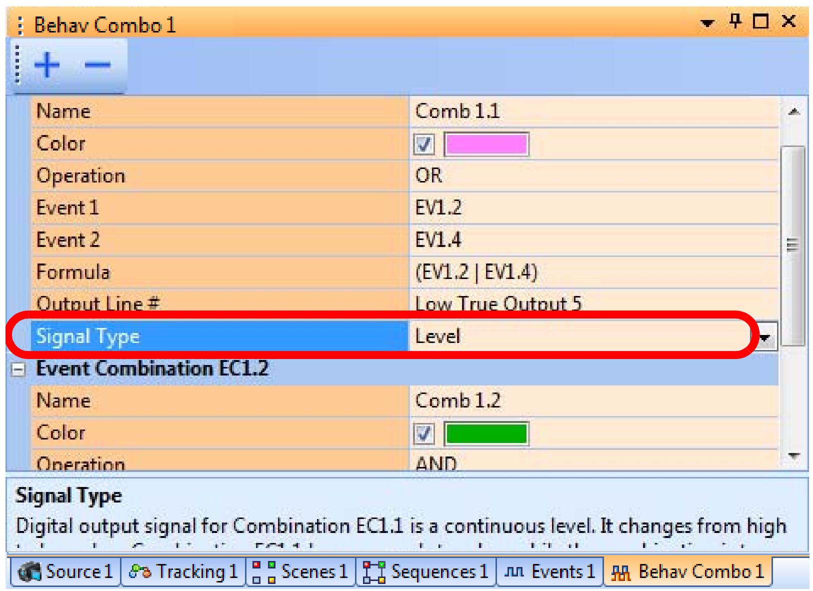


7 If the output **Signal Type** is **Pulse**, specify the **Pulse Duration (s)**.



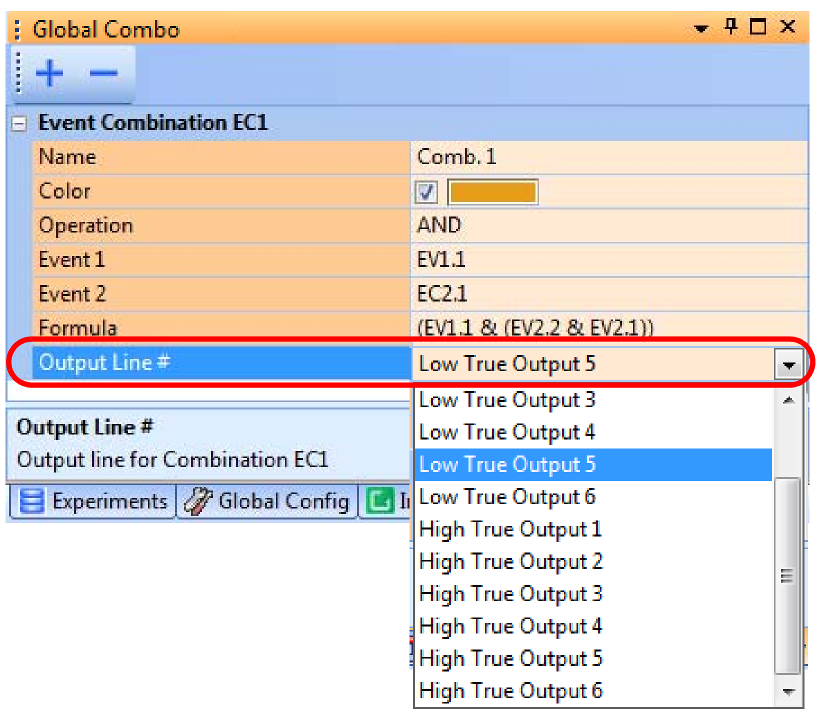
8 If the output **Signal Type** is set to **Level**, the system will assert the output continuously while the condition is true.

Note: The signal logic is described in Appendix C-USB Digital Input/Output Interface.



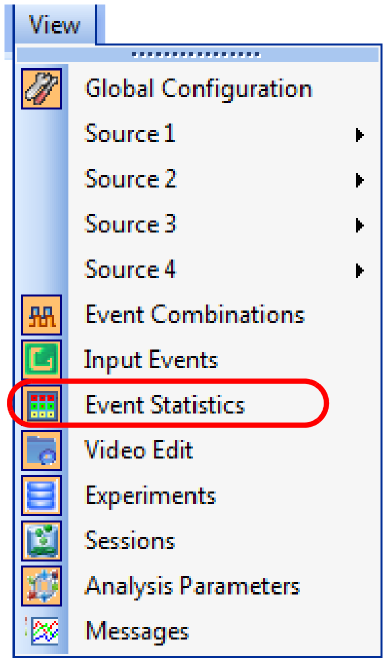
Specifying digital outputs for global combination events

You can specify digital outputs for global combination events in the same manner as for the source-specific events (as described above).

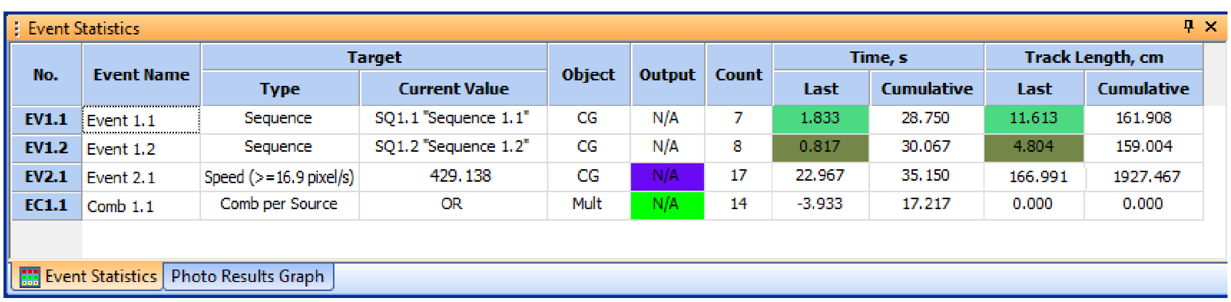


7.18 Displaying Behavioral Event Statistics As They Occur

To open the Event Statistics window, select **Event Statistics** from the **View** dropdown menu.

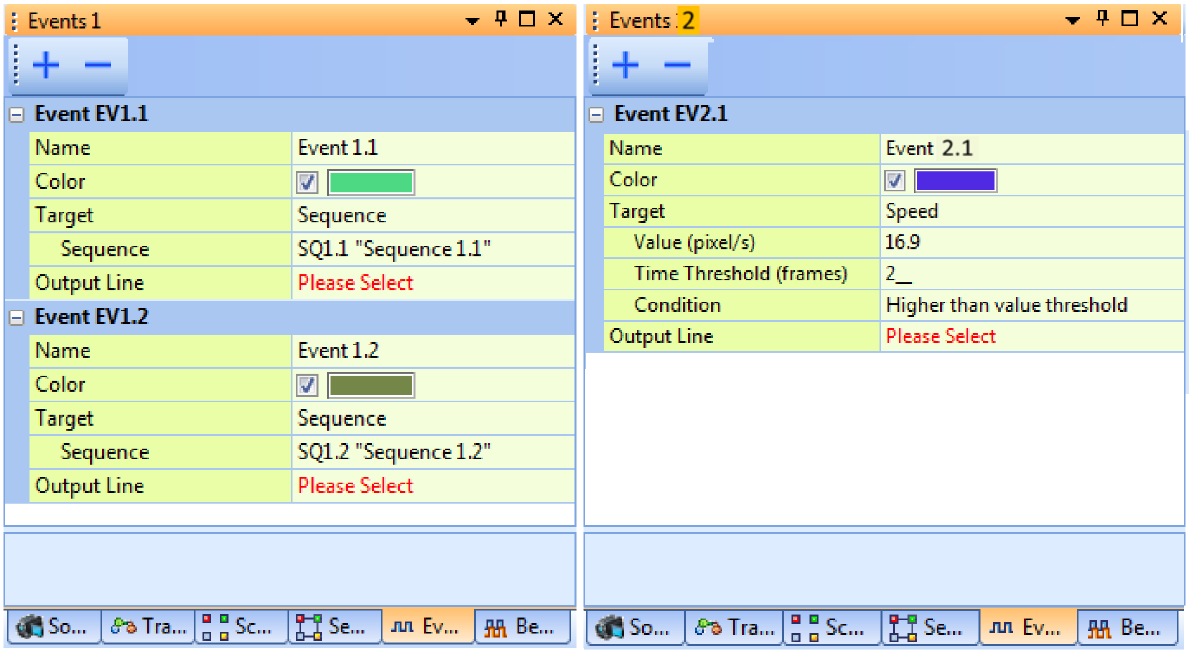


The image below is an example of an event statistics display for events based on zone sequences (Event 1.1 and Event 1.2), a Speed event (Event 2.1) and a combination event (Comb 1.1). The colored cells in the display indicate that an event was occurring at the moment this image was captured.

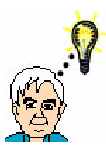


No.	Event Name	Target		Object	Output	Count	Time, s		Track Length, cm	
		Type	Current Value				Last	Cumulative	Last	Cumulative
EV1.1	Event 1.1	Sequence	SQ1.1 "Sequence 1.1"	CG	N/A	7	1.833	28.750	11.613	161.908
EV1.2	Event 1.2	Sequence	SQ1.2 "Sequence 1.2"	CG	N/A	8	0.817	30.067	4.804	159.004
EV2.1	Event 2.1	Speed (>=16.9 pixel/s)	429.138	CG	N/A	17	22.967	35.150	166.991	1927.467
EC1.1	Comb 1.1	Comb per Source	OR	Mult	N/A	14	-3.933	17.217	0.000	0.000

Following is the configuration that generated the Event Statistics example 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.

7.19 Where to Go Next

If you have purchased the Photometry Option, go to Chapter 8, Configuring the Photometry Parameters.

When you are ready to start video recording, see Chapter 9, Recording and Monitoring Video.

Chapter 8
Configuring the Photometry Parameters

8.1 Introduction

This chapter explains how to configure the Plexon® CineLyzer® Multifiber Photometry System (Photometry System). The photometry hardware and software license are optional, and can be added to the standard CineLyzer System.

If you have the standard CineLyzer System (without the Photometry option), this chapter does not apply.

Optical monitoring of neural activity

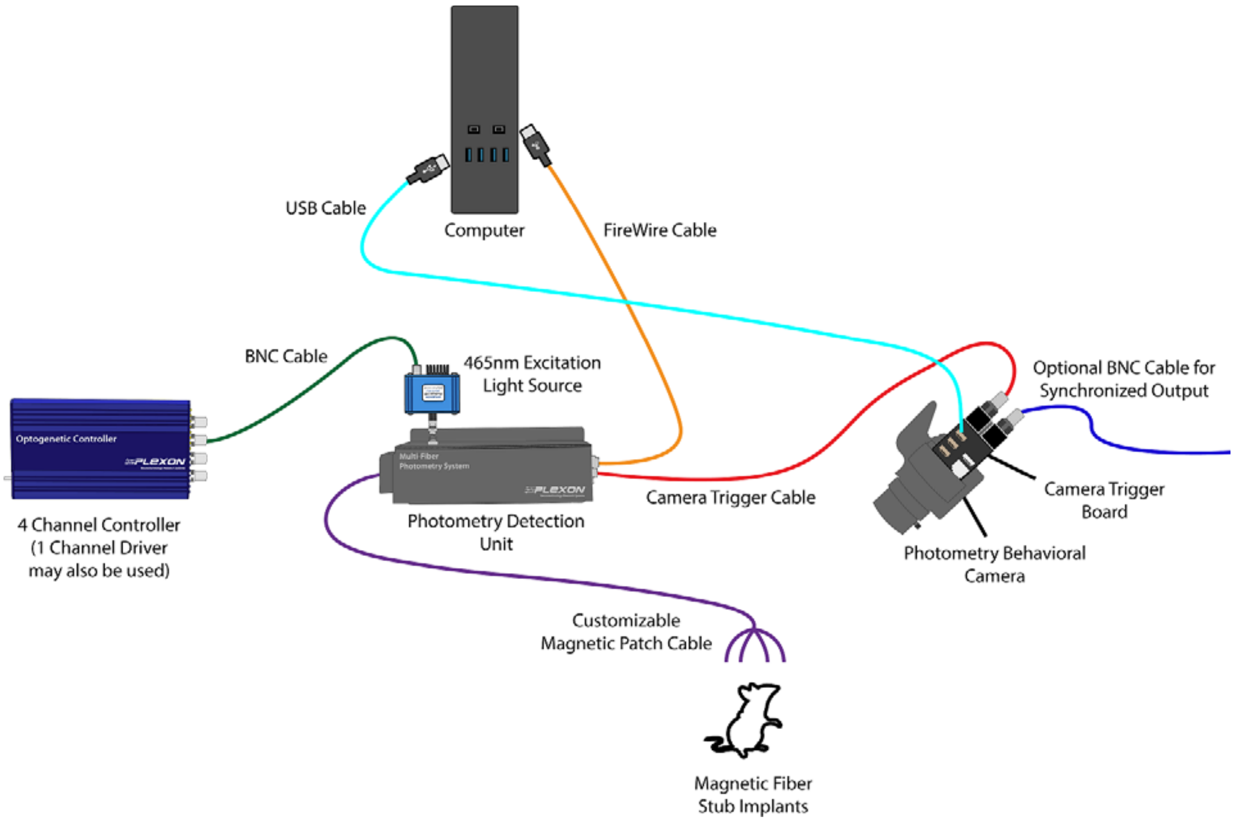
The fluorescence of genetically encoded calcium indicators (GECIs), such as GCaMP proteins, enables real-time optical monitoring and recording of neural activity of deep brain structures in behaving animals. You can monitor multiple brain regions simultaneously, which is useful in studying the connection between different brain regions, the behaviors that are produced, and the neural correlates of social interaction.

Photometry System function

The Photometry System measures and records the activity-dependent light emitted by fluorescent reporter cells in the brain in response to an applied excitation light. The system allows you to simultaneously record the activity-dependent reporter light and the animal’s behavior.

8.2 Photometry System and Component Overview

The Photometry System components and connections are shown below.



The individual components are described in the pages that follow.

The Photometry System includes the following components:

- Photometry Detection Unit
- Photometry Behavioral Camera Kit
- Camera Trigger Cable
- Excitation Light Source
- Computer

The following items are available for purchase separately:

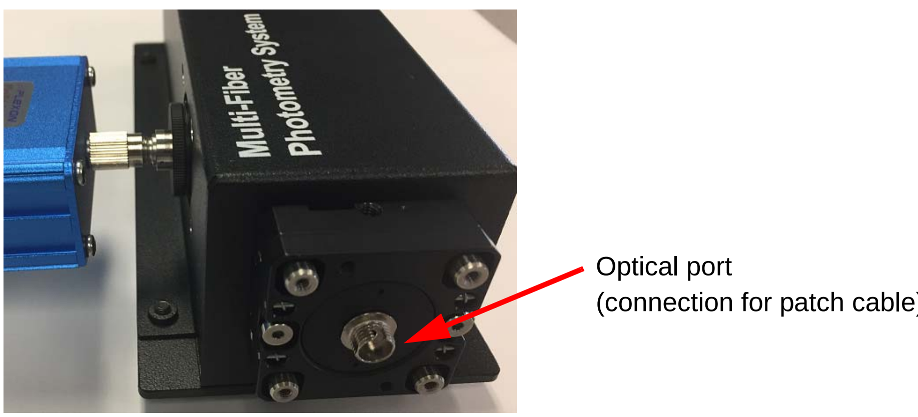
- Current-driven optical excitation system—
  (4 Channel Controller or 1 Channel Driver)
- Patch cable
- Fiber stub implants
- Synchronization output cable

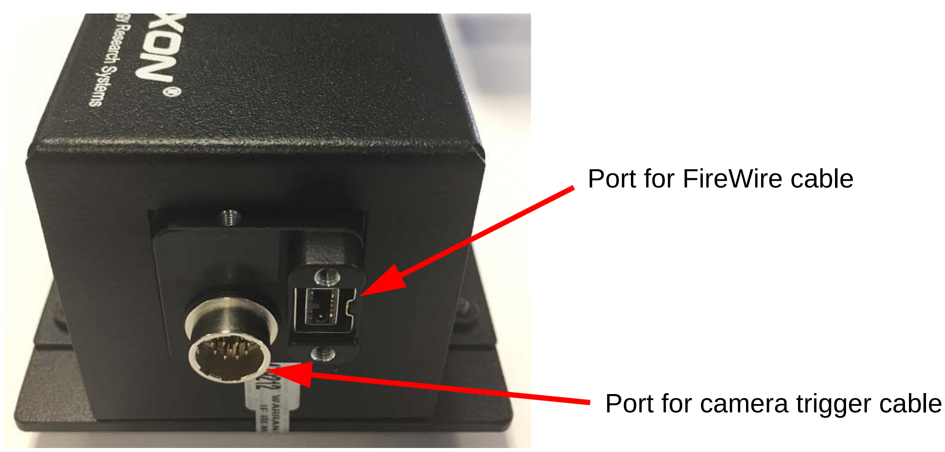
8.3 Photometry Detection Unit

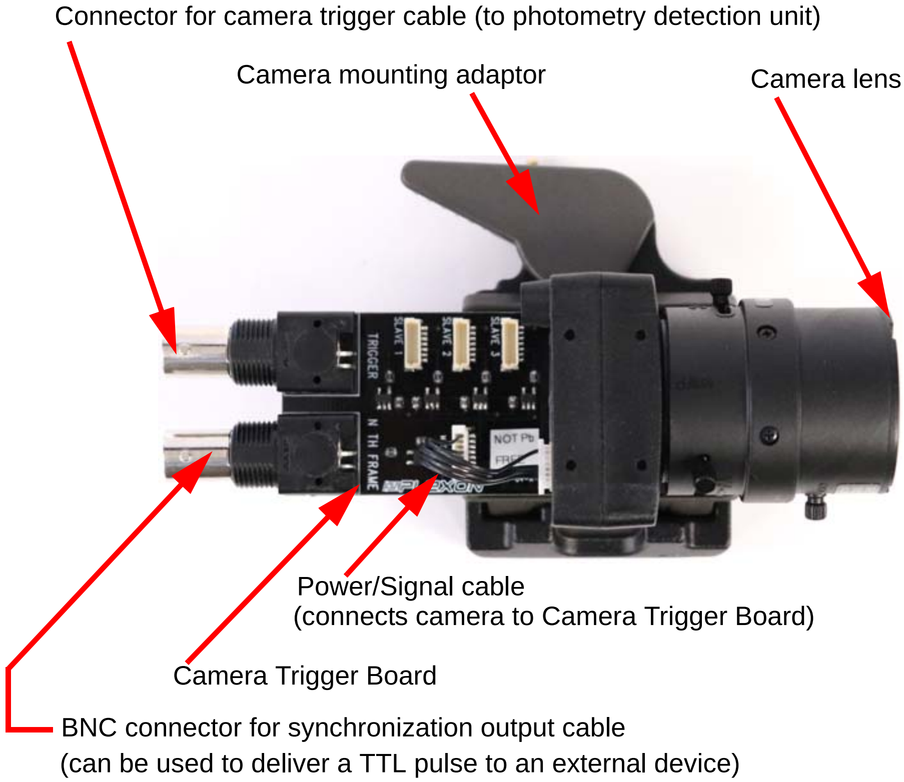
A single unit connected to and powered from a FireWire card installed in the PC with a type B FireWire cable. The assembly consists of optical components (filters, dichroic mirrors and lenses) for filtering the incoming excitation light and directing it down the multi-branch optical cable and for filtering the emission light coming back up the optical cable and directing it into the optical sensor for analysis.

Top view

End views







8.4 Photometry Behavioral Camera Kit

The kit includes the following items (see the image above):

- A Point Grey Research® Firefly® color USB camera with lens and USB cable—This camera connects to a dedicated USB PCI express card in the PC for optimal performance. It operates at a resolution of 640x480 pixels when used in conjunction with the photometry system. (A black and white camera can be provided in place of the color camera, if requested.)
- A mounting plate and adjustable pan tilt.
- A Camera Trigger Board, and a dedicated power/signal cable that connects the camera to the board.

8.5 Camera Trigger Cable

The camera trigger cable (not shown in the image above, described later in this section) connects the shutter output from the Camera Trigger Board to the trigger input port on the photometry detection unit. It ensures that the optical sensor in the detection unit is fully synchronized with the behavioral camera.

8.6 Excitation Light Source

A blue (465nm) excitation LED (a Plexon table-top LED module) which can be energized by a current driver.

Note: Plexon offers current-driven systems for use as excitation sources for this purpose (see “**Current-driven optical excitation system**” in Components Available for Purchase Separately, below).

8.7 Computer

The Photometry System includes a specially configured PC prepared by Plexon and delivered as part of the system. The PC contains dedicated USB and FireWire expansion cards that interface to the behavioral camera and photometry detection unit, respectively.

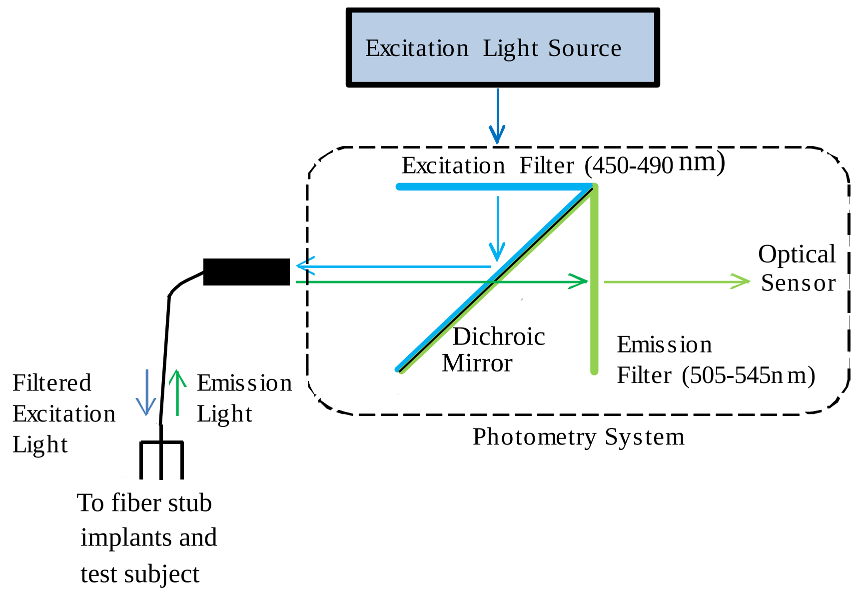
8.8 Components Available for Purchase Separately

- **Current-driven optical excitation system**—A current-driven system to drive the excitation light source, preferably one with a software GUI for defining and playing excitation patterns. You can select one of the Plexon PlexBright® Optogenetic Stimulation System controllers for this purpose, either the single-channel driver or the four-channel controller. See the Plexon website and speak with your Plexon sales engineer to determine which system will meet the needs of your experiment.
- **Patch cable**—An optical cable 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. The branched cable end is shown in the image below.

- **Fiber stub implants**—Magnetic or non-magnetic LC fiber stub implants built in accordance with customer requirements.
- **Synchronization output cable**—Can be used to deliver a TTL pulse from the trigger board to an external device. The pulse rate is configurable as described in the procedure later in this chapter.

8.9 Theory of Operation

The system operates as shown in the diagram.

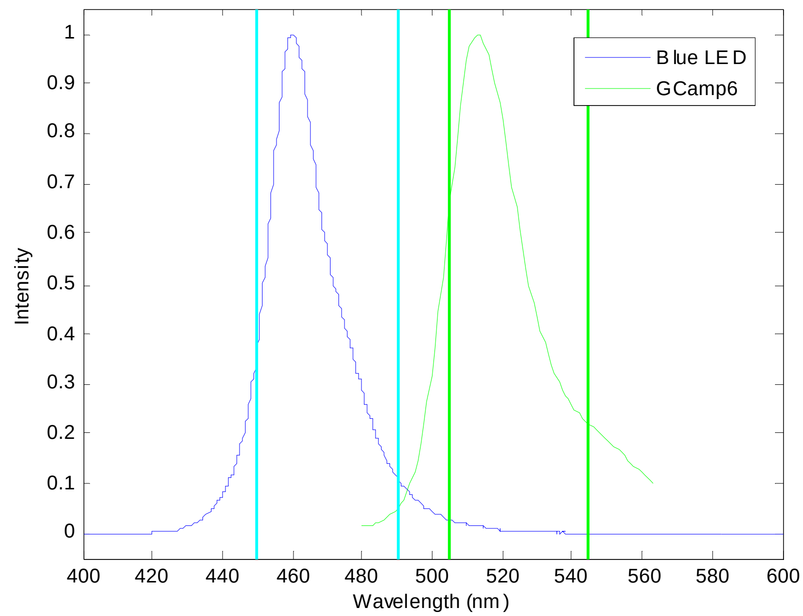


The dichroic mirror in the Photometry System directs the two light beams. Each fiber branch carries light in two directions:

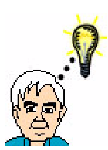
- Filtered excitation light is carried from the Photometry System to the subject.
- Green colored fluorescence emitted by GCaMP cells is carried to the optical sensor in the Photometry System.

The excitation light source is a blue LED with a relatively broad spectral output centered around 465 nm. This broad light is bandpass filtered from 450 to 490 nm and reflected off a dichroic mirror into the optical path. The dichroic mirror reflects wavelengths below 490 nm. 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 505 nm. The emission light is filtered from 505 to 545 nm to increase specificity of the signal before it is quantified by the optical sensor.

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 sensor 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 blue LED unit. Be sure to measure this power level at the fiber ends as described in Section 8.11, “Measuring and Adjusting Output Power of the Excitation Source” on page 210 to be sure it is appropriate for your specific experiment.

8.10 Photometry Hardware Setup and Pretest

Before you start—If not already done, follow the procedure in Chapter 2, Installing and Starting the System to connect and test the behavioral camera. Then turn off (power down) the computer again.

CAUTION

The computer MUST be powered off
before connecting or disconnecting a FireWire cable

Never connect or disconnect the FireWire cable while the computer is powered as FireWire devices are not designed for hot swapping and damage can result.

Use this procedure to connect the Photometry System to the PC. Refer to the diagrams earlier in this chapter.

Computer connections

1. Verify that the computer is turned off (powered down).
2. Use the supplied USB cable to connect the behavioral camera to a USB port in the expansion card on the back panel of the PC. It is important to connect the camera to one of these ports for optimum handling of the video stream; do not connect the camera to any other port.

   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. If you have any questions regarding cable connections, please contact Plexon Support.

3. Connect the keyboard, mouse and monitor to the PC.
4. Plug the CineLyzer license key into any available USB port on the PC.
5. Use the FireWire cable to connect the Photometry System to the computer as follows:

   5a Connect one end of the FireWire cable to the FireWire port in the Photometry System.

   5b Connect the other end of the cable to one of the computer FireWire ports.

   5c Use a screwdriver to secure both ends of the cable.

6. (Optional) If you are using input or output lines on the USB DIO interface, connect the interface to any available USB port on the PC.

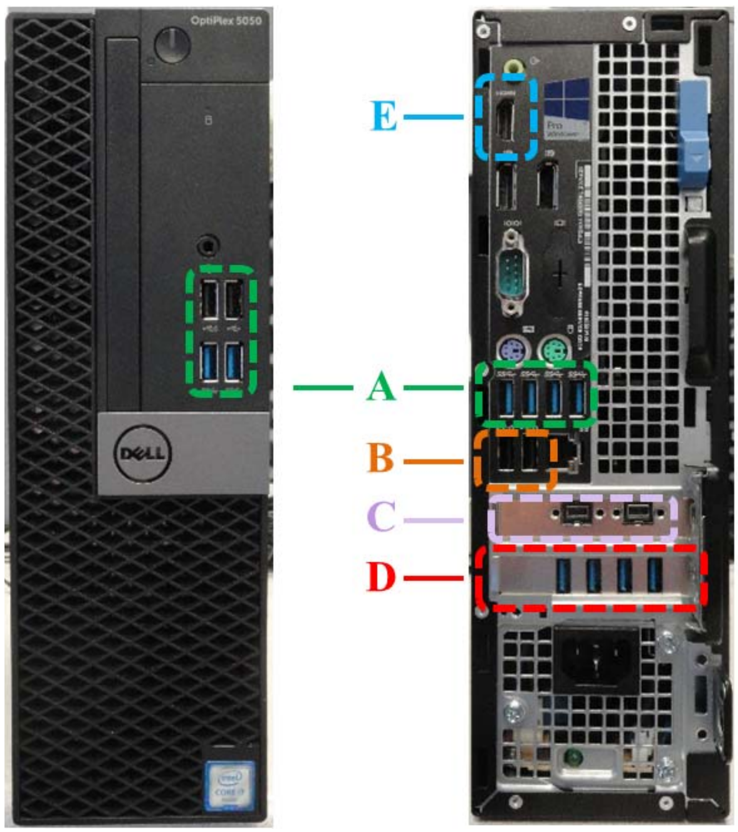
A — Built in USB ports:
For license key(s), USB DIO interface, and Optogenetic Controller

B — Built in USB ports: Suggested for keyboard and mouse

C — Expansion card FireWire ports: Use one of these for connection to the Photometry System

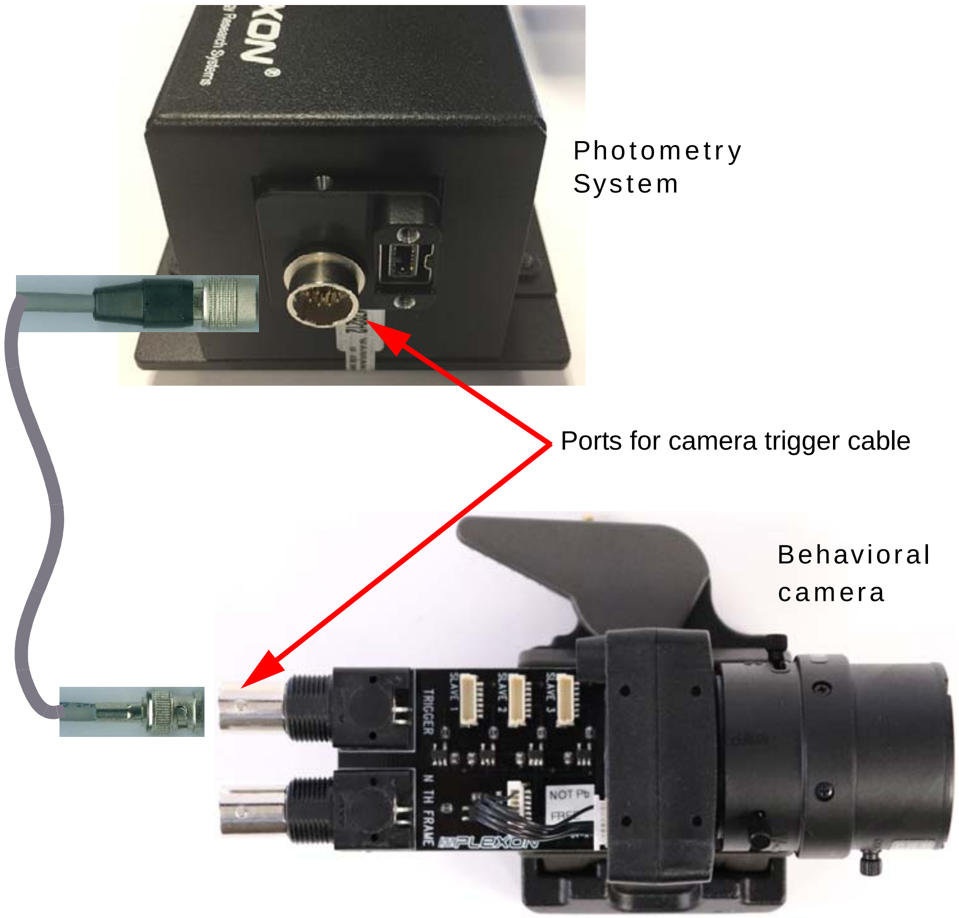
D — Expansion card USB ports: Use one of these for connection to the behavioral camera

E — Built in HDMI port: For monitor



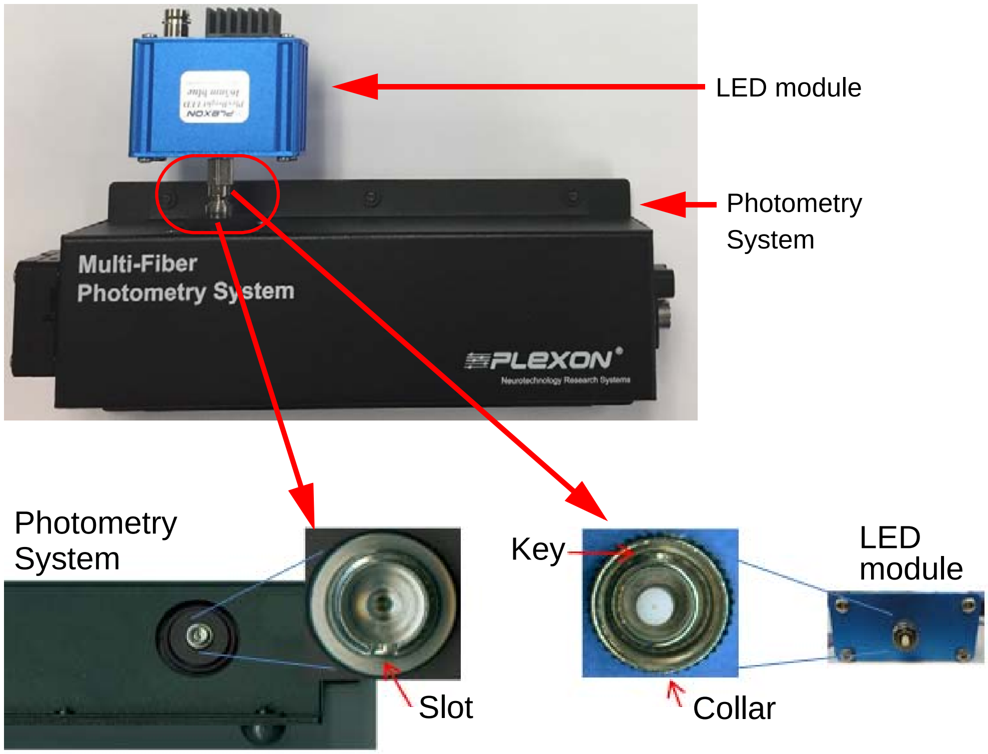
Trigger connection

Use the camera trigger cable (PN 06-06-A-07 or later) to connect the Photometry System to the “Trigger” BNC on the behavioral camera. This connection is used to synchronize the photometry data to the behavioral video.



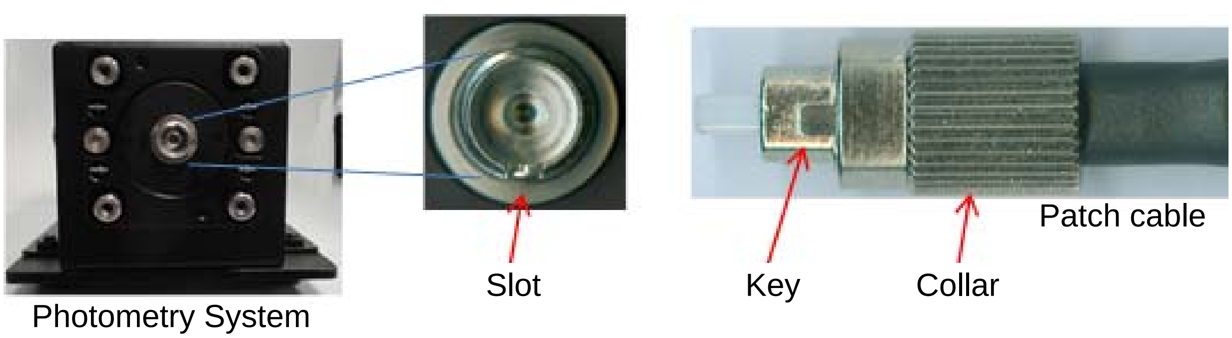
Optical connections

1 Connect the Plexon LED module to the Photometry System. Note that there is a “key” on the male FC connector on the Plexon LED module that must fit into the corresponding “slot” on the female FC connector on the Photometry System. Make sure the key is aligned in the slot and then push the “collar” forward and screw it down to secure the connection. See the image below.



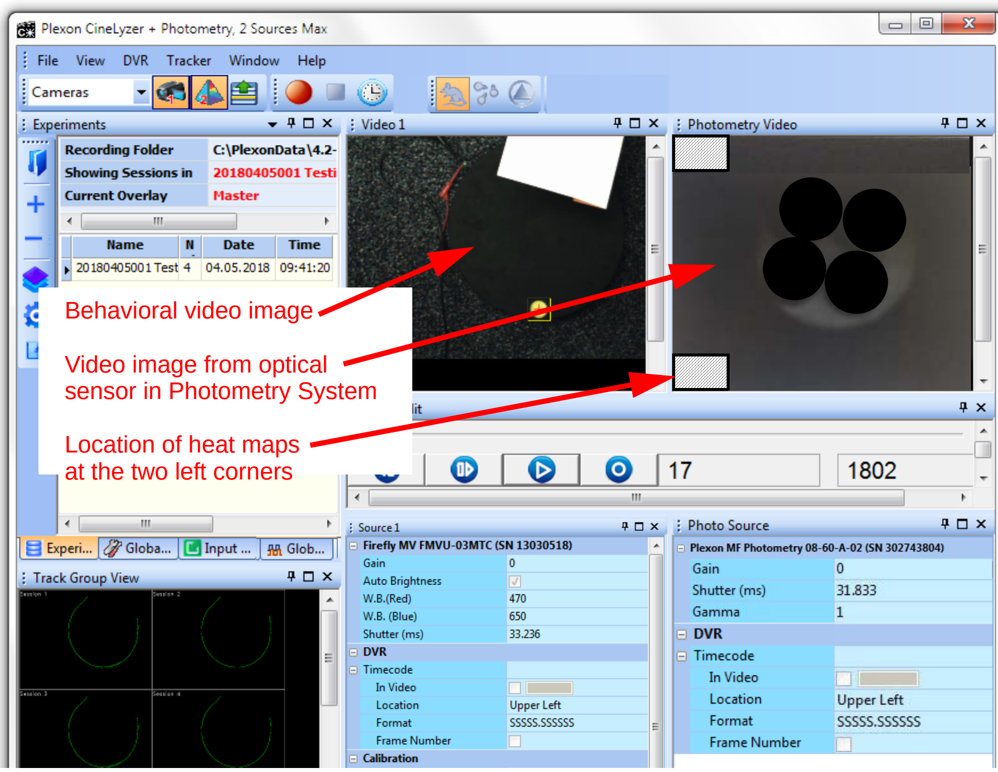
2 Connect the LED module to the LED driver following the instructions provided with the LED driver.

3 Connect the patch cable to the optical port of the Photometry System. Note that there is a “key” on the male FC connector on the patch cable that must be aligned with a “slot” on the female FC connector on the Photometry System. Make sure the key is aligned in the slot and then push the “collar” forward and screw it down to secure the connection. See the image below.



System Startup

1. Turn on the PC and launch the CineLyzer application. This will provide power to the Photometry System through the FireWire cable.
2. Verify the LED (excitation light source) is energized by the current-driven power unit and emitting blue light.
3. Verify that the patch cable is connected to the photometry detection unit.
4. In the CineLyzer GUI, verify that the system is in **Cameras** mode.
5. Verify that the **Video 1** and **Photometry Video** windows are displaying the appropriate video streams. The **Photometry Video** window should be displaying a faint image caused by the small amount of light present in the fibers (this is ambient or reflected light, not fluorescence). The image below shows a four-branch fiber cable with a small amount of visible light in the cable.
6. If the fiber image is not visible, turn up the power level in the current-driven power unit. If there is still no image, verify the blue LED excitation light is on and the patch cable is connected properly to the Photometry System according to the instructions provided earlier in this chapter.



As seen in the image above, the individual fiber ends (when not exposed to ambient light) will always appear dark, even if fluorescent light is present. The amplitude of the fluorescent light is too low for the human eye to see in the **Photometry Video** window. However, when the heat maps are on, the sensitive detector in the photometry detection unit is able to detect it so it can be displayed. Heat maps are discussed in Section 8.14, “Working with the Photometry Visualization Options” on page 220.

7 **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 detection unit 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 unit.

Note: The screen layout is customizable. If you want to restore the default layout, select **Window | Layout | Reset to Default Layout** from the main menu.

8.11 Measuring and Adjusting Output Power of the Excitation Source

Prior to starting a photometry experiment, it is essential to use an optical power meter (e.g. Plexon light measurement kit) to measure the level of excitation light coming out of the branches 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 sensor.

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.

Power measurement

1 Verify that the blue LED and multi-branch optical cable are connected to the Photometry System, and the LED is powered up (emitting blue light).

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

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

4 Verify that the light intensity is sufficient for your experiment. (Keep in mind that the intensity will be further reduced when you connect the branch to the fiber implants.)

Mapping individual patch cable branches to images in Photometry Video window

5 If you are using a multi-branch patch cable—If the fiber end(s) are exposed to ambient light, you should see some of this light in the Photometry Video window. You can use this ambient light to help you identify which circle in the **Photometry Video** window corresponds to each individual branch 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 label on the branch and the circle that appears in the **Photometry Video** window.

8.12 Configuring the Initial Recording Parameters

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

8.13 Defining Photometry Fibers

This section explains how to define and adjust the photometry fibers in the CineLyzer GUI (one fiber for each branch in a multi-branch optical cable).

8.13.1 Selecting and Configuring Behavioral Camera Source Parameters

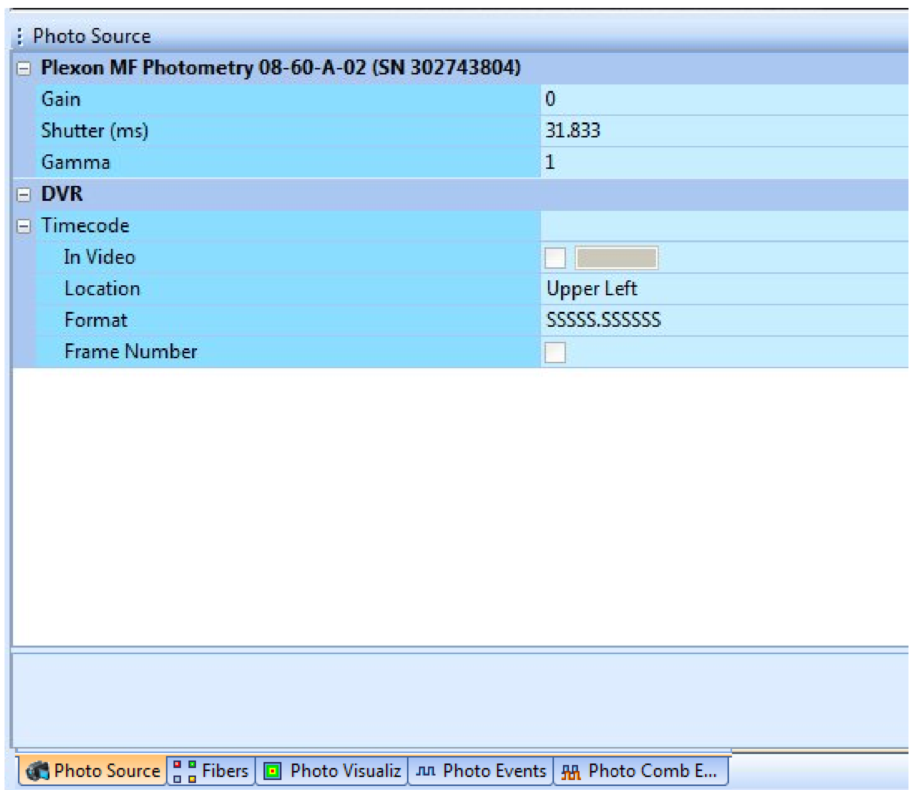
Follow the steps in Section 5.7, “Configuring the Source Parameters” on page 77 to configure the camera settings in the **Source** tab for the behavioral camera.

8.13.2 Selecting and Configuring Photometry Source Parameters

Follow the steps below to configure the parameters in the photometry source tab (**Photo Source**).

1 In **Cameras** mode, select or create an experiment.

2 Click on the **Photo Source** tab. This tab is displayed.



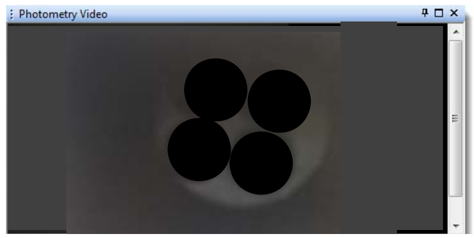
Note: Leave the **Gain**, **Shutter** and **Gamma** settings at factory default.

3 If desired, you can display the **Timecode** and **Frame Number** in the video stream by selecting the applicable checkboxes.

8.13.3 Selecting and Configuring Fibers

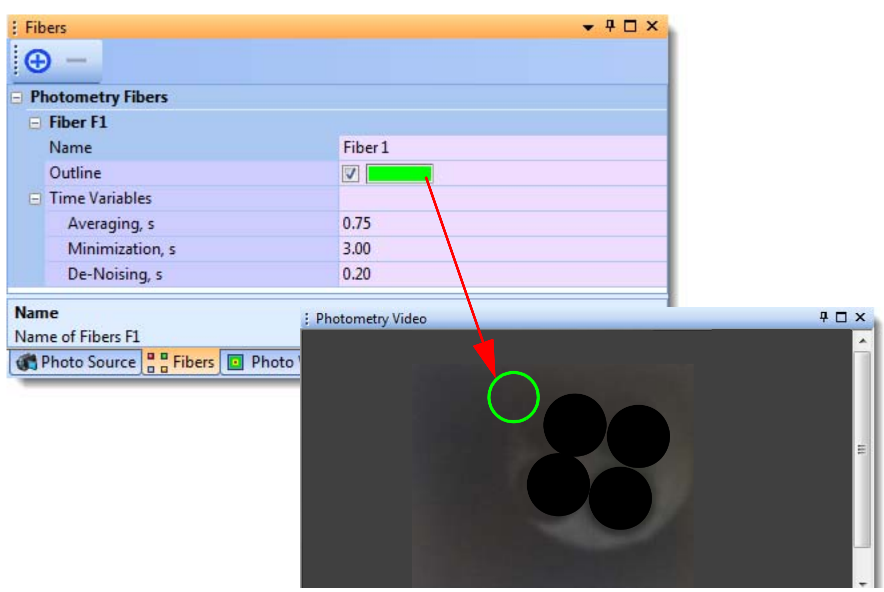
Follow these steps to configure the photometry fibers. In the **Fibers** tab (shown in the images below), each item you configure (Fiber F1, Fiber F2, ...) corresponds to a single fiber branch of the multi-branch optical cable.

1 **IMPORTANT**—Verify the blue LED is connected to the photometry detection unit, is powered on and bright enough to be detected in the **Photometry Video** window.



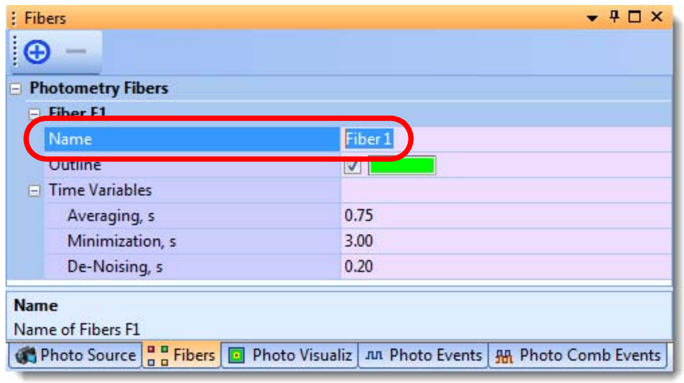
2 Click on the **Fibers** tab, then click the "+" in the upper left corner to add a new fiber.

The system will display parameters for a new fiber in the window, and it will place a default circle in the **Photometry Video** window. (The default circle might not match the size or location of the image the system is receiving from the actual fiber; you will adjust the circle in the next step.)

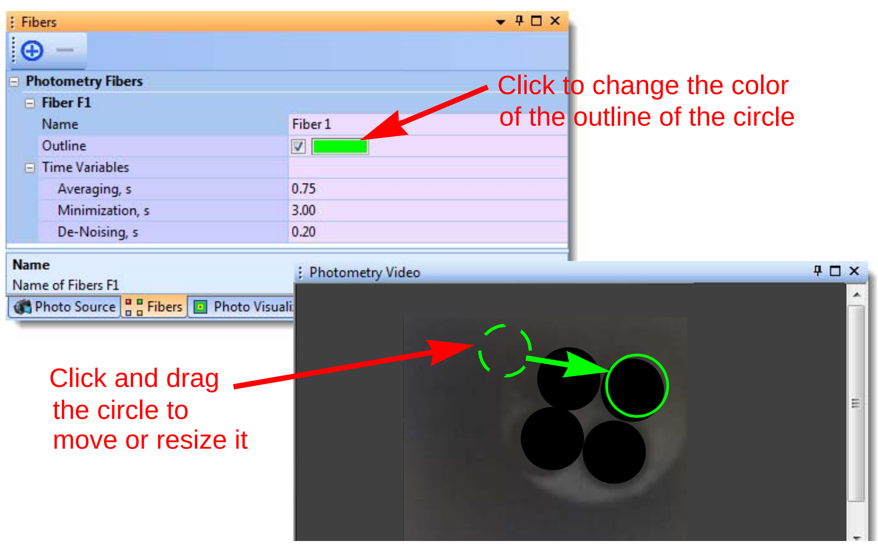


3 To define Fiber F1 (or any other fiber), double click in the field on the right side of the **Name** row for that fiber (see the image below) and enter the name you want to apply to that fiber. For example, you might enter a name that corresponds to the label attached to the fiber branch.

Note: There is no need to adjust the **Time Variables** at this point in the procedure. These parameters will be discussed later.



4 You can modify the position, diameter and color of the circle in the **Photometry Video** window so it matches the circular region of the fiber.

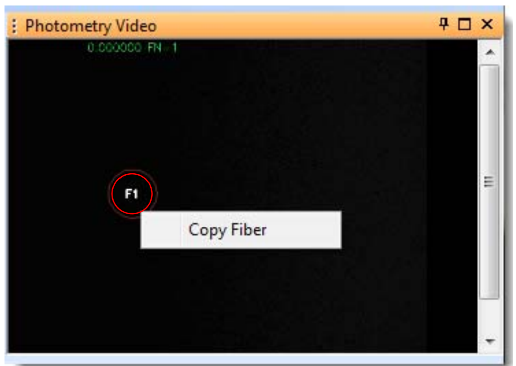


5 To configure additional fibers, repeat Step 1 through Step 4. (See the tip, below.)

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

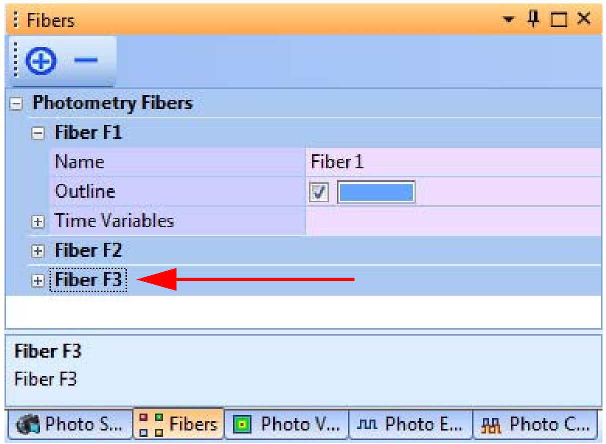
Do not overlap fibers

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

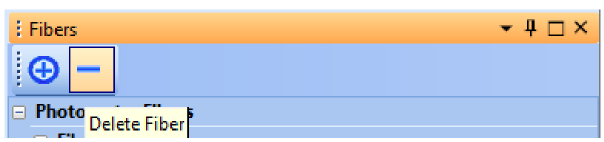
8.13.4 Deleting Fibers

You can delete individual fibers as described below.

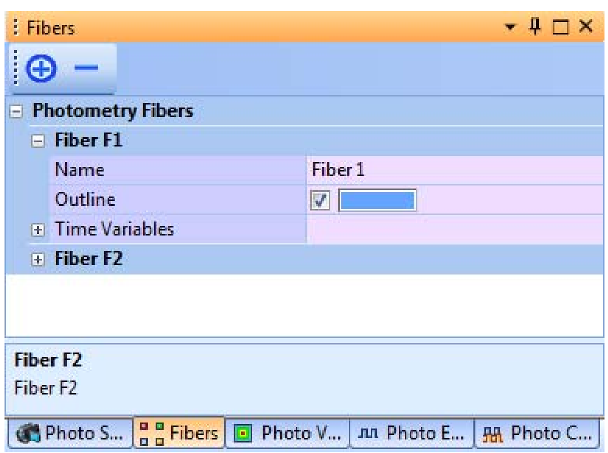
1. Click on the **Fibers** tab, then click on the fiber that you want to delete. In the example below, the user has clicked on Fiber F3 (notice the black dotted line around this fiber name).



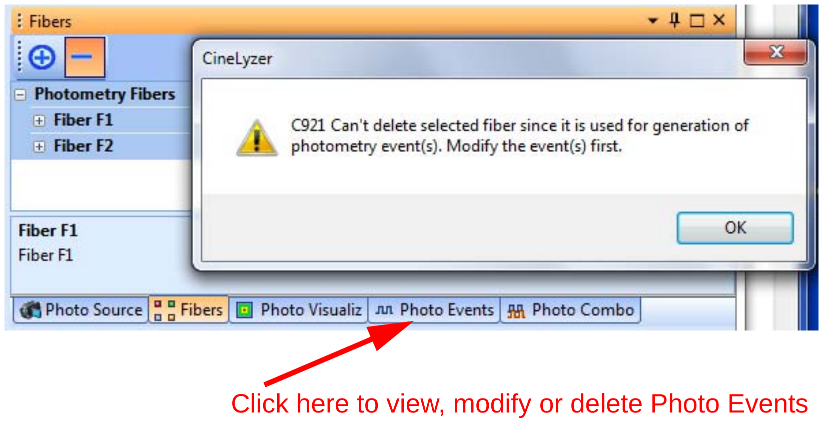
2. Click the “—” (**Delete Fiber**) icon.



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

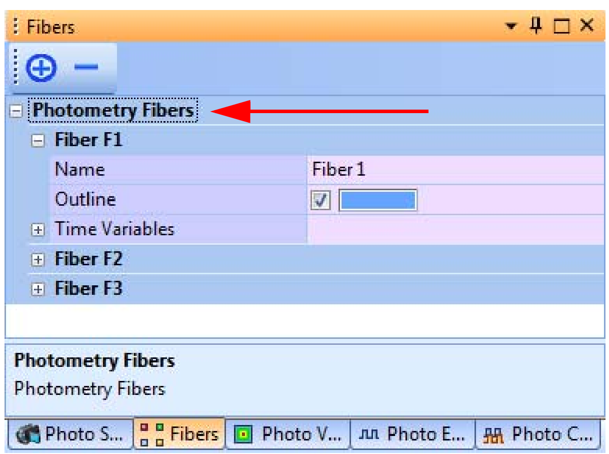


3 If you have a **Photo Event** configured for the fiber you are trying to delete, the system notifies you that the fiber cannot be deleted because the fiber is associated with a photometry event. In that case, modify the photometry event (disassociate the fiber from this event) or delete the photometry 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.



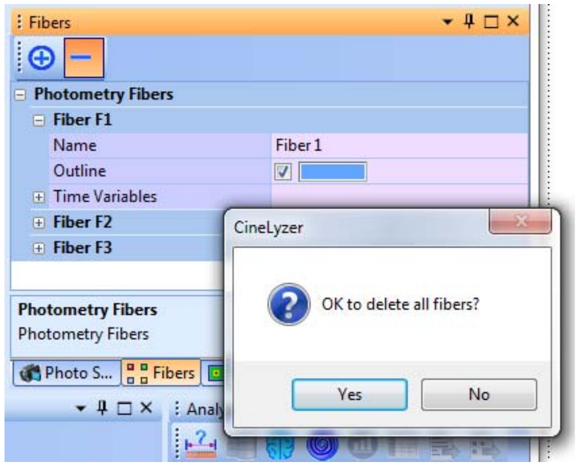
You can delete all fibers as described below.

1 Click on the **Fibers** tab, then click on the **Photometry Fibers** row. In the example below, notice the black dotted box displayed in this row).



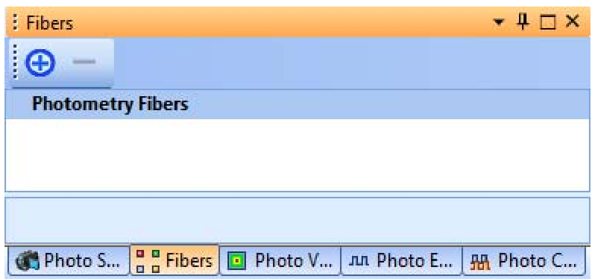
2 Click the "—" (**Delete Fiber)** icon.

The system displays a caution notice.

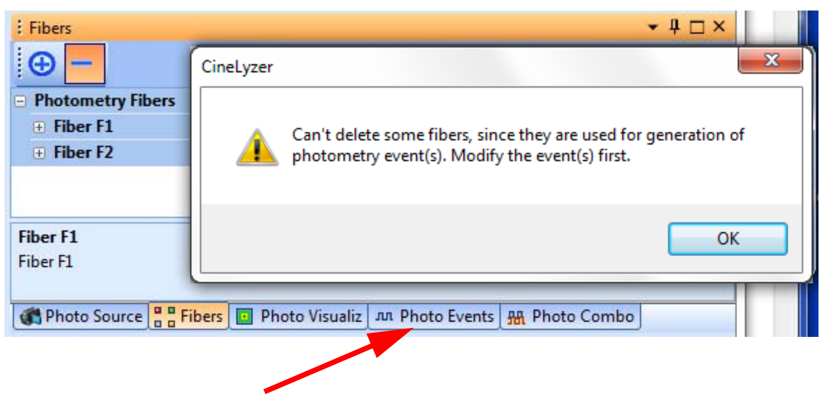


3 If you are sure you want to delete all fibers, click **Yes**. Otherwise click **No**.

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



4 If you have a **Photo 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.



Click here to view, modify or delete Photo Events

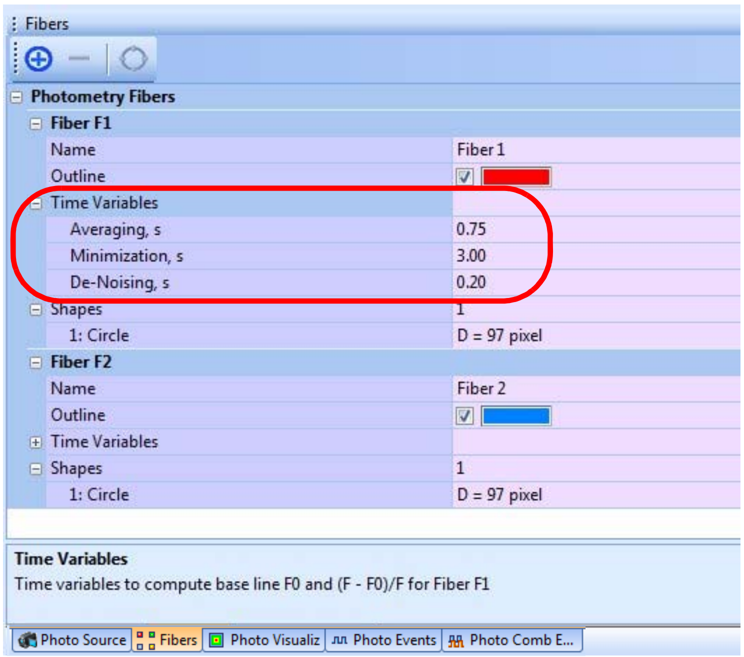
8.13.5 Configuring the Time Variables

The default photometry analysis parameters are taken from the reference H. Jia, N.L. Rochefort, X. Chen, A. Konnerth as listed in Section 8.23, “Photometry Reference” on page 234. All of the analysis parameters can be adjusted on a per fiber basis. The controls to adjust the analysis parameters are configurable in the **Time Variables** section in the **Fibers** window.

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.

Note: For additional discussion of these values (τ_1,τ_2 and τ_0), see Section 8.23, “Photometry Reference” on page 234.

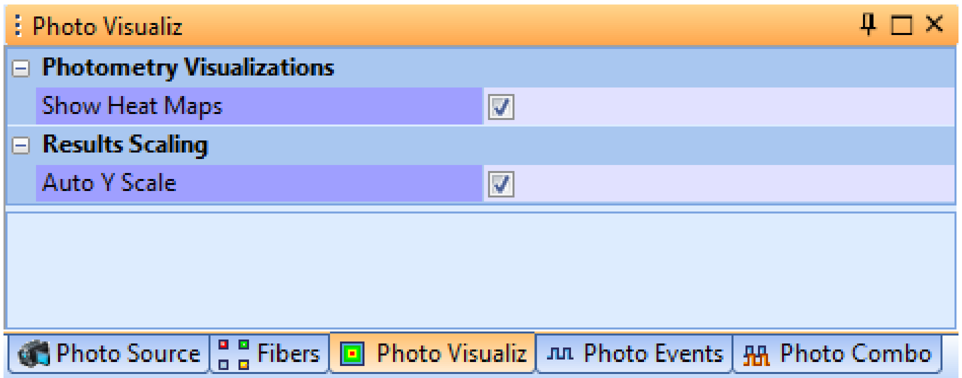


8.14 Working with the Photometry Visualization Options

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

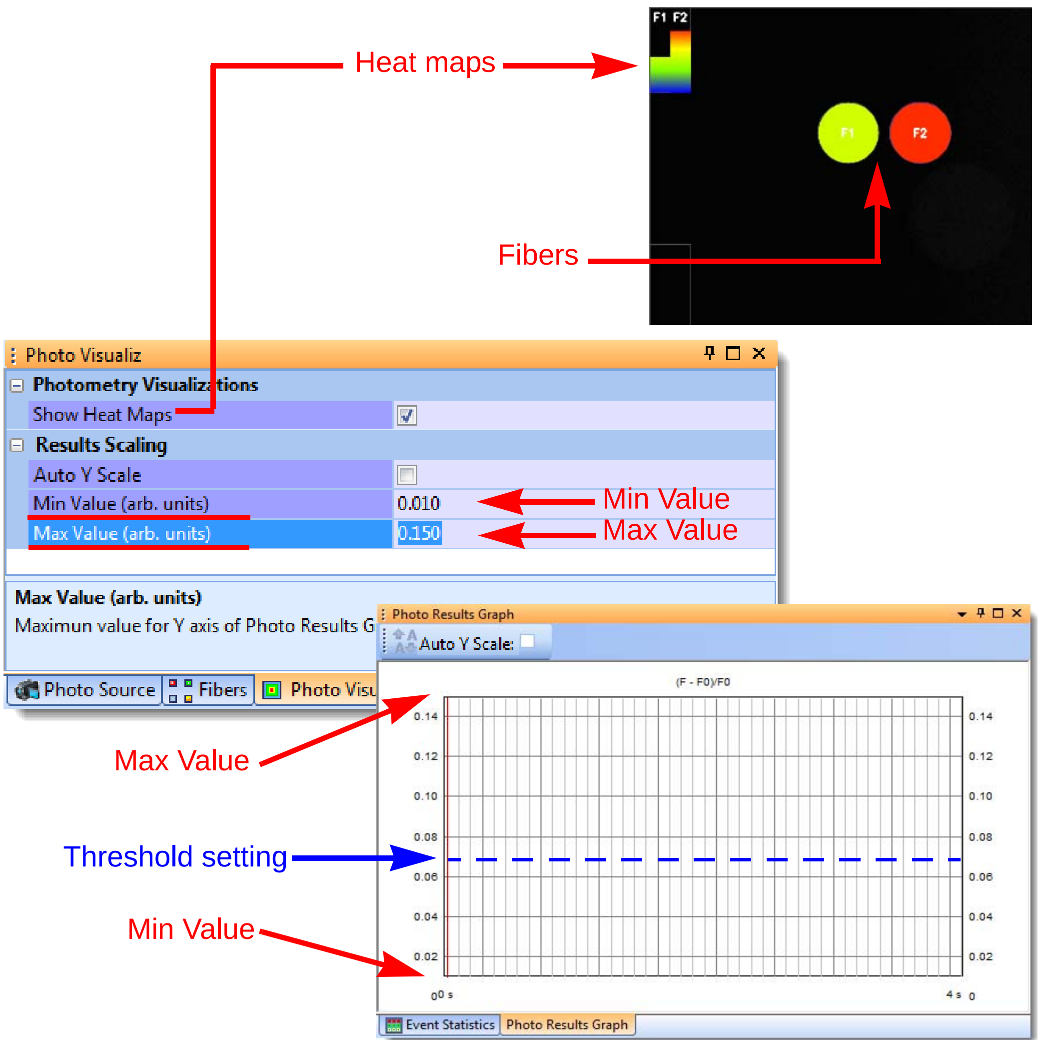
- The appearance of the heat maps in the **Photometry Video** window
- The y-axis scaling in the **Photo Results Graph**.

Click on the **Photo Visualiz** tab to access these options. By default, both of the checkboxes in this dialog are selected.



- **Show Heat Maps**—Select this checkbox to show the heat maps for the fibers displayed in the **Photometry Video** window. The intensities of the heat maps displayed in the **Photometry Video** window correspond to the intensity levels shown in the **Photo Results Graph** (see the screen image shown below).
- **Auto Y Scale**—Select this checkbox to allow the system to determine the y-axis scaling and labeling for the **Photo 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 8.16, “Creating Photometry Events” on page 224. 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.

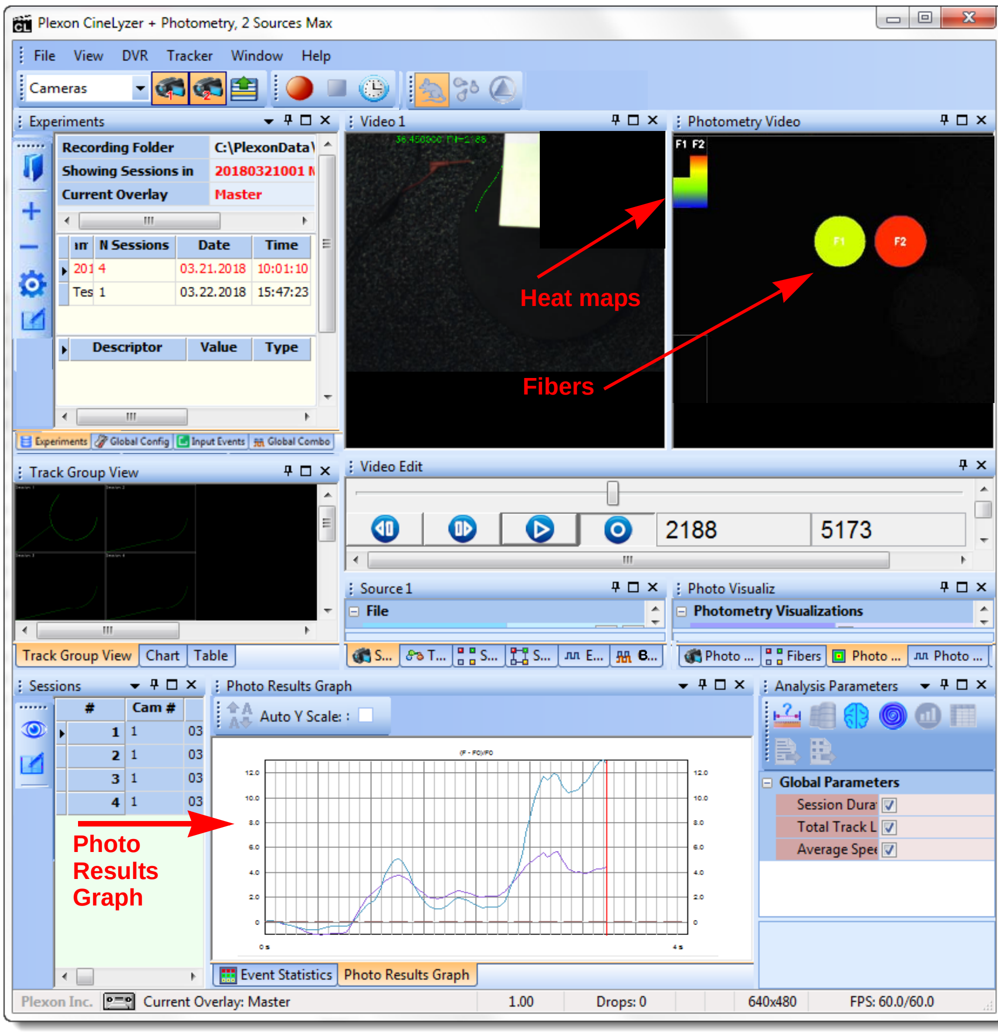
  In most cases the Y axis of the graph is scaled automatically considering the minimum and maximum of the signals that have been observed. If there is a large transient change in fluorescence, the maximum or minimum Y values of the graph may take on abnormally large values with the result that the data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



Heat maps
Fibers
F1 F2
Photo Visualiz
Photometry Visualizations
Show Heat Maps
Results Scaling
Auto Y Scale
Min Value (arb. units)
0.010
Min Value
Max Value (arb. units)
0.150
Max Value
Max Value (arb. units)
Maximun value for Y axis of Photo Results G
Photo Source
Fibers
Photo Visu
Photo Results Graph
Auto Y Scale:
(F - F0)/F0
Max Value
Threshold setting
Min Value
Event Statistics
Photo Results Graph

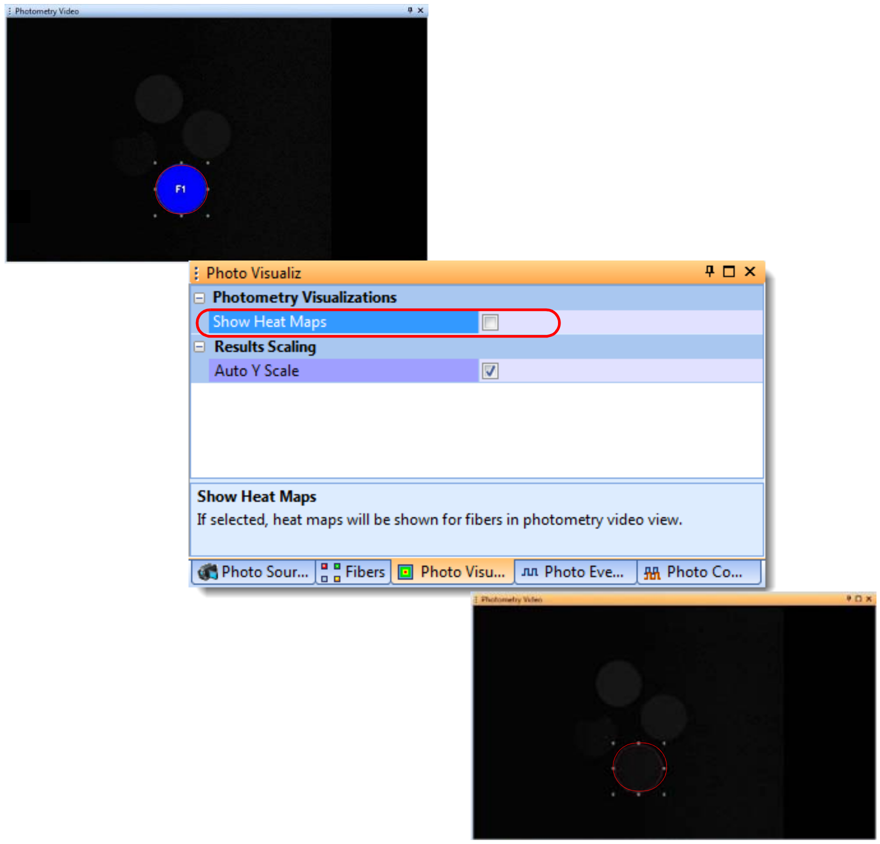
8.15 Adjusting Fiber Locations and Size During Operation

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 GECI cells 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.



Shortly after you first create a fiber, the system displays a circle and begins to fill the circle 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. 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 on the **Photo Visualiz** tab, then deselect the **Show Heat Maps** box.



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

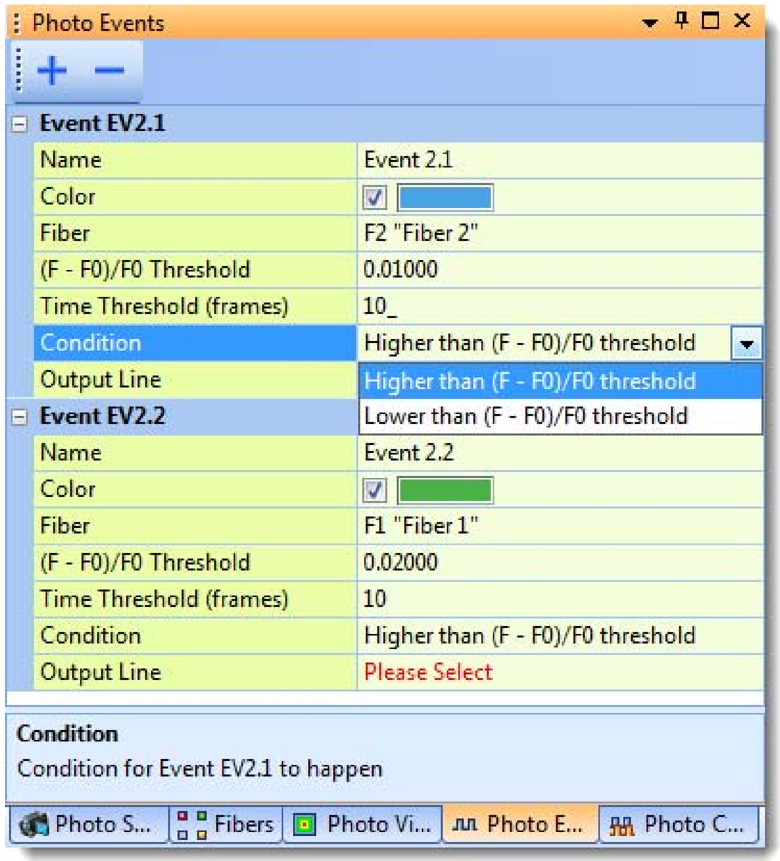
3 Check the **Show Heat Maps** box again to display the heat maps.

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

8.16 Creating Photometry Events

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

- **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.
- **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 8.20, “Displaying Behavioral and Photometry Event Statistics” on page 228.
- **Fiber**—Select the fiber for this event.
- **(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.
- **Time Threshold (frames)**—Specify the number of frames for which the **Condition** must be met for the event to be triggered, 0 to 999 (default 10).
- **Condition**—Specify when the event is triggered, that is, when the measured relative fluorescent intensity is **Higher than (F -F0)/F0 threshold** or **Lower than (F -F0)/F0 threshold**.
- **Output Line**—Specify the line(s) you are using to send a signal from the CineLyzer System to an external device (optional). For information on configuring the output lines, see Section 7.17, “Specifying Digital Outputs” on page 190

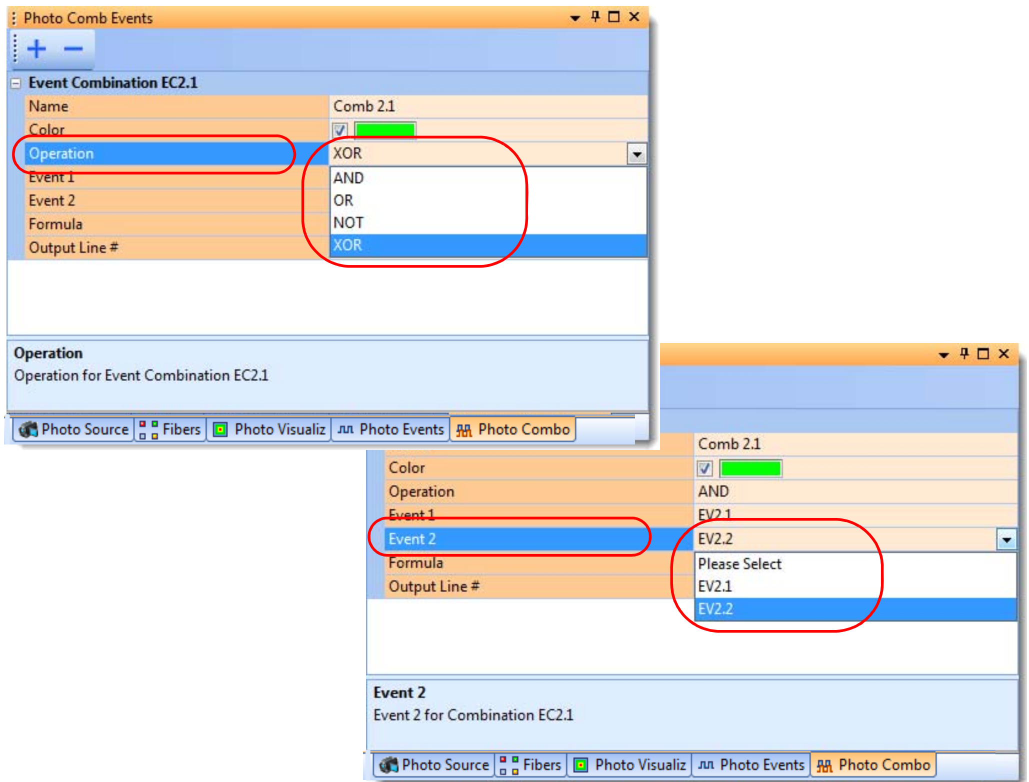


8.17 Understanding the Relationship of Fibers and Sessions

For any session, photometry events can only be created for fibers that have been defined for that specific session. An example is presented in AppendixF, Photometry Examples.

8.18 Creating Photometry Combination Events

You can create combination photometry events based on a combination of the intensity of the fluorescence detected in multiple fibers. Click the **Photo Combo** tab and create the desired events. The parameters in this dialog are the same as for the behavioral Combination Events. The images below illustrate some of the options.



8.19 Creating Global Combination Events with the Photometry Option

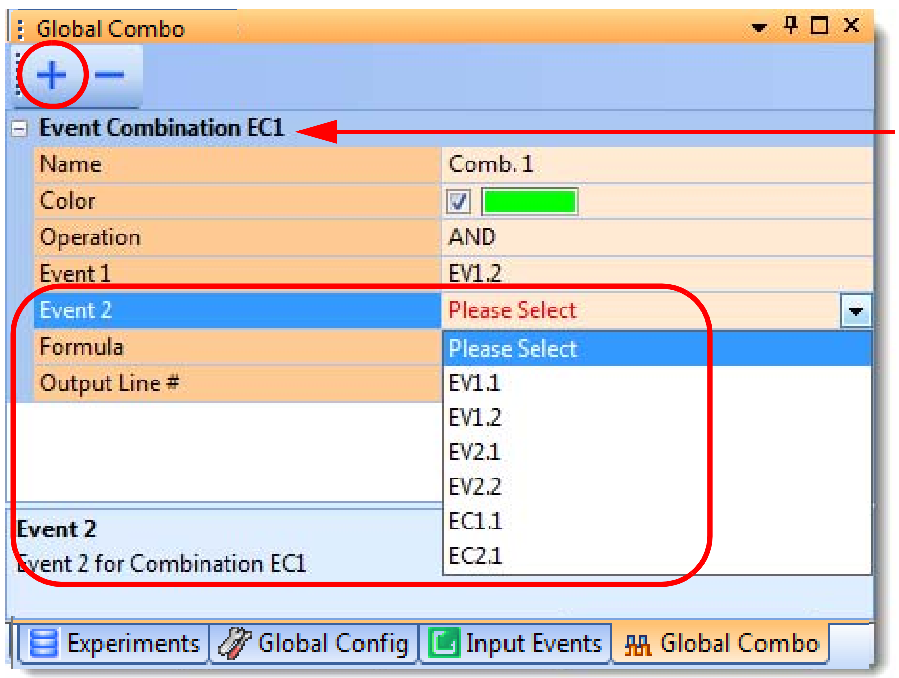
You can create global combination events based on a combination of fluorescence intensity and behavioral events. Click on the **Global Combo** tab in the global settings window (see the image below), then click the **+** icon to create a new event. In this example, we are creating a global combination event "**Event Combination EC1**."

- **Event 1** is specified as EV1.2, which is a behavioral event.
- For **Event 2** of this global combination event, we have the option of selecting, for example, EV2.1 (from Fiber 1) or EV2.2 (from Fiber 2).
  We can also select EC2.1, which is a *combination* event from the two fibers.

Note: In general:

The "1." in " **EV1.***x*" or "**EC1.**x" identifies an event or combination event as being associated with the **Video 1** window.

The "2." in "**EV2.***x*" or "**EC2.**x" identifies an event or combination event as being associated with the **Photometry Video** window.

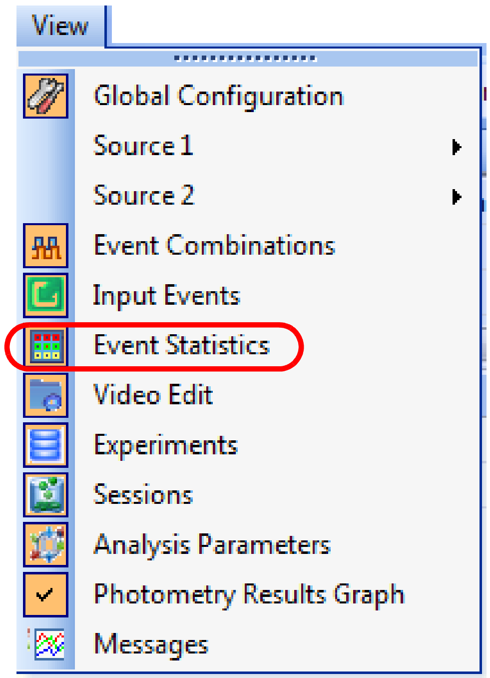


Configure values for the other parameters as needed—**Name**, **Color**, **Operation** and **Output Line #:**

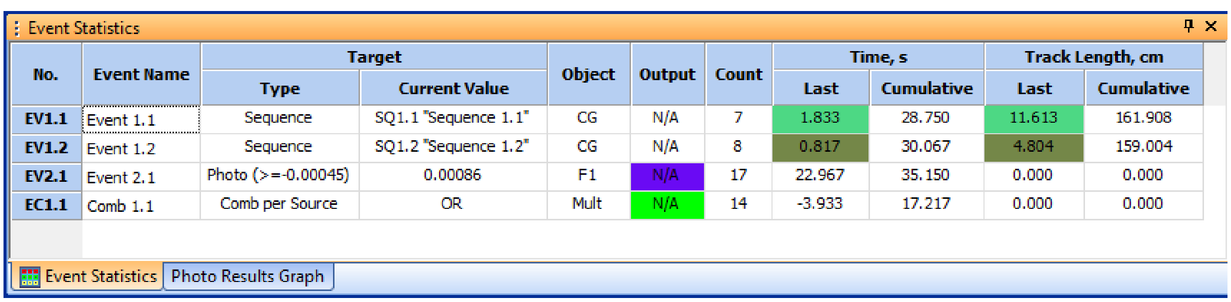
- The **Operation** row parameter is described in Section 7.14, "Defining Combination Events per Video Source" on page 179.
- The **Output Line #** parameter is described in Section 7.17, "Specifying Digital Outputs" on page 190.

8.20 Displaying Behavioral and Photometry Event Statistics

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 events based on zone sequences (Event 1.1 and Event 1.2), photometry level (Event 2.1) and a combination event (Comb 1.1). The colored cells in the display indicate that an event was occurring at the moment this image was captured.

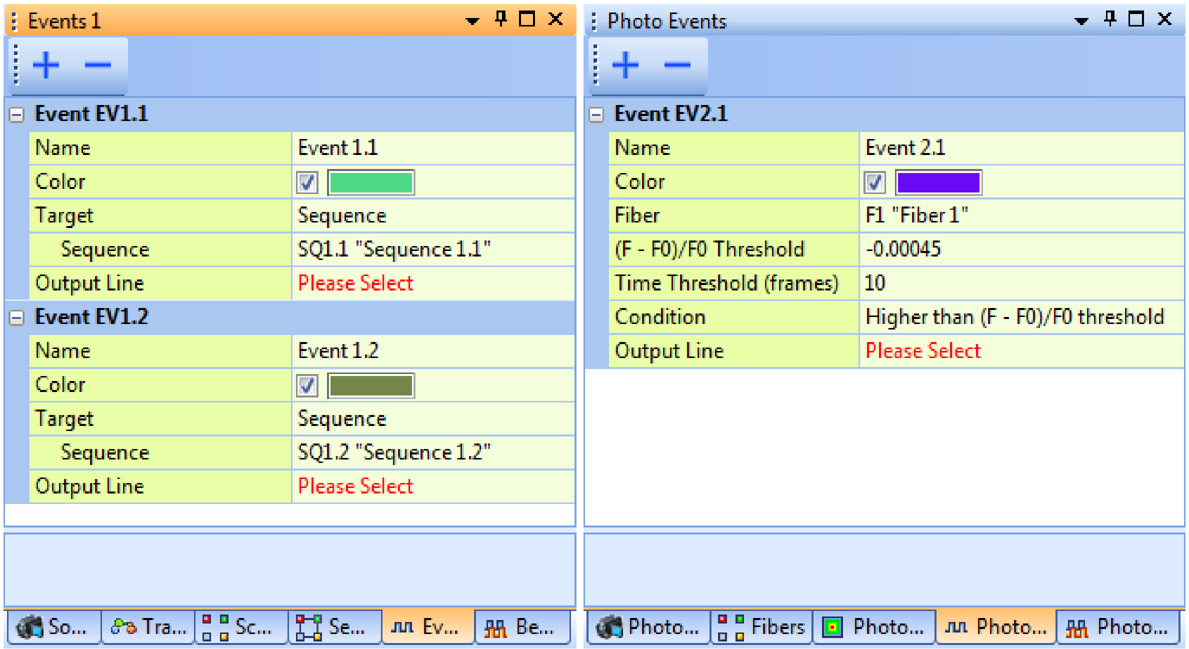


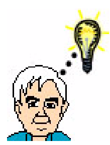
Event Statistics

No.	Event Name	Target		Object	Output	Count	Time, s		Track Length, cm	
		Type	Current Value				Last	Cumulative	Last	Cumulative
EV1.1	Event 1.1	Sequence	SQ1.1 "Sequence 1.1"	CG	N/A	7	1.833	28.750	11.613	161.908
EV1.2	Event 1.2	Sequence	SQ1.2 "Sequence 1.2"	CG	N/A	8	0.817	30.067	4.804	159.004
EV2.1	Event 2.1	Photo (>=-0.00045)	0.00086	F1	N/A	17	22.967	35.150	0.000	0.000
EC1.1	Comb 1.1	Comb per Source	OR	Mult	N/A	14	-3.933	17.217	0.000	0.000

Event Statistics | Photo Results Graph

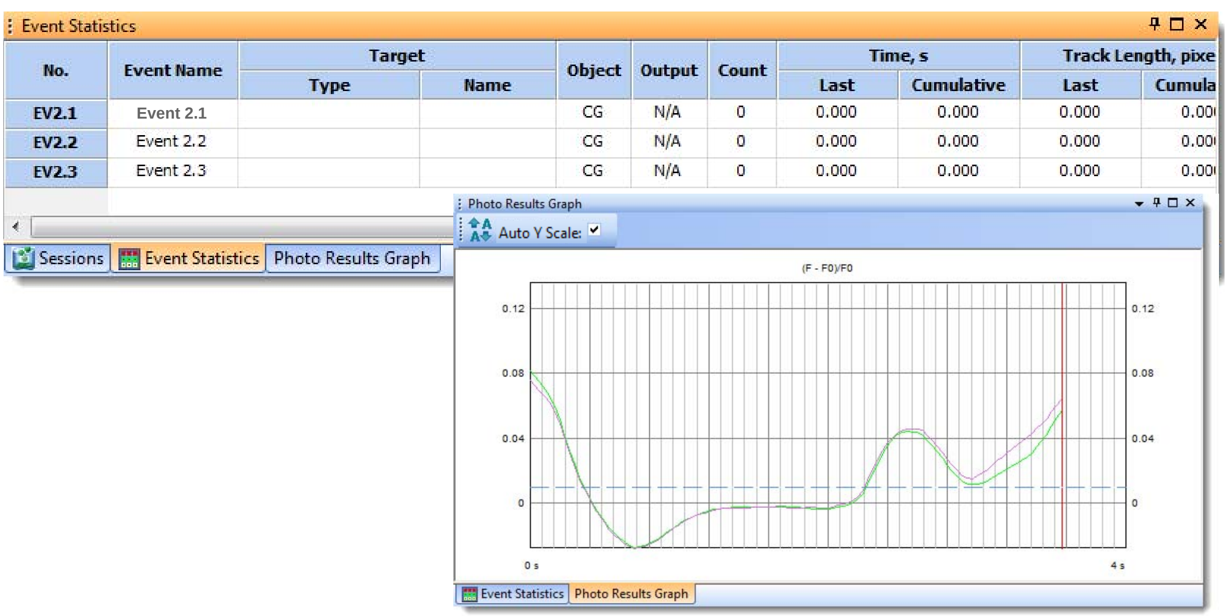
Following is the configuration that generated the Event Statistics example shown above.





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. Examples of the **Event Statistics** and **Photo Results Graph** tabs are shown below.

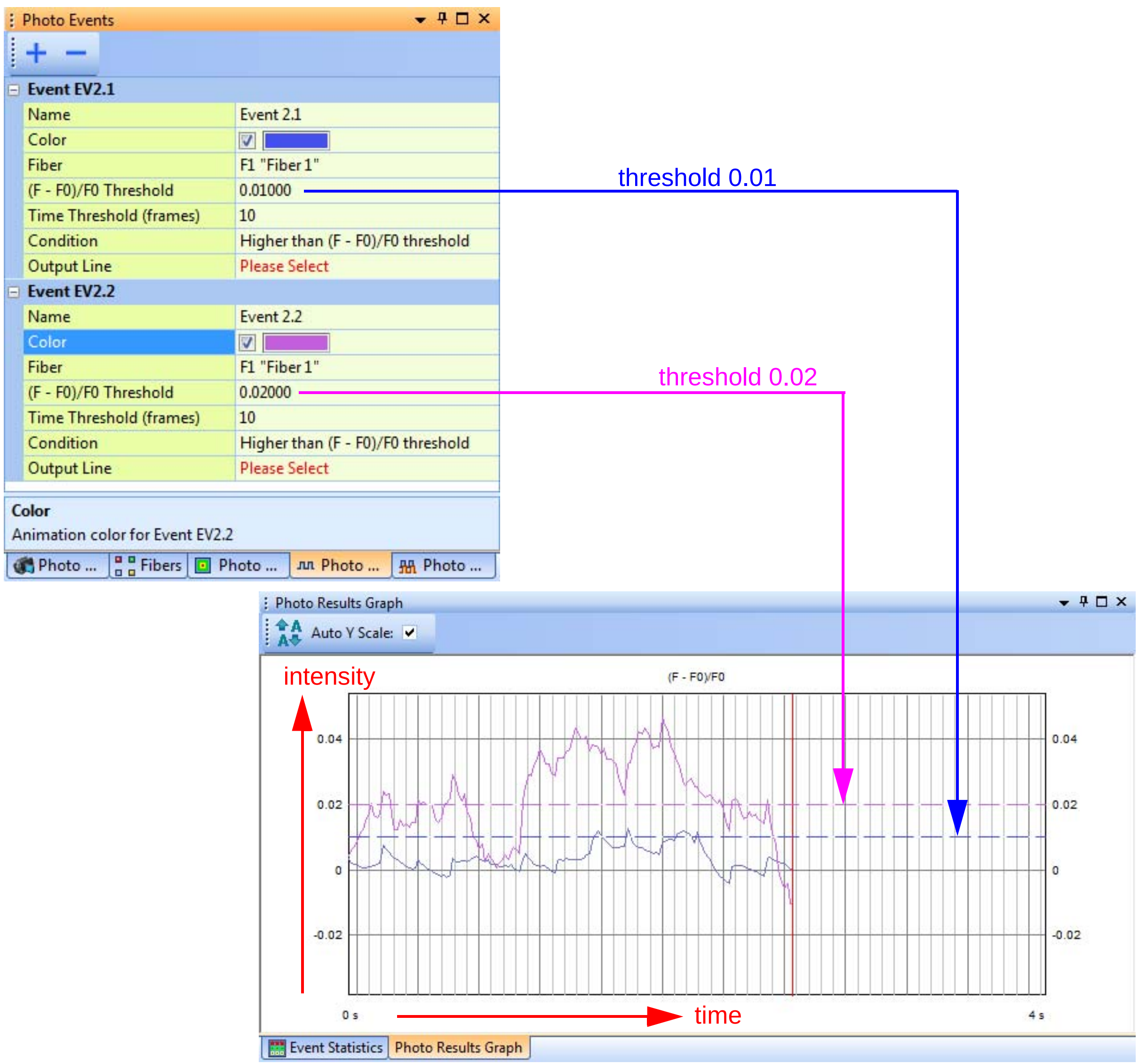


No.	Event Name	Target		Object	Output	Count	Time, s		Track Length, pixe	
		Type	Name				Last	Cumulative	Last	Cumula
EV2.1	Event 2.1			CG	N/A	0	0.000	0.000	0.000	0.00
EV2.2	Event 2.2			CG	N/A	0	0.000	0.000	0.000	0.00
EV2.3	Event 2.3			CG	N/A	0	0.000	0.000	0.000	0.00

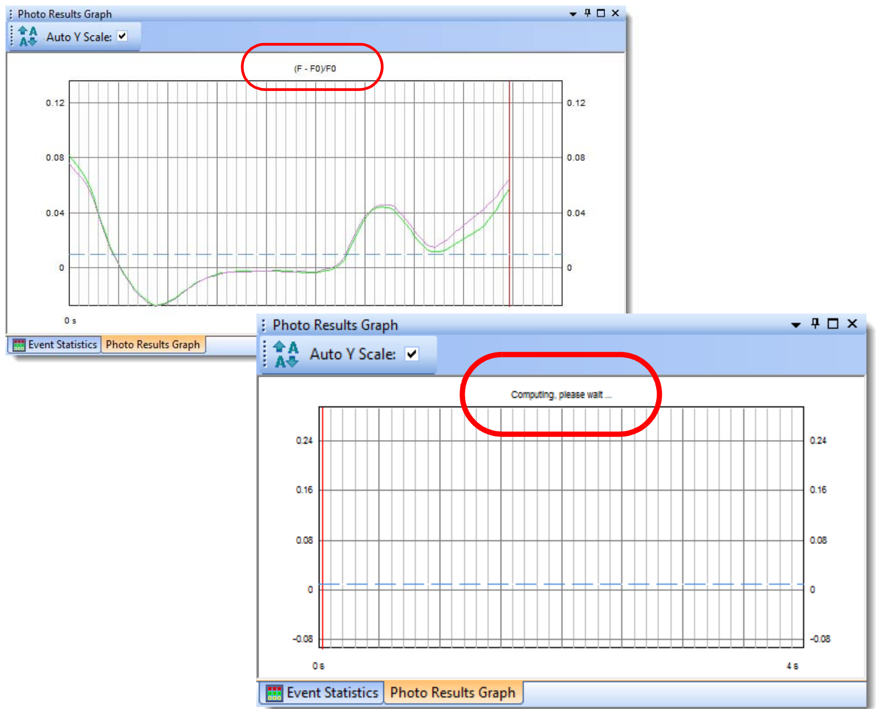
8.21 Interpreting the Photometry Results Graph

The image below shows an example of the **Photo Results Graph**. The graph displays information about the fluorescence intensity of each fiber and the user specified threshold value for each fiber, specifically:

- A moving graphical display of the relative fluorescence intensity of each fiber. The color of each line in this graph matches the color specified for the corresponding fiber in the **Fibers** tab.
- A horizontal dotted line identifying the relative intensity threshold value, **(F–F0)/F0 Threshold**, specified in the **Photo Events** tab.



The heading in the **Photo Results 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...**.

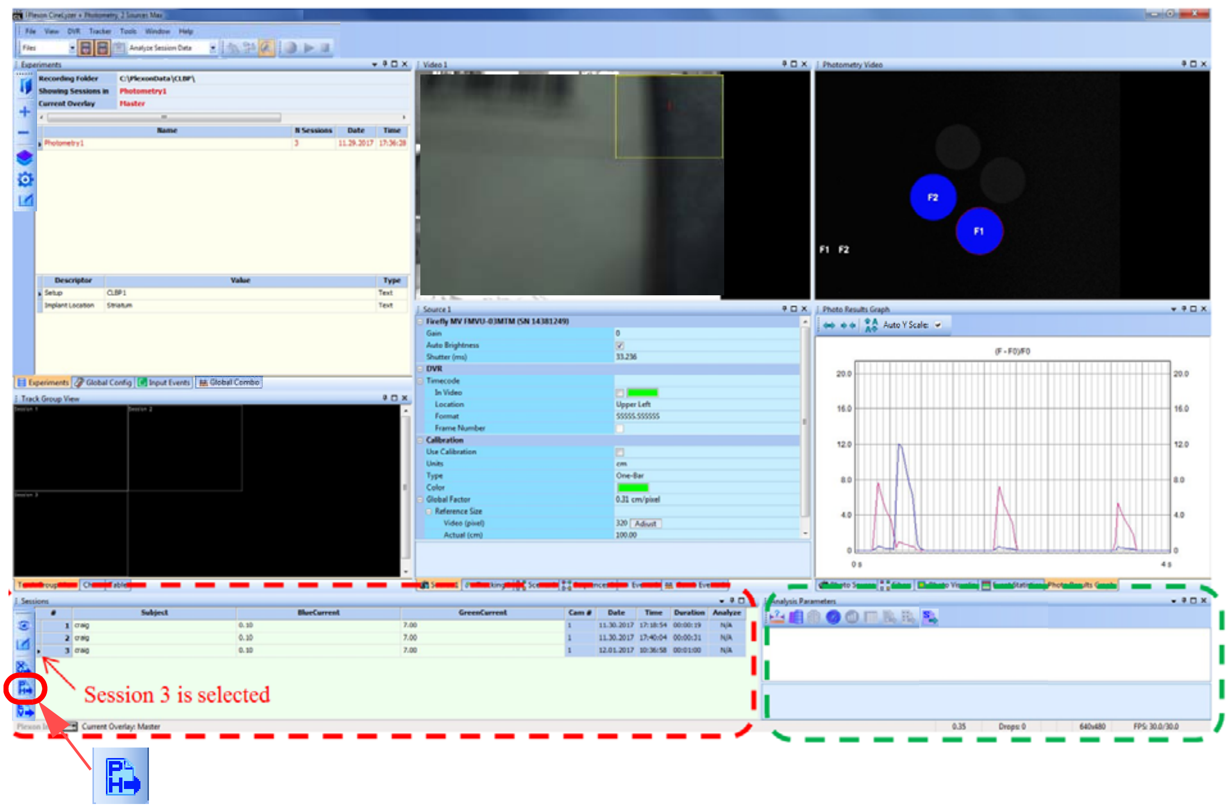


The sample experiment that follows this section will guide you through a typical process of exporting photometry data. See also the information in Section 8.23, “Photometry Reference” on page 234.

8.22 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

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 photometry results per frame single session** 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 photometry results .csv file has the following format.

<== Data for Fiber 1 ==> <== Data for Fiber 2 ==>

	A	B	C	D	E	F	G	H
1	Frame	time	RawF_1	F0_1	R_1	RawF_2	F0_2	R_2
2	1	0	14.338	14.281	0.009	10.29	10.344	0.003
3	2	0.03351	14.446	14.281	0.011	10.243	10.344	-0.001
4	3	0.06687	14.372	14.281	0.01	10.519	10.344	0.003
5	4	0.10024	14.383	14.281	0.01	10.343	10.344	0.002
6	5	0.13362	14.274	14.281	0.006	10.366	10.344	0.001
7	6	0.16699	14.438	14.281	0.009	10.393	10.344	0.003
8	7	0.20037	14.171	14.281	0.004	10.294	10.344	0.001
9	8	0.23374	14.415	14.281	0.006	10.468	10.344	0.005
10	9	0.26712	14.533	14.281	0.009	10.597	10.344	0.012
11	10	0.30062	14.409	14.281	0.01	10.359	10.344	0.009
12	11	0.334	14.344	14.281	0.009	10.355	10.344	0.008
13	12	0.36736	14.198	14.281	0.006	10.403	10.344	0.008
14	13	0.40076	14.386	14.281	0.006	10.344	10.344	0.006
15	14	0.43413	14.31	14.281	0.006	10.425	10.344	0.008
16	15	0.46749	14.098	14.281	0.001	10.315	10.344	0.005
17	16	0.50087	14.392	14.281	0.001	10.451	10.344	0.005
18	17	0.53425	14.476	14.281	0.004	10.578	10.345	0.011
19	18	0.56774	14.221	14.281	0.002	10.392	10.348	0.01
20	19	0.60112	14.39	14.288	0.004	10.376	10.354	0.009

There are columns for the frame # and frame time. Then for each photometry fiber, there are three columns, RawF, F0, and R. 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 8.23, “Photometry Reference” on page 234.

The number of data sets “RawF, F0, and R” can be different for each session, depending on how many fibers were defined at the time the session was recorded. For example, if you record a session with one fiber defined, there will be one data set. If you record a session with four fibers defined, there will be four data sets. For further information on fibers and sessions, see AppendixF, Photometry Examples.

8.23 Photometry Reference

General photometry concepts are discussed in Section 8.9, "Theory of Operation" on page 203. The CineLyzer photometry calculations 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 8.13.3, "Selecting and Configuring Fibers" on page 213). 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:

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

The default value of τ_2 = 3s.

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

Changing photometry analysis parameters (Time Variables)

The default photometry analysis parameters are taken from the reference article listed below. 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. The controls to adjust the analysis parameters are described in Section 8.13.5, “Configuring the Time Variables” on page 219.

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.

Chapter 9
Recording and Monitoring Video

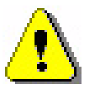
9.1 Before You Start

Verify that you have completed the procedures in the previous chapters as applicable to your experiment.

Note: (If your experiment includes photometry) Set the parameters for photometry as described in Chapter 8, Configuring the Photometry Parameters.

9.2 Starting the System

Perform these steps to start the system and ensure that the cameras are ready to begin video recording.



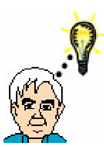
CAUTION

Lighting Conditions

If lighting conditions change enough during recording that 'objects too large' or some other condition occurs, the position data may be meaningless.

1. Ensure that the system is connected as described in Chapter 2, Installing and Starting the System.

2. Double-click the CineLyzer® icon. Wait for the application user interface to appear on the monitor.



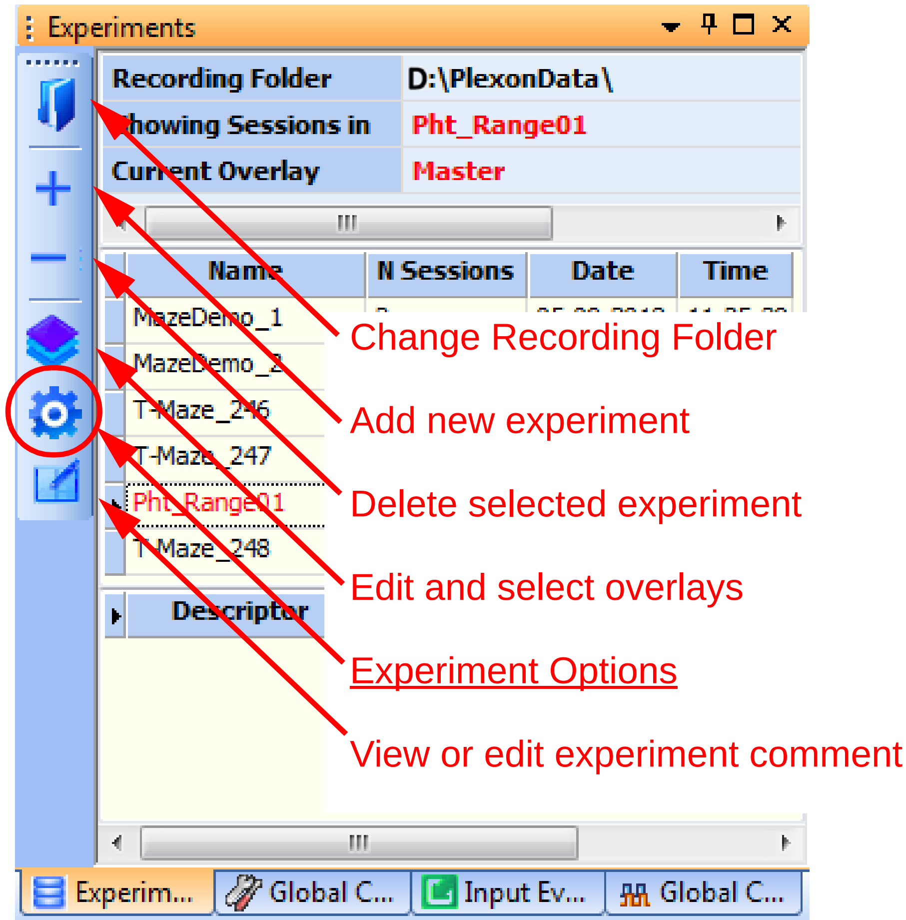
TIP

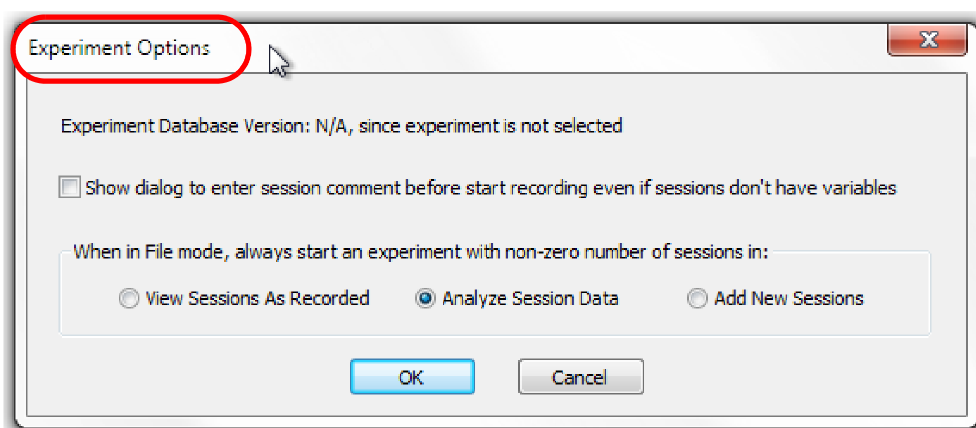
Reset to Default Layout

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**.

9.3 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, described below.





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 9.5, “Configuring, Starting and Stopping the Recording” on page 241.

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.

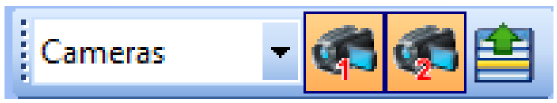
The **When in File mode...** options are discussed in Chapter 10, Analyzing Data and Adding Sessions.

9.4 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 3.9, “Ensuring Consistent Parameter Settings in an Experiment” on page 51.

9.5 Configuring, Starting and Stopping the Recording

1 Verify that **Cameras** is selected in the dropdown list and that a camera image is displayed for each of the camera(s) that will be used in the experiment. The example below shows that both Camera 1 and Camera 2 are selected (indicated by the orange color of the icons), so the live images for both of those cameras should appear in the video windows (Video 1 and Video 2).



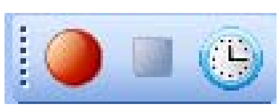
2 In the **Experiments** tab, open the **Recording Folder** browser by clicking the **Change Recording Folder** icon  then navigating to the folder that contains the individual subfolders for your experiments.

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. (The system allows you to select an experiment only if its frame resolution matches the current frame resolution settings in the Experiments tab.) 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 you have the option to change settings for the frame rate (**Frame Rate, fps** parameter) and **Frame Resolution**. If desired, make changes to these settings.

Note: It is important to set the frame rate (**Frame Rate, fps** parameter) and **Frame Resolution** to the values you want to use for all sessions in this experiment. After you start your first recording (session), you cannot change the values for these two parameters in that experiment. This feature is designed for consistency across all sessions.

7 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.

Note: If you select an experiment that was created with a **Frame Rate, fps** or **Frame Resolution** that differs from the **Frame Rate, fps** or **Frame Resolution** of the experiment (as set previously in the Experiments tab), the system does not open the file, but displays an informational dialog box. In this case, you will need to create a new experiment.

8 In the Global Config tab you have the option to change settings for Compression (default value 4) and Layers Transparency (default value 0.5) The default values are suitable for most experiments.

9 Verify that you have activated (selected the icons for) all of the cameras on which you want to record, and the at the system is displaying all the corresponding video images (Video 1, 2, 3 and/or 4).

10 Click on the **Source** tab under the Video window to view these parameters. The settings for these parameters were entered in the procedures in Section 5.7, “Configuring the Source Parameters” on page 77. If any of these settings need to be changed, make those changes now.

11 In the **Scenes** tab for each 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 for one or more of the cameras.

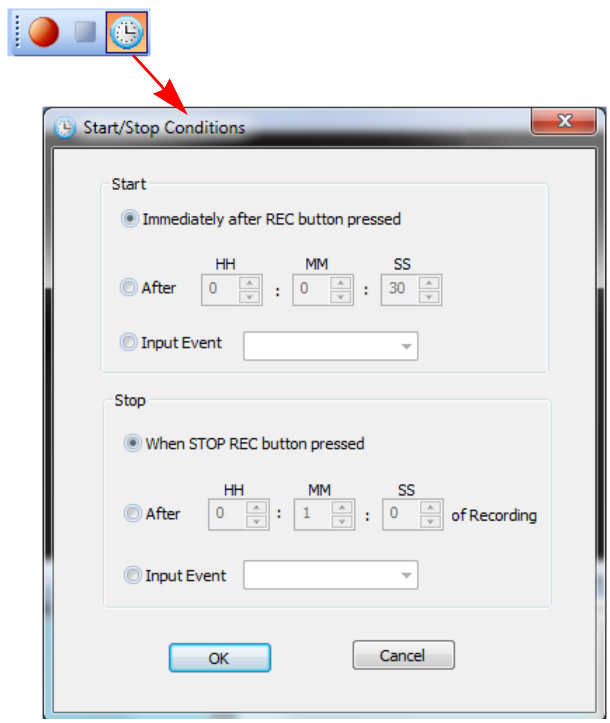
12 In the **Tracking** tab for each camera, ensure that the subject is being tracked properly. Note that the status bar flashes red if one or more cameras is not ready to track the subject.

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

13 (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.



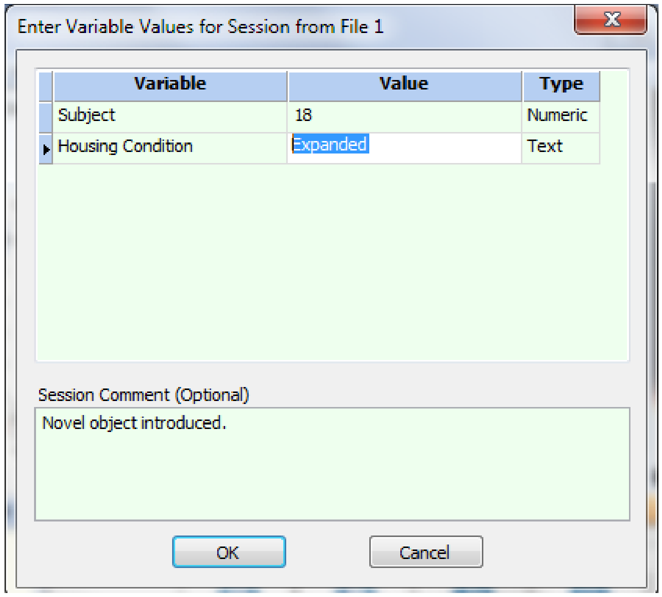
For more details about using this option, see Section 9.6, “Setting Start and Stop Conditions” on page 248.

Starting and stopping the recording of a session (manual/immediate)

14 To start recording video manually, click the **Start recording** icon  on the toolbar.

15 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.



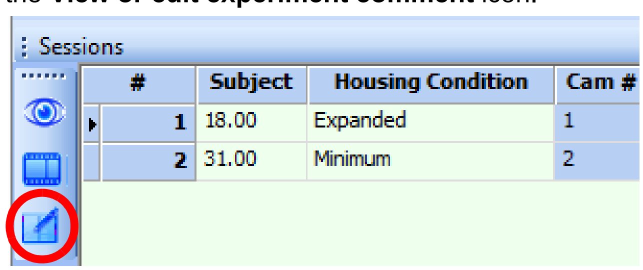
Note: You can also set the system to open the above dialog even if there are no variables for the session. You might want to do this if you plan to enter a Session Comment for some of your sessions. See Section 9.3, “Setting Experiment Options” on page 239.

16 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.

17 Click **OK**.

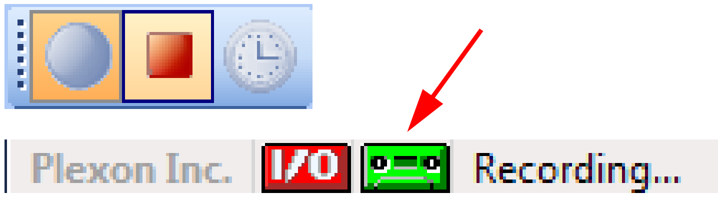
Note: The system presents a dialog box for each video stream (camera) in the session.

Note: At any time, you can view or edit the **Session Comment** by clicking the **View or edit experiment comment** icon.



If you are viewing an experiment that was created with a *previous version* of the CineLyzer software, and if the Comment field was not present in the previous version, the **View or edit experiment comment** icon for this experiment is disabled (greyed out). If the previous version allowed multiple comment fields, only the first comment field is preserved.

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.

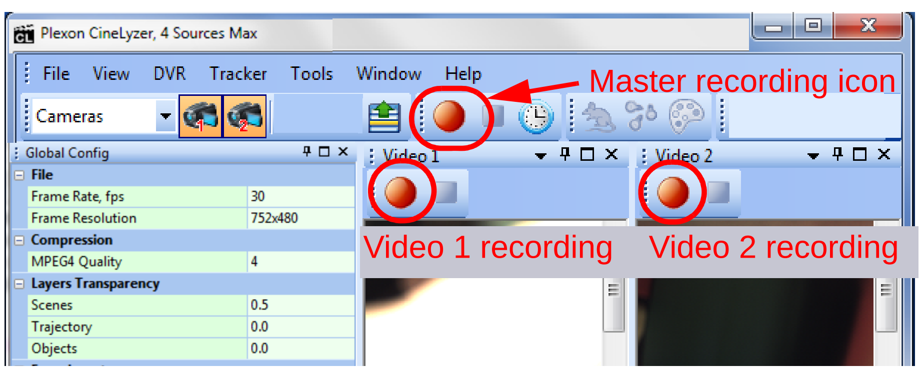
Note: The I/O icon, as seen in the image above, is not related to the recording function. (It is described in Section 9.8, “Monitoring the Video During Recording” on page 253.)

18 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.

Controlling individual cameras

In systems with multiple cameras, each camera can be individually controlled. As shown in the example below, you can record on Video1 or Video2 (use the icons in the individual Video windows), or both simultaneously (the master recording icon in the main toolbar).



If desired, you can

– Start multiple recordings simultaneously using the icon in the main toolbar, and then stop each of the recordings by pressing the **Stop** icon in the individual Video windows.

- Start multiple recordings individually using the icons in the Video windows, then stop all recordings simultaneously by pressing the **Stop** icon in the main toolbar.

Understanding synchronization of multiple cameras

CineLyzer cameras are not actively synchronized like the cameras used in the CinePlex® Studio and CinePartner™ systems. Although the CineLyzer cameras may seem to start recording together when the master recording icon is pushed (see the image above), they do not actually do that. The actual time the shutter opens for Frame 1 for each camera will vary by milliseconds and possibly even frames. In addition, the cameras are not triggered by a single central timing signal, but by their own internal clocks. This means that the frames for each camera will gradually drift apart in clock time.

The intended use of the multiple cameras in the CineLyzer System is to record multiple sessions using different animals in different arenas within the same experiment. This goal is reflected in certain coordinated features for the system, including the following.

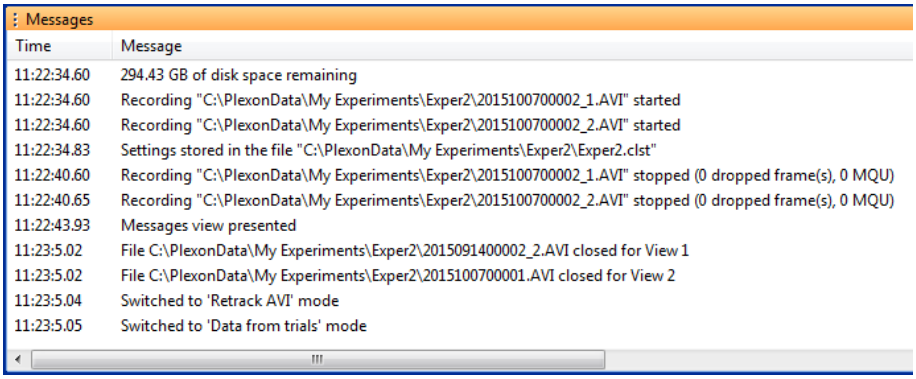
- The cameras can be started separately or together (if all animals are ready in their respective arenas).
- The system coordinates the creation and deletion of arena and zone shapes across all cameras. Arena and zone shapes that are added to one camera view are added automatically to all of them. The shapes can be resized and manipulated to accommodate different camera angles and viewing distances within each camera view, but the same basic shapes will be present in all video windows. For details, see Section 5.8, “How to Duplicate Parameter Settings to All Cameras” on page 82.

Synchronization example

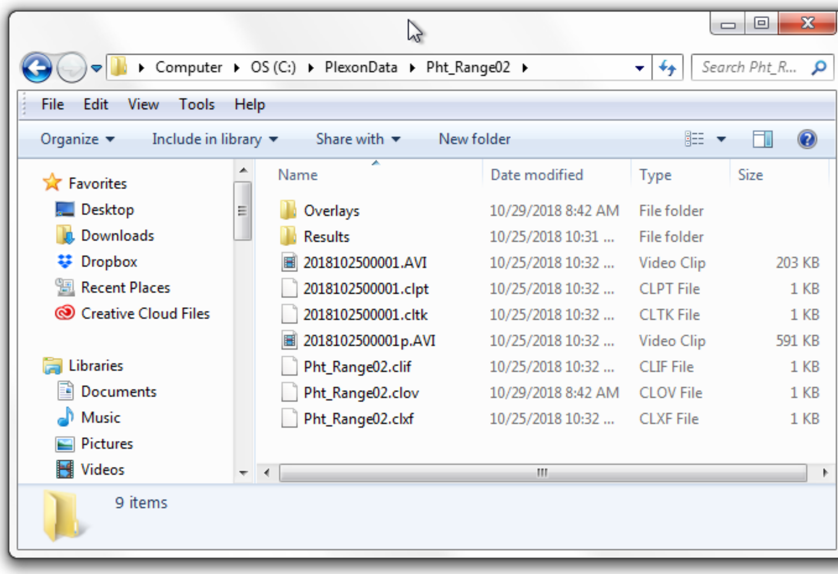
Although the CineLyzer cameras are not actively synchronized, you can use the system’s capabilities to improve the coordination of multiple cameras. For example, you could place an LED in the experimental area and ensure that it is visible in the fields of view of all the active cameras. Then, after you press the master recording icon (to start all cameras), you can flash the LED once. Then flash the LED again just before you stop recording. The two LED flashes provide an opportunity to synchronize the video streams. Some frame coordinate data will have to be repeated or dropped to make the file lengths among the various cameras match up. You can trigger the LED flashes manually, or you can use frame pulses generated by the Camera Trigger Board (see Section 1.5, “System Hardware, Software and Cameras” on page 10 and Section 5.7, “Configuring the Source Parameters” on page 77) to trigger the LED flashes.

Viewing messages and files after recording a session

The Messages window (which you can access from the main menu as **View** > **Messages**) displays information similar to the example below. In this example, the system checks for remaining disk space and creates new sessions (new recordings) for Video1 and Video2.



19 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 in the CineLyzer System or with Windows® Media Player® or many other available video players.



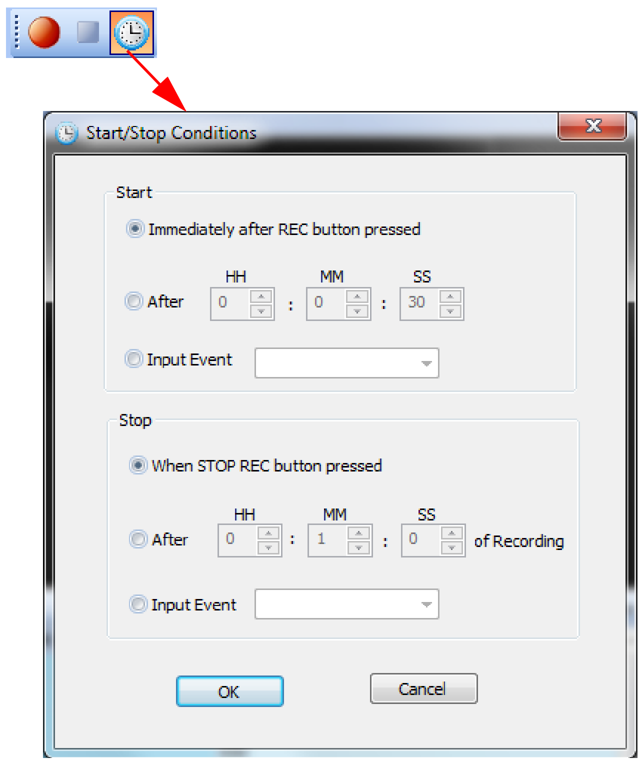
20 To transfer files to an analysis computer (if different than the recording computer), follow standard Windows procedures. A flash drive with a valid CineLyzer user license is required to open CineLyzer files on the analysis computer.

9.6 Setting Start and Stop Conditions

This section explains how the **Start/Stop Conditions** dialog box works and how to use it.

9.6.1 Understanding the Start/Stop Conditions

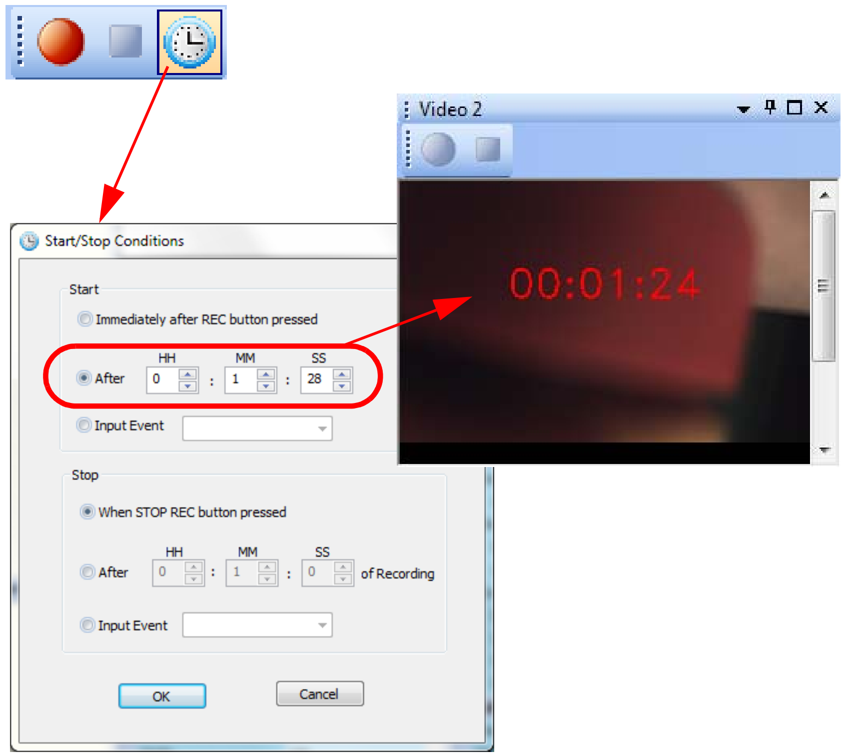
Clicking the clock icon  opens the dialog box.



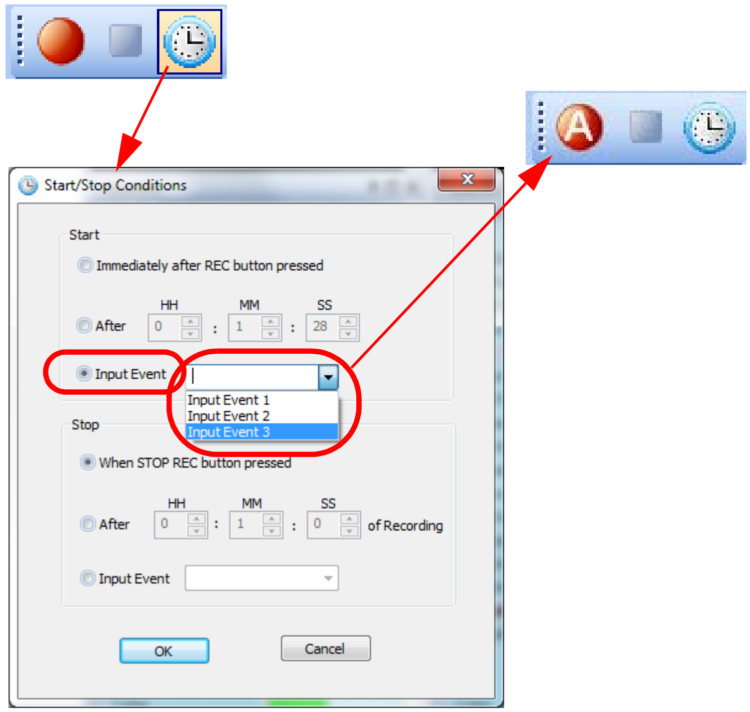
There are three options for starting a recording and three options for stopping the recording. The default settings are to immediately start and stop recordings when you press the **Start** and **Stop** buttons.

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 configured in the Input Events tab and are received through the CineLyzer USB Digital Input/ Output (DIO) Interface (see Section 2.4.6, “Input Events Tab” on page 27). After you click **OK**, the Start button displays the letter “A” to indicate it is ready to be armed.



9.6.2 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 DIO Interface, perform the following steps:

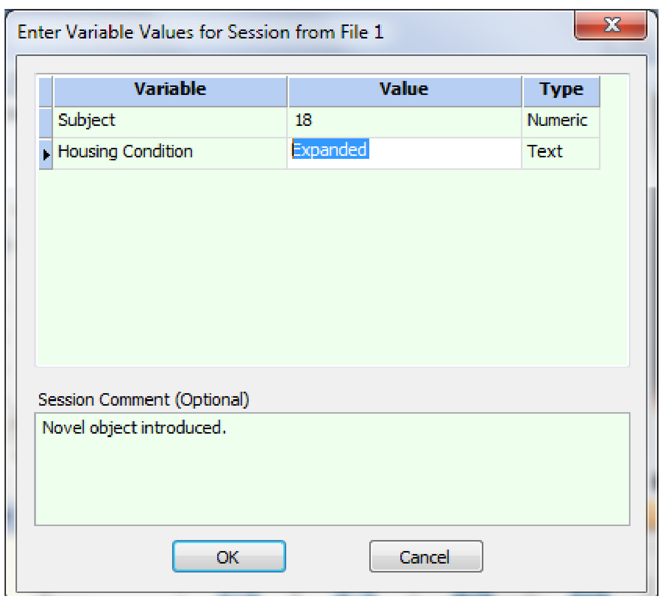
a Click on the **Input Event** radio button and select an **Input Event** from the dropdown list. After you click **OK**, the **Start** button displays the letter “**A**” to indicate it is ready to be armed.

b Click on the **Start** button. The following events occur:

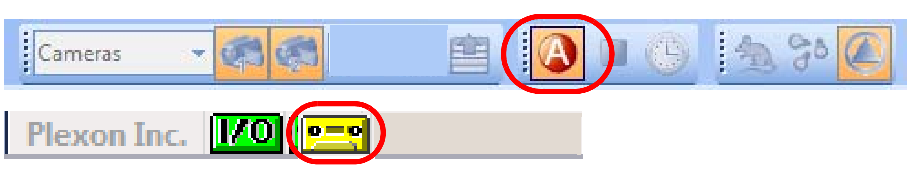
– The background of this icon turns orange to indicate that the system is armed and will start recording when the specified Input Event is received.

– 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 9.8, “Monitoring the Video During Recording” on page 253.)

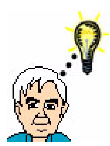
1 If you want to return the system to manual start mode, click the **Start** icon to disarm the system. then open the **Start/Stop Conditions** dialog box and select **Immediately after REC button pressed**. Then click **OK**.

Adjusting the Stop time

2 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.)

3 If you want the recording to stop after the system receives an input event through the DIO 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.

9.7 File Naming Process

When you click the **Start** icon to start a new session (a new recording), the system prompts you to enter values for the programmed variables. After you enter the values, the system starts the recording. The system creates an AVI file, one for each camera, and saves the file in the folder for the current experiment within the **Recording Folder** that you specified in the **Experiments** tab.

Although AVI file names are not normally needed for analysis, the following information is provided to the user for reference when file names are mentioned throughout this document. The AVI file names are as follows:

- If there is only one active camera, the file name format is *yyyymmddnnnnn.avi.*
- If there are multiple active cameras, the file name format is *yyyymmddnnnnn_N.avi*

where:

yyyymmdd is the year, month and day.

nnnnn is a 5-digit number, 00001, 00002, ... 99999 assigned automatically by the system.

N is the camera number for which the file is created – 1, 2, 3 or 4.

CAUTION

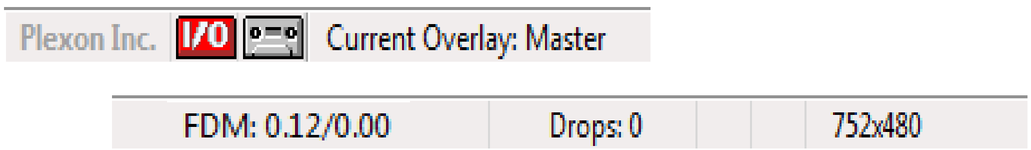
Do not manually change the names of AVI files

Do not attempt to change the file name of an AVI file in the Windows 7 environment, because that will cause the CineLyzer System to be unable to locate it automatically in the future.

9.8 Monitoring the Video During Recording

Before recording has started

When recording has not started, the status bar in the CineLyzer user interface displays the parameters shown in the example below. (The image is split into two sections to fit the page.)



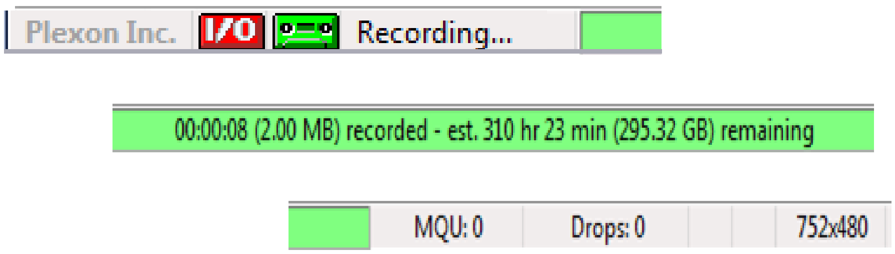
The contents of the status bar include

- The I/O icon (see the note, below).
- 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.) In the image above, the FDM is 0.12 for Camera 1and 0.00 for Camera 2.
- 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 CineLyzer 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).

Note: The **I/O** icon is red I/O when the USB Digital I/O unit is not connected, and green I/O when the unit is connected. (Details of the USB Digital I/O unit are covered in Appendix C, USB Digital Input/Output Interface.)

When recording starts

The status bar in the CineLyzer user interface displays information that allows you to monitor the video recording process. This drawing shows the status bar during a typical recording. (In the example below, the image is split into three sections to fit the page.) The frame resolution of the cameras is shown on the right side of the status bar.



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.
- The maximum number of video frames queued for compression and waiting to be written to the AVI file at any time during the current recording—maximum queued units (MQU).
- 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).

9.9 Managing CPU Demand and Avoiding Dropped Frames

The standard configurations of the CineLyzer 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:

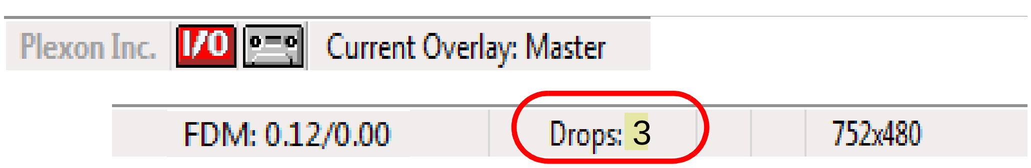
- Higher frame rates
- Large numbers of colors being tracked
- Difficulty of tracking colors all of the time due to darkness or blocking
- Large numbers of complex events

Other applications running on the computer may interfere with CineLyzer operation. These include:

- MATLAB®
- Windows Update
- Backup software
- User installed software
- Browsers
- Slower disk activity (can occur as a disk comes close to 100% full)

For example, when you are recording from cameras, it is possible to exceed the computational bandwidth of CineLyzer computer by provisioning settings near their limits. For example, you could configure the system to track 12 color markers at 60 frames per second on multiple cameras, and generate multiple simultaneous and complex events. In such a case, the events might not all be detected or trigger outputs in a timely fashion if they occur on the same video frame. Additional challenges to the computer processor can occur if objects are lost due to darkness or exiting the arena.

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 CineLyzer 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.

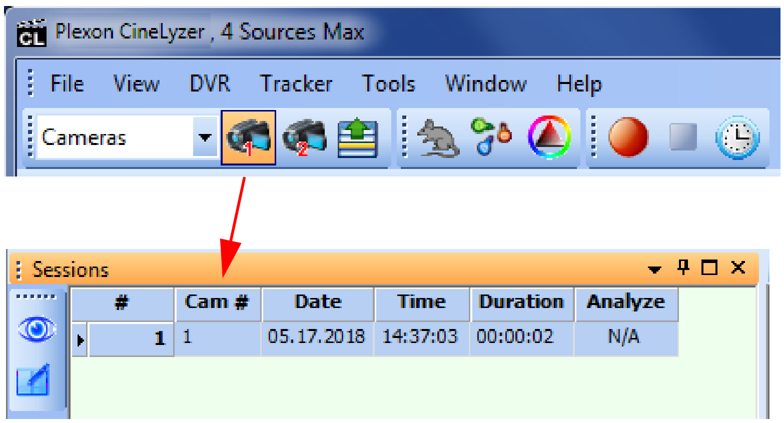
9.10 Managing Sources per Session in Cameras Mode and Files Mode

This section describes how the system responds when you change the number of video sources from one session to the next. In this usage, “source” can refer to either of the following:

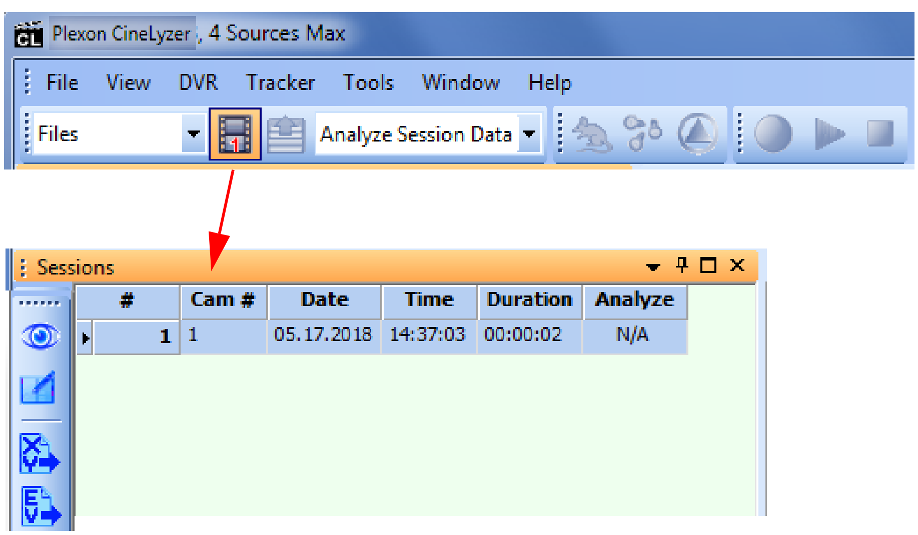
- An active camera in **Cameras** mode
- A file that you use to add a session in **Files** mode

Example 1

In this example, one camera is enabled, so a single session is recorded when we press the record button.



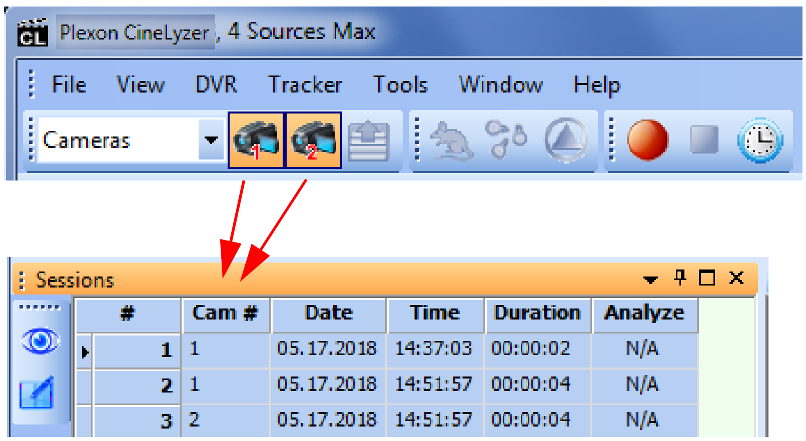
If we switch to **Files** mode and select this same experiment, the display shows that one source is available. The **Sessions** window shows the file we just recorded.



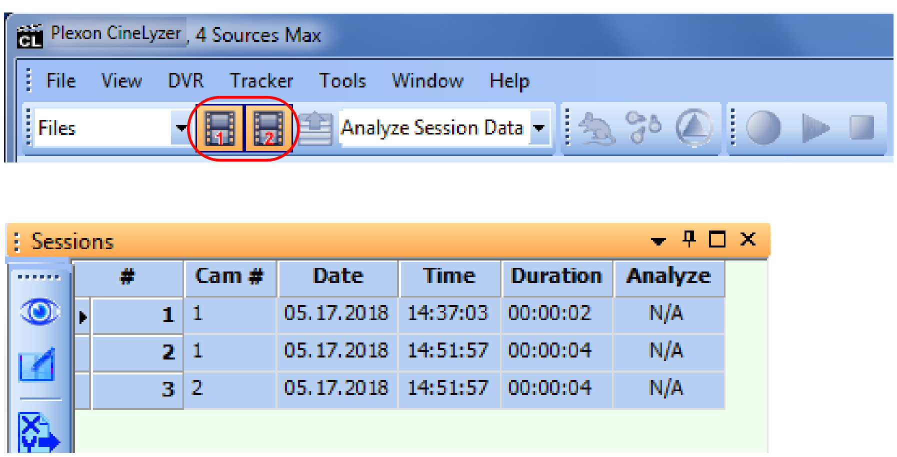
Example 2

We return to **Cameras** mode and select the same experiment as in Example 1.

In this example, we enable two cameras, so two sessions (Sessions #2 and #3) are recorded when we press the record button. Notice that the **Sessions** window displays the camera number (**Cam #**) that recorded each of the sessions—Session #2 was recorded with Camera 1 and Session #3 was recorded with Camera 2.

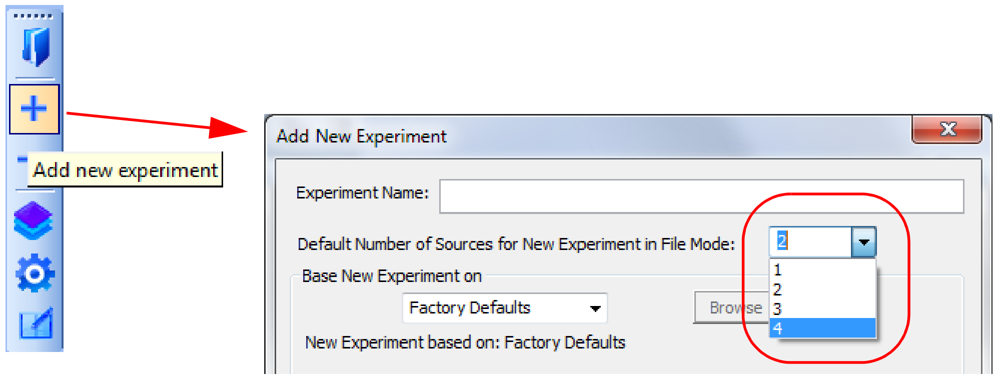


If we switch to **Files** mode and select this same experiment, the display shows two sources are available. In general, the number of sources in **Files** mode is equal to the maximum number of sources (video streams in **Cameras** mode or previously recorded files in **Files** mode) that have ever been used to create recordings (sessions) for this experiment. Notice that the list of recorded sessions for this experiment is the same as above; switching from **Cameras** mode to **Files** mode does not, of course, change this list.

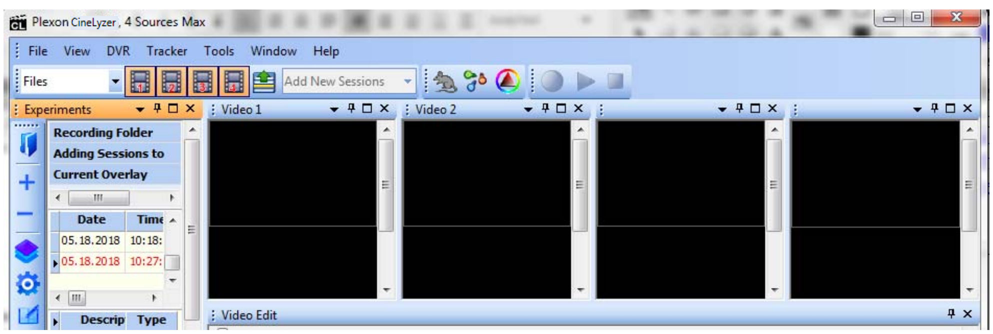


Example 3

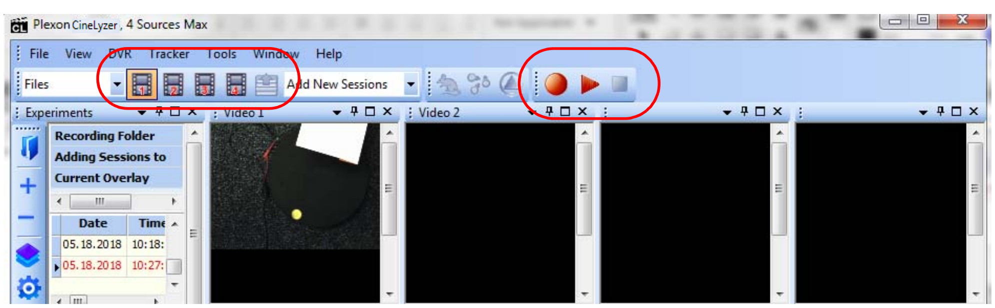
Next, we remain in **Files** mode and create a new experiment. The dialog has a dropdown list and we can choose the default number of sources for the new experiment, from 1 up to the number of sources in our CineLyzer license, in this example up to 4.



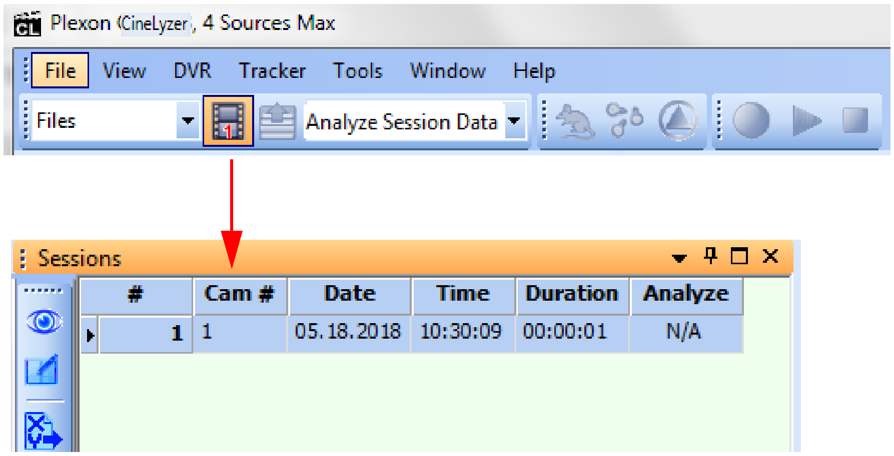
We select 4, and the system displays four sources.



If we only want to record a single session, we can deselect all but one of the sources, select the source file for the active video stream, then press the record button. Only one session will be created.



If we close the CineLyzer application, then reopen it and select this same experiment, the display will show only one source in **Files** mode, not the original four sources. That is because we recorded the first session in **Files** mode with one source. We cannot add more sources in this experiment.



⚠	**CAUTION** **Do not connect or disconnect cameras with the PC on** Always shut down the PC completely before you connect or disconnect a camera. Otherwise, the camera could be damaged,

Example 4

We create an experiment with one active source but do not record any sessions. We open this experiment again in **Files** mode and we see only a single session can be recorded now for this experiment (only one video source is allowed) in **Files** mode

We create an experiment with one active source but do not record any sessions. We open this experiment again in **Cameras** mode with four cameras connected and we see that the system allows us to record on all four cameras. We have the option then of recording on any number of cameras, 1 ... 4.

With four cameras connected, we create an experiment with one active source and this time we do record one or more sessions. We close the CineLyzer application, then reopen it again and select this same experiment in **Cameras** mode. We see that all four cameras are available for further recording. Then we switch to **Files** mode, select this same experiment, and see that all four sources are still available.

9.11 Where to Go Next

If you have a CineLyzer license and would like to view and analyze your data, go to Chapter 10, Analyzing Data and Adding Sessions.

Chapter 10
Analyzing Data and Adding Sessions

10.1 Introduction

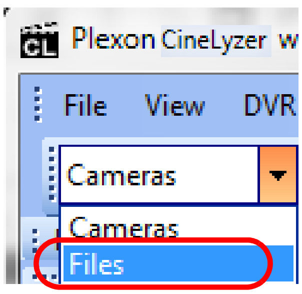
This chapter explains how to manage your Experiment folders and session video files, how to perform analyses and view the results, how to input and output behavioral events data, and how to add more sessions to an existing experiment.

Note: (If your experiment includes photometry) Set the parameters for photometry as described in Chapter 8, Configuring the Photometry Parameters.

10.2 Placing the System in Files (Offline/Analysis) Mode

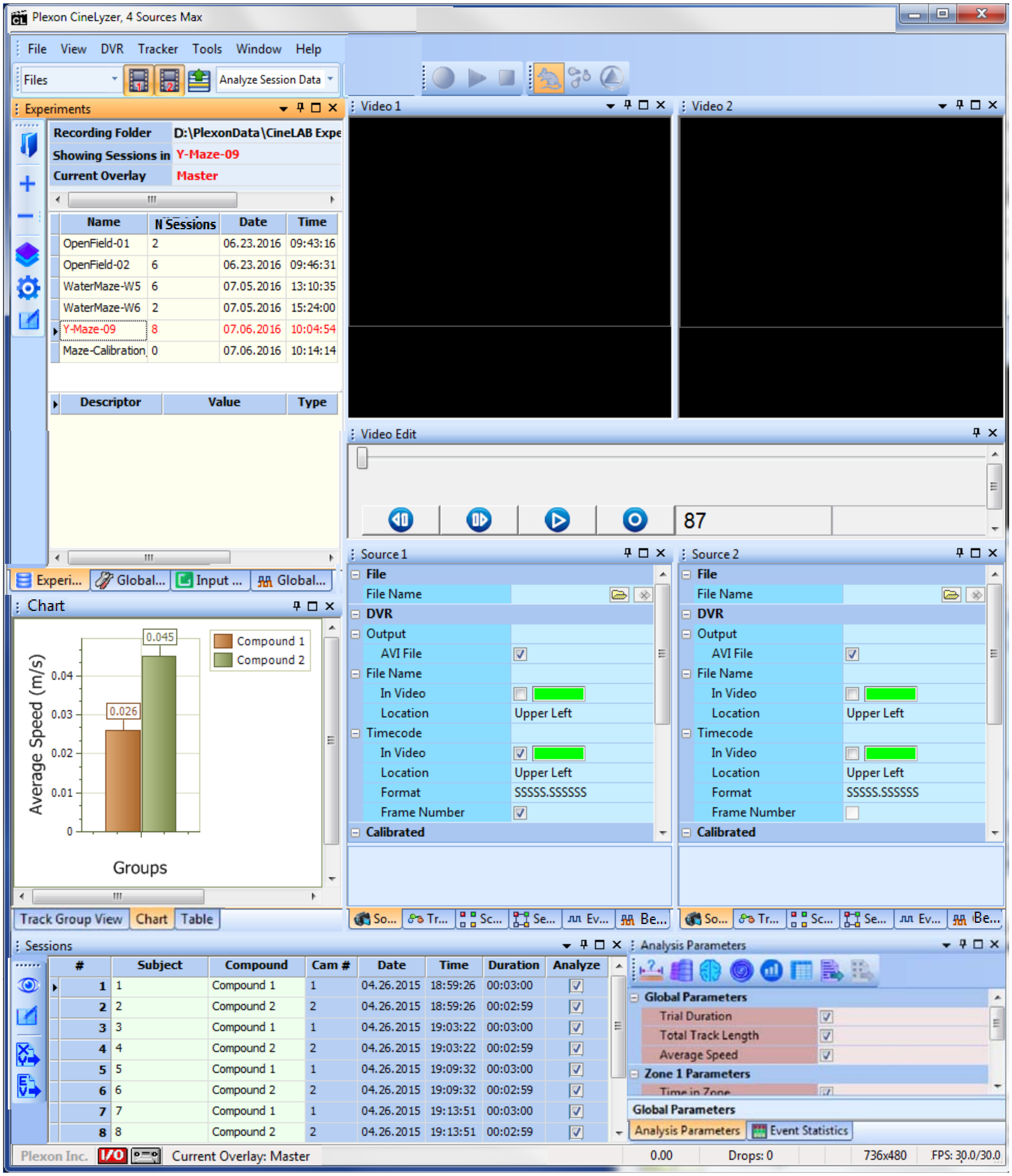
The system has two modes of operation: **Cameras** (online/recording) mode and **Files** (offline/analysis) mode.

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.



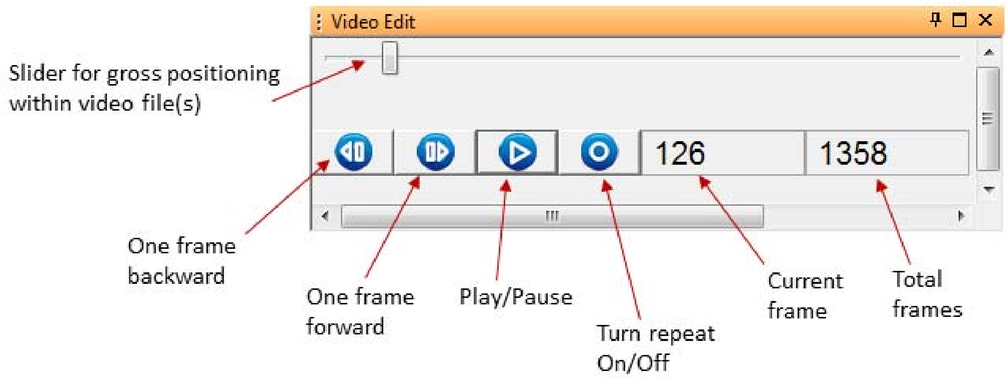
10.2.1 CineLyzer User Interface Layout

The image below shows a typical CineLyzer® user interface layout with files from Camera 1 and Camera 2 enabled.



10.2.2 Video Edit Window

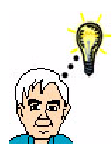
You can use the icons in the Video Edit window to replay the video file frame by frame. If the Video Edit window is not displayed, you can display it by selecting **Video Edit** from the **View** dropdown menu in the main toolbar.



Note: The Video Edit functions are disabled while a recording is in progress.

10.3 Viewing the Main Toolbar in Files Mode

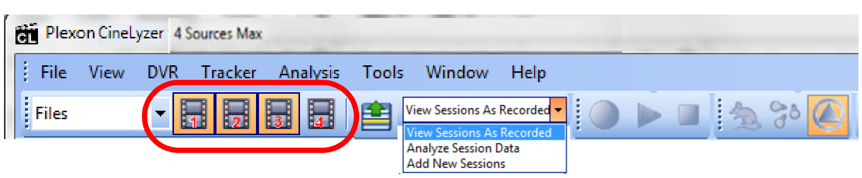
The image below shows a close-up view of the CineLyzer® main toolbar in Files mode. In this example, there are four file sources, one for each of the four cameras used in the original recording. Three of the files have been selected by the user for this analysis.



TIP

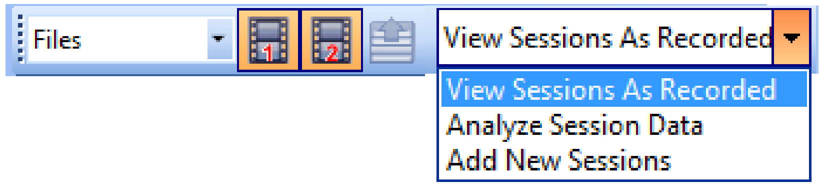
Number of Files icons is based on number of video sources

The number of **Files** icons shown in the toolbar is equal to the number of video sources used to create the experiment data.



10.4 Understanding Files Submode Settings

There are three submodes you can select when the system is in **Files** mode.



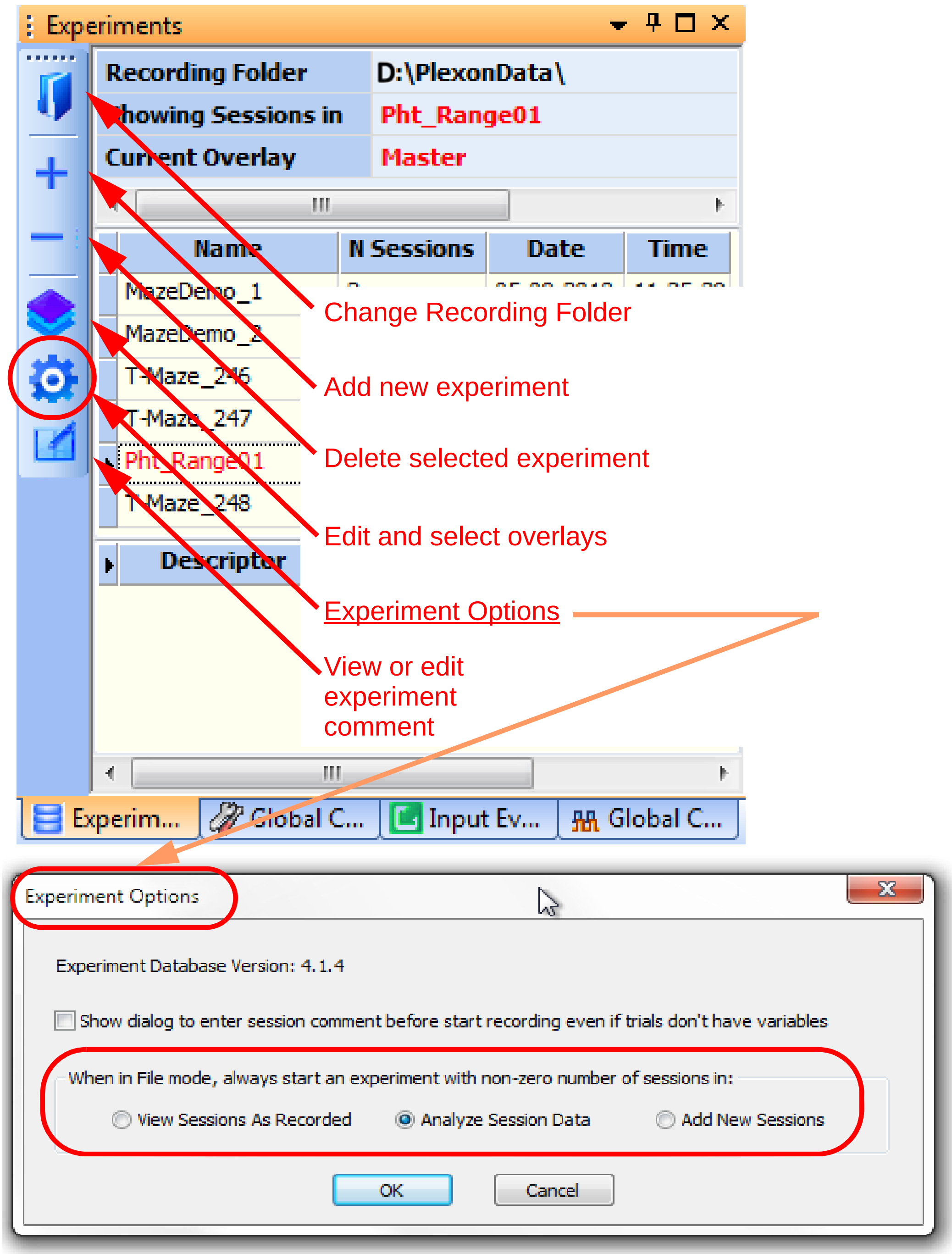
- **View Sessions As Recorded—**For each individual session, you can view the original settings of the session source and global settings, that is, the settings that were in place when the session was recorded. You can export coordinate data, photometry data (if present) and recorded events (per frame values and intervals). No changes to the original settings are allowed in this mode.
- **Analyze Session Data—**For each individual session that has been recorded, you can modify the arena and zone shapes and the original parameter settings, and recalculate coordinate data, photometry data (if present) and recorded events (per-frame values and intervals).

  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. Empty experiments (experiments with no sessions recorded) always open in **Add New Sessions** mode.

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.

Note: The “Experiment Database Version” number (shown in the above image) 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.

10.5 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 32
- Section 3.2, “Planning the Database” on page 32
- Section 3.3, “Setting Parameters for an Experiment with Multiple Sessions” on page 33
- Section 3.4, “Setting the Recording Folder Location” on page 36
- Section 3.5, “Creating a New Experiment” on page 37
- Section 3.6, “Selecting a Previously Saved Experiment” on page 45
- Section 3.7, “Editing the Experiment Name, Descriptor and Variable Values” on page 46

10.6 Viewing Videos of Completed Sessions

This section explains how to use the system in **Files** (offline/analysis) 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 9.5, “Configuring, Starting and Stopping the Recording” on page 241.

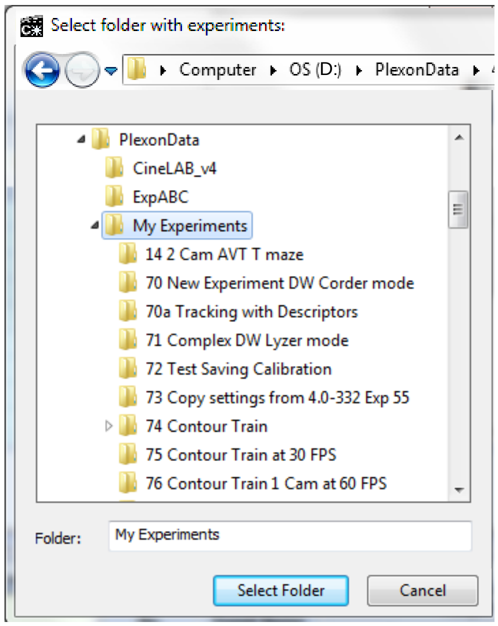
The offline functions of the CineLyzer System can be used on the host PC where the camera(s) are installed, or on an appropriate standalone computer (see Section 1.8, “Using a Standalone Computer for Data Analysis” on page 12). To perform offline functions on a standalone PC, the required Experiment 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 CineLyzer license must be plugged into that computer. You can view files from up to four sources according to the number of cameras allowed by your license.

Note: A specially configured Plexon® supplied computer is required for supporting the four-camera license.

10.6.1 Selecting Files Mode and Navigating to the Experiments Folder

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. 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. (Optional) In the **Global Config** tab you have the option to change settings for **Compression** (default value 4) and **Layers Transparency** (default value 0.5) The default values are suitable for most experiments.

   (The values for **Frame Rate** and **Frame Resolution** cannot be changed in an existing experiment. Those values were set prior to the recording of the file.)

6. In the Experiments tab, click in the row for the experiment you want to analyze.

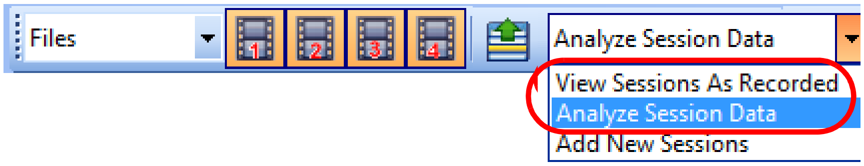
   Note: After you click on the row for your experiment, notice that the list of sessions for this experiment appears in the Sessions window.

7. 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 arena or zone shapes, or any parameter settings.

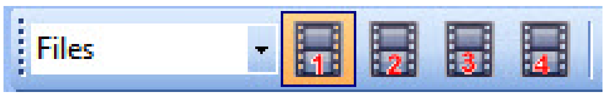
- **Analyze Session Data** submode—This is a viewing and reanalysis mode. You can change arena and zone shapes and any parameter settings, then analyze and export the new data.

Note: The number of file icons displayed in the main toolbar is equal to the number of cameras used in the original recording for the experiment. The image below shows an experiment in which video was recorded on all four cameras.



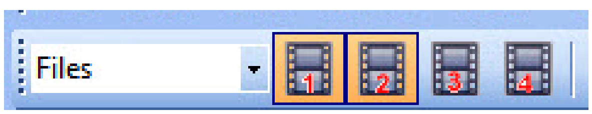
10.6.2 Selecting Sessions to Be Viewed and/or Analyzed

The existing video files are represented by icons in the toolbar. You can select from one to four files to process in offline mode, up to the number of cameras licensed for the system.

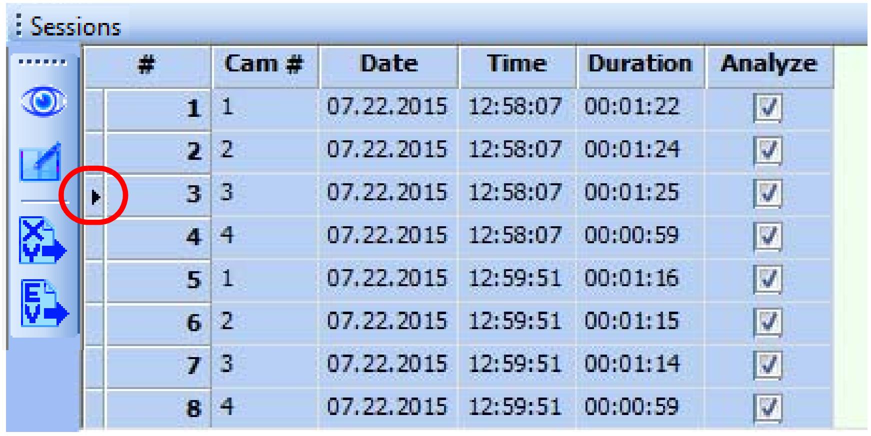


The following procedure organizes the user interface and loads the desired files. It also displays the Video Edit window, which can be used to replay the video file frame by frame.

1 Typically, you will want to view all of the recorded video files from all the cameras that were used in the experiment. However, you can deactivate one or more of the files by deselecting them in the main toolbar. For example, in the image below, the files from Camera 3 (**File 3**) and Camera 4 (**File 4**) have been deactivated.

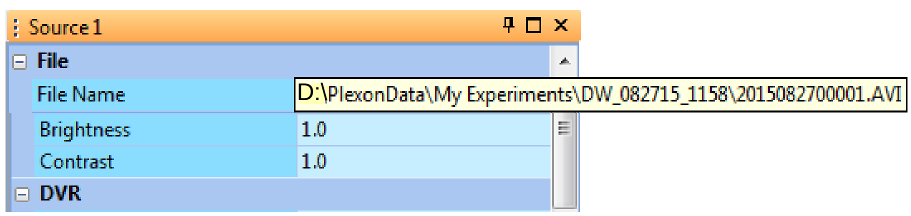


2 In the **Sessions** window, click in the row containing a session for which you want to view the video. In the example below, it is Session 3.

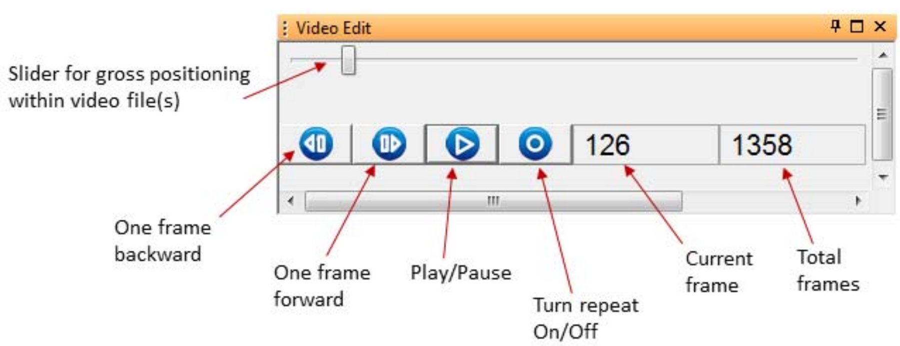


The system displays the Video Edit window and the selected video. The display appears in the tabs associated with the camera that originally recorded the session; for example, if the session was recorded with Camera 1, the display appears in the Video 1 and Source 1 tabs.

In the image below, Source 1 is displayed. 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.

10.7 Modifying Arenas and Zones

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 10.14, “Using the Overlay Feature during Analysis” on page 305, and Appendix E, Modifying Arenas and Zones.

You need to select **Files/Analyze Session Data** mode to modify arenas, zones and settings in the previously recorded sessions.

CAUTION

Do not delete 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 modify 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.

10.8 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.

10.8.1 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 9.5, “Configuring, Starting and Stopping the Recording” on page 241.

10.8.2 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.

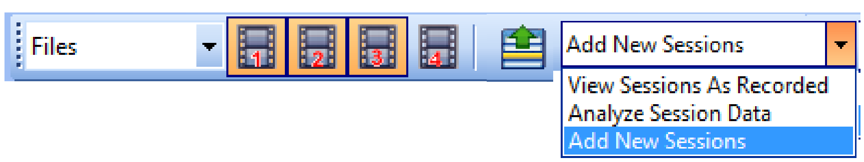
5 (Optional) In the Global Config tab you have the option to change settings for **Compression** (default value 4) and **Layers Transparency** (default value 0.5) The default values are suitable for most experiments.

(The values for **Frame Rate** and **Frame Resolution** cannot be changed. Those values were set for the experiment prior to the recording of the file.)

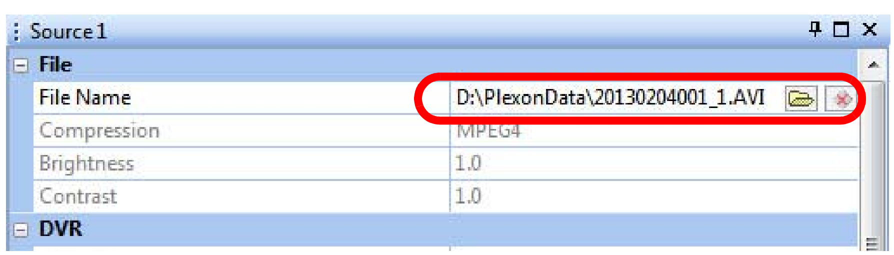
6 In the Experiments tab, click in the row for the experiment to which you want to add sessions.

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.

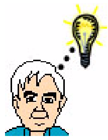


8 In the Source tab, in the **File Name** row, click on the folder icon to navigate to the folder containing the desired AVI file and select the file.



Note: If you select a file that was created with a frame rate **(Frame Rate, fps** parameter) or **Frame Resolution** that differs from the **Frame**

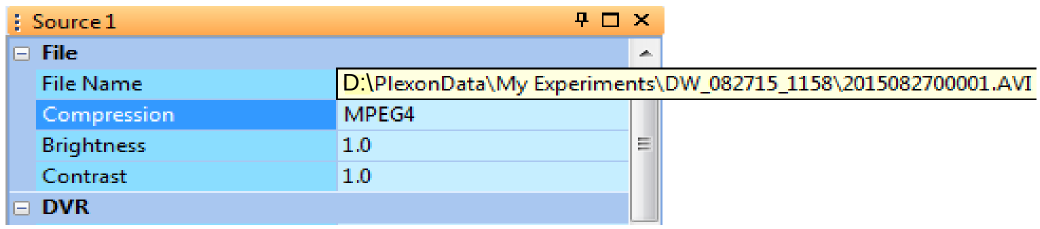
Rate, fps or **Frame Resolution** of the experiment (as set previously in the Global Config tab), the system does not open the file, but displays an informational dialog box. In this case, you will need to create a new experiment in the **Files** mode from the video files.



TIP

View the full file name and path in Files mode

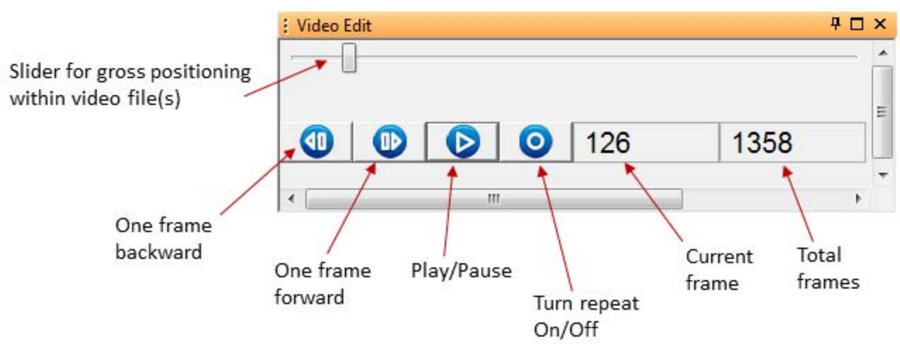
In **Files** mode, you can view the full path and file name by positioning your mouse over the cell on the right side of the File Name row, as shown in the following image.



9 Repeat Step 8 for each of the files you want to include in the session.

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

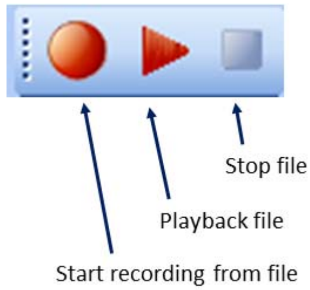
10 (Optional) You can use the icons in the Video Edit window to replay the video file frame by frame. Note that the Video Edit controls are active when you are viewing a previously saved video file, but inactive when a new video is being recorded.



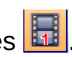
11 Verify that the system is displaying all the video files (Video 1, 2, 3 and/or 4) that you want to include in the session. If a video window is open but you have not opened a video file in that window, deselect the corresponding **File** icon.

For example, if the Video 3 window is open but there is no video file open in that window, deselect the third **File** icon in the main toolbar.

After the active video files (and only the active files) are displayed, the system enables the recording toolbar.



Preparing to start recording a session

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 and open a file in the Source tab for each of the active files , or [2] when you select a previously saved experiment, select **Add New Sessions** from the dropdown menu, and open a file in the Source tab for each of the active files .

12 Create or select an experiment from the list in the Experiments tab.

13 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 Section 5.7, “Configuring the Source Parameters” on page 77. If any of these settings need to be changed, make those changes now.

14 IMPORTANT—
If you plan to make any changes in the **Tracking**, **Scenes** or other tabs: First be sure to read and understand the information in Section 10.15, “Caveats—Understanding Tracking Data in System Computations” on page 309.

Starting the recording of a session

15 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.

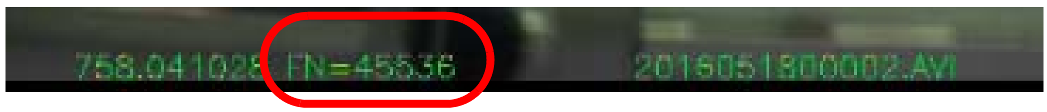
16 You can stop the recording by clicking the **Stop file** icon. Or you can allow the video to continue until it is finished.

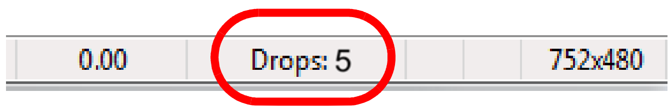
Note: The Video Edit functions are disabled during a recording.

Monitoring dropped frames when adding a session (re-recording a file)

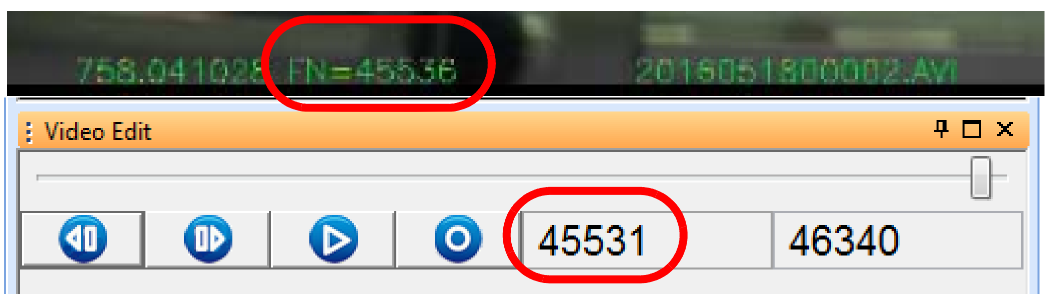
One or more video frames can be dropped (fail to be recorded to an AVI file) during a recording session if the computer is experiencing partial congestion, which is typically caused by non-Plexon applications. It is important to understand how the system displays information about dropped frames when your system is in **Files** mode vs. **Cameras** mode.

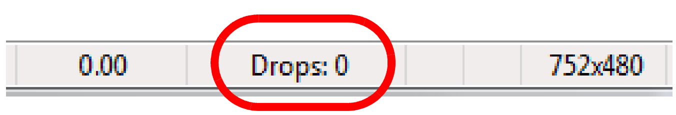
During original recording in Cameras mode—In the example below, notice that the frame number displayed in the Video window is 45536 and the status bar at the bottom of the CineLyzer interface shows that five frames have been dropped. This means that only 45531 frames have been successfully recorded.





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 CineLyzer interface is disabled—it will always display **Drops: 0** in **Files** mode.





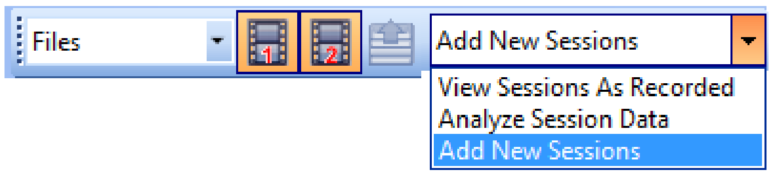
Note: In the event that processing ever experiences problems, see “Dropped Frame Count Non-zero While Recording” on page G-7. If problems persist, please contact Plexon® Support for help in identifying and resolving the issue.

10.9 Examples—Sessions and Combination Events in Files Mode

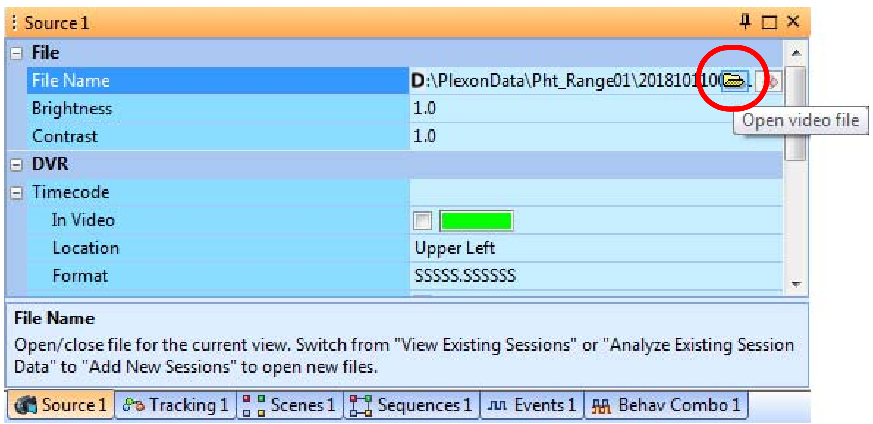
The use cases in this section show how the system handles the addition and analysis of sessions in **Files** mode when combination events are involved.

10.9.1 Introduction and Terminology

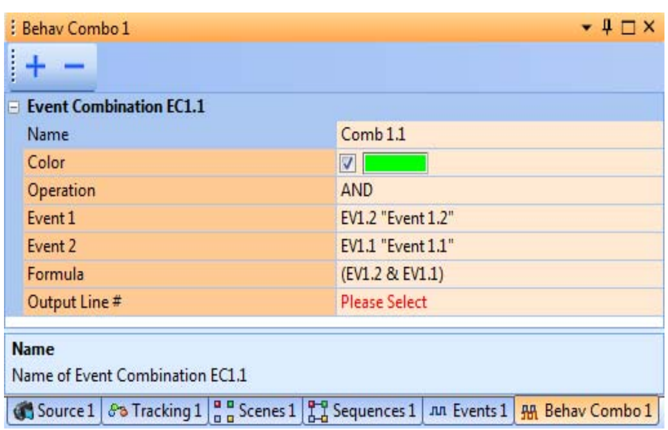
Here are the submodes you can select when the system is in **Files** mode.



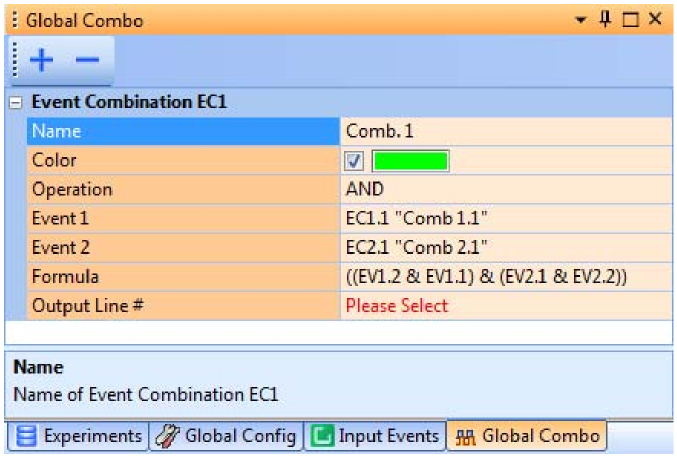
To load a specific AVI file, click the **Open video file** icon in the **Sources** tab.



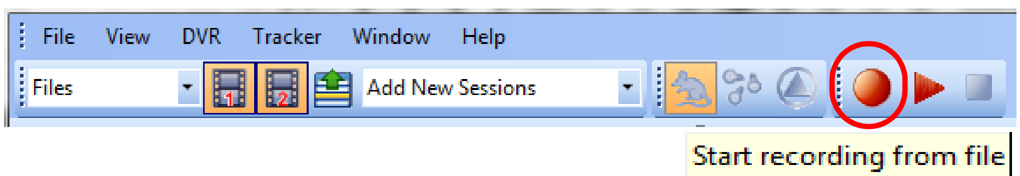
Combination events can be created for each video source in the **Behav Combo** tab.



Global combination events can be created across multiple video sources in the **Global Combo** tab.

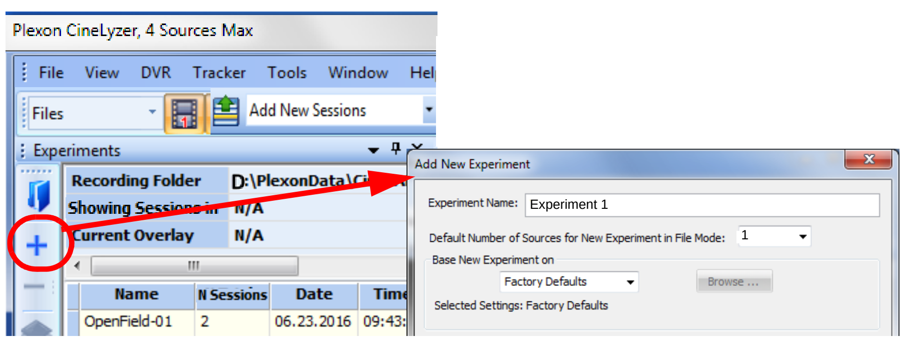


You can add sessions by clicking the **Start recording from file** button.



10.9.2 Example—One Video Source

1 Switch to Files/Add New Sessions mode and create Experiment 1.



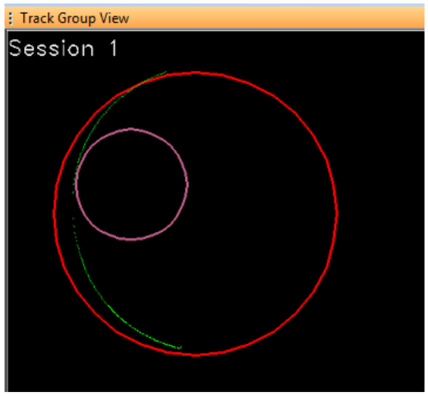
2 Load the AVI file in the Source 1 tab.

3 Create an arena and one zone (Zone 1.1).

4 Set up whole body tracking.

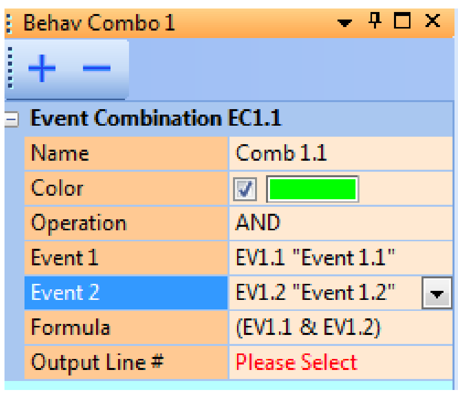
5 Create Zone Event 1.1 associated with Zone 1.1.

6 Record Session 1.



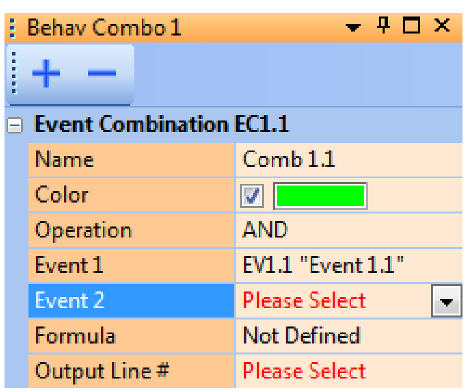
7 Now add another zone (Zone 1.2) and create Zone Event 1.2 associated with this zone.

8 Create the behavioral combination event Behav Combo 1.



9 Record Session 2.

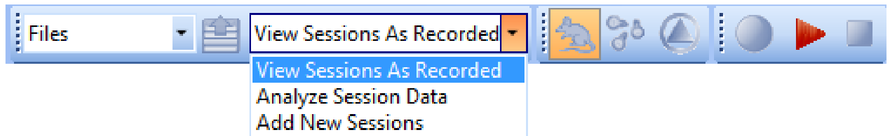
10 Now deselect Event 2 in Behav Combo 1.



11 Remove Event 1.2.

12 Record Session 3.

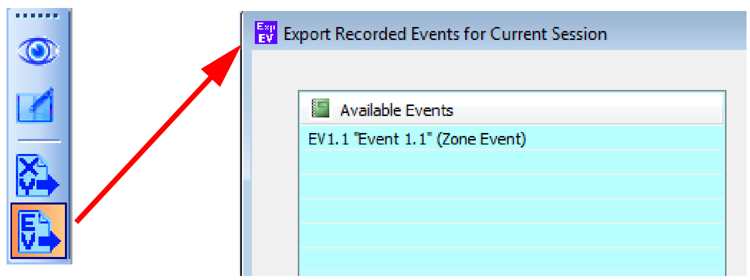
13 Switch to result viewing (View Sessions As Recorded submode)



Your currently selected session at this point will be Session 1.

<u>Notes:</u>

- The Track Group View will show arena and zones for Session 1 only, just as it was originally recorded. There will be no Zone 2.
- There will be only one zone and one event defined, as originally recorded.
- If you click on the Export Events icon, you will see that there is only one event defined.



14 Click on Session 2 to select it. You will see two zones, <u>two</u> behavioral zone events, and one behavioral combo event.

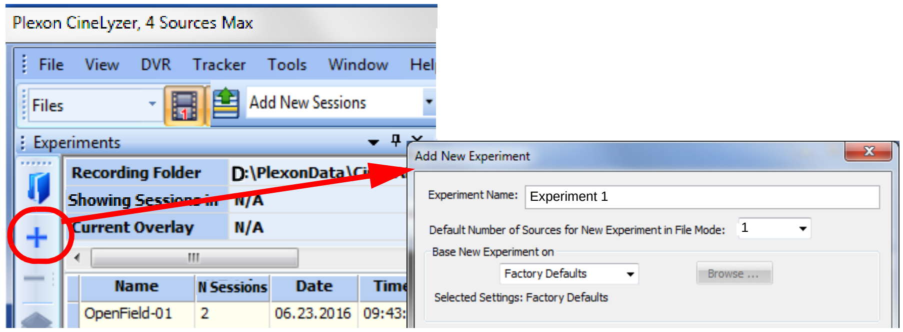
15 Click on Session 3 to select it. You will see two zones, <u>one</u> behavioral zone event, and one behavioral combo event.

<u>Notes:</u>

- **Modifying a zone in one session**—When you create a new zone for in any session (as we did for Session 2, above), that new zone will appear in the Task Group View for all sessions in that experiment (including Sessions 1 and 3 in this example). However, if you later resize or move that zone for Session 2, that zone will stay at the original size and position in all other sessions.
- **Events cannot be applied retroactively**—In the example above, Event 1.2 was created after Session 1 was already recorded. Therefore, if you need Event 1.2 to be included in Session 1, you will need to create a new session with Zone 1.2 and Event 1.2 set up, load the AVI file from Session 1, then record the new session.

10.9.3 Example—Multiple Video Sources

1 Switch to Files/Add New Sessions mode and create Experiment 2 with four video sources.



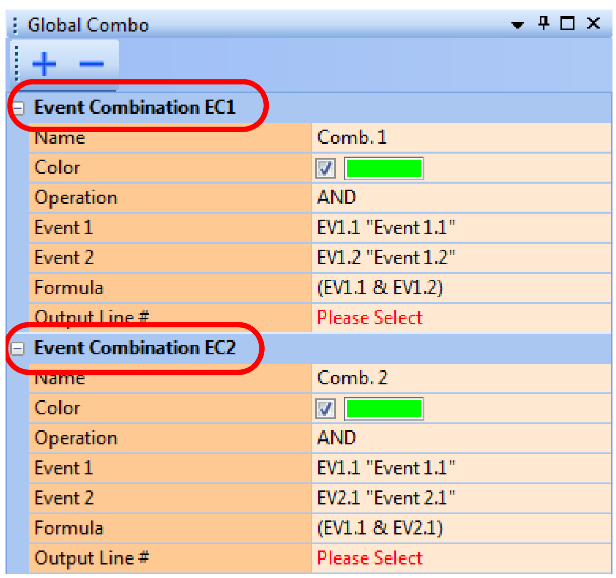
2 Load the AVI files in the four Source tabs.

3 Set up whole body tracking.

4 Create an arena two zones and two events.

5 Add Global Combo Event EC1, and configure its items as Event 1.1 (the first event from File 1) and Event 1.2 (the second event from File 1).

6 Add another Global Combo Event EC2, and configure its items as Event 1.1 (the first event from File 1) and Event 2.1 (the first event from File 2).

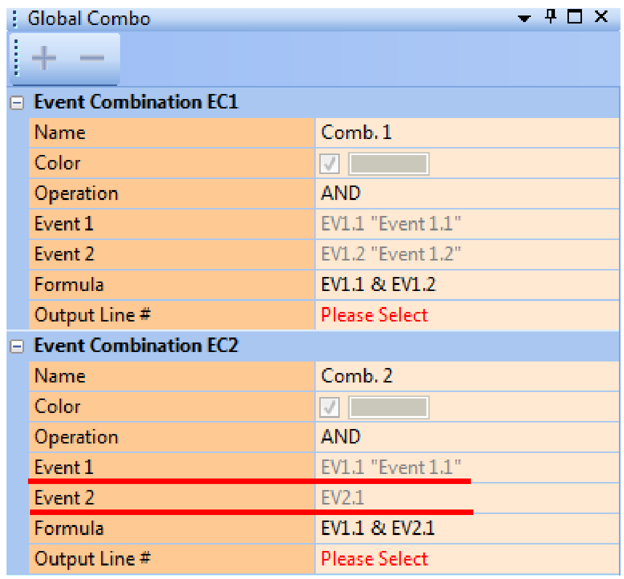


7 Record.
The system will record four sessions (files), one for each of the video sources. You will have two zones and two behavioral events for each session.

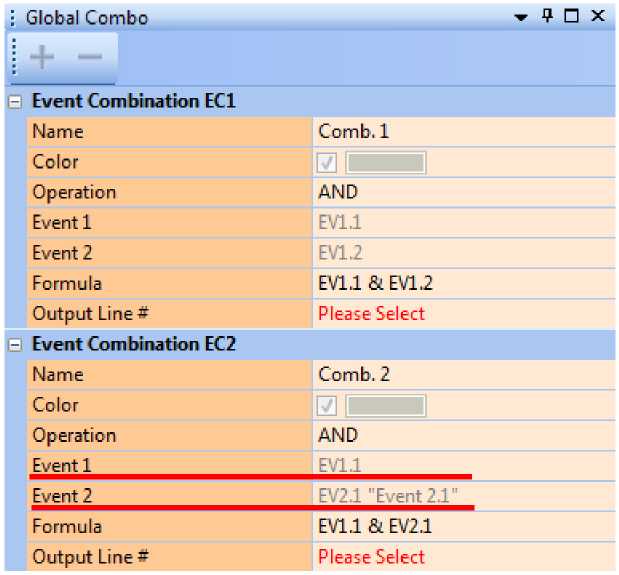
8 Now switch to View Sessions As Recorded submode to view the two Global Combo events, EC1 and EC2, for Session 1. (Note that most of the event options are grayed out in this mode, which is simply a reminder that these options are not configurable in this submode.)

In the image below, take a look at Event 1 and Event 2 for Event Combination EC2. Notice that Event 1 is displayed as EV1.1 “Event 1.1,” but Event 2 is displayed only as EV2.1 without a name. This situation occurs because we are in View Sessions As Recorded submode and focusing only on Session 1. We can only know the names of events for Session 1 (from video source 1), not the names of events in Sessions 2, 3 and 4 (video sources 2, 3 and 4).

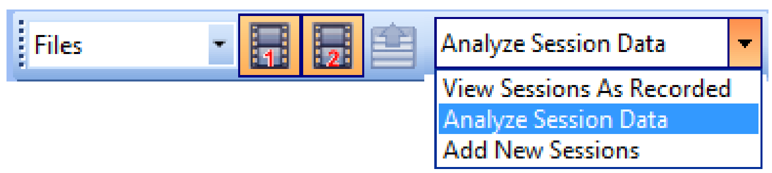
In terms of the Overlay for this experiment, we cannot get the name of EV2.1 from the Master overlay, because the Master overlay could be different during Session 2 recording vs. Session 1 recording. In addition, we cannot get the name of EV2.1, because we have loaded only Session 1 in this case, and it contains no information about the views for videos 2, 3 and 4.

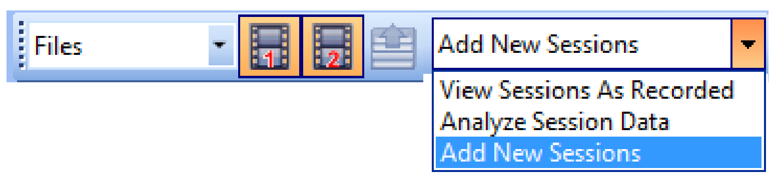


9 A similar situation occurs for any of the other sessions. For example, if we select Session 2 and view the Global Combo tab again, we will see the image below. Now Event 1 is displayed as EV1.1 with no name, but Event 2 is displayed as EV2.1 "Event 2.1."

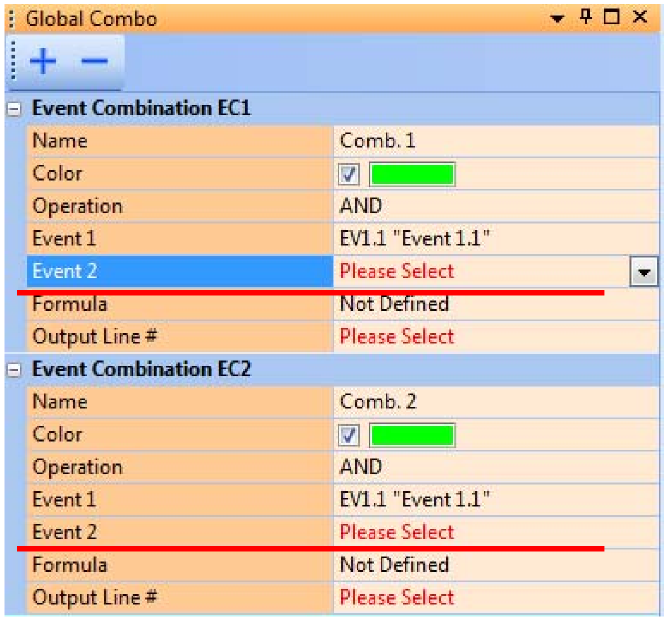


10 Now switch back to either Analyze Session Data or Add New Sessions submode.



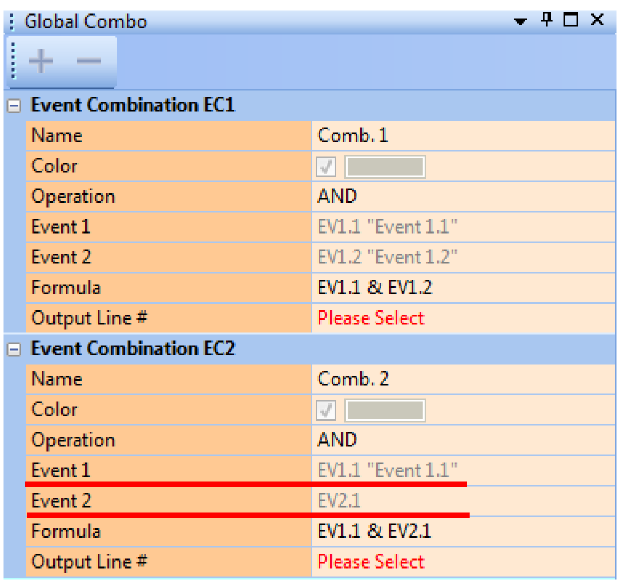


You will see that Event 2 is undefined for both EC1 and EC2. This means that the currently selected overlay (which is the Master overlay) has been modified accordingly.

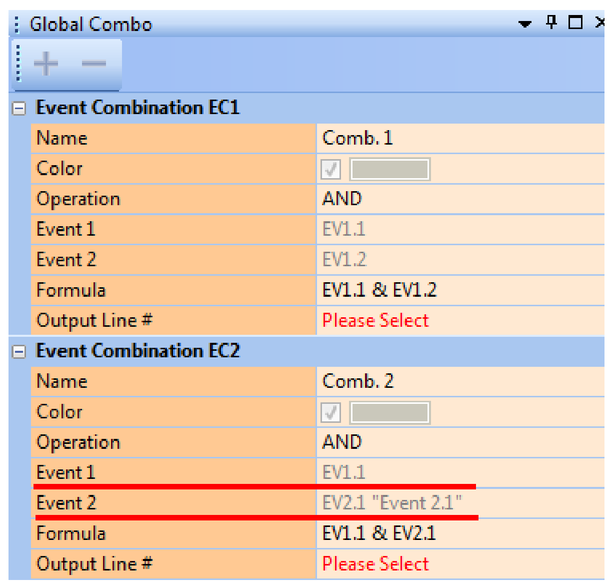


11 Now switch back to **View Sessions As Recorded sub**mode and notice that EC1 and EC2 look the same as they were in Step 8 and Step 9.

Global Combo for Session 1

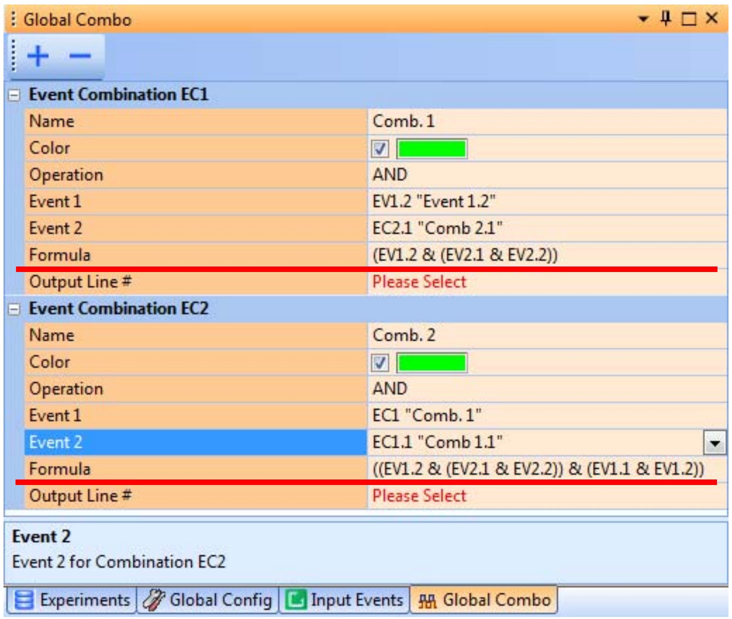


Global Combo for Session 2



10.9.4 Example—Formula Display in Global Combo Tab

There is one additional feature to notice in the Global Combo tab.
In this tab, if a Formula for an Event Combination includes other combination event(s), the full lower-level combination event is displayed. In the example below, EC1 Event 1 contains the event combination EC1.2 from video source 1, and EC2 Event 2 contains EC1 from this same Global Combo tab. Notice that the Formula lines expand the events all the way down to the lowest level.



10.10 Setting the Analysis Parameters and Analyzing the Data

10.10.1 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.

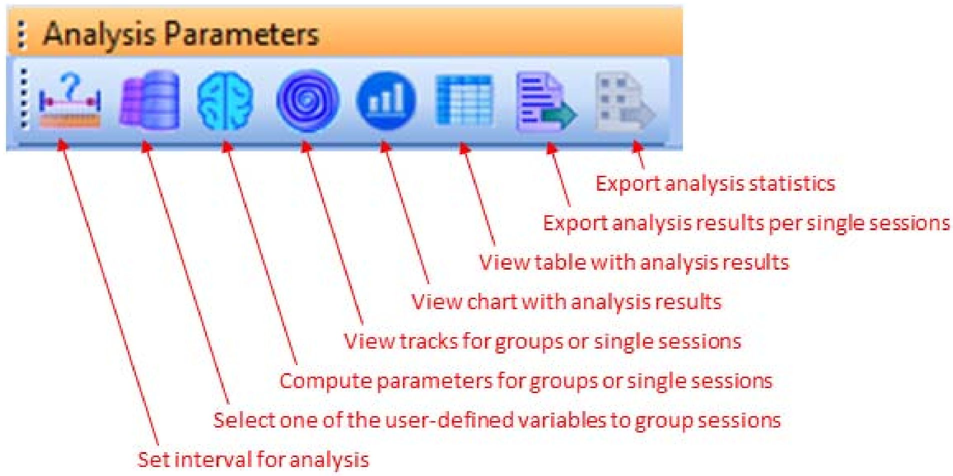
1. Set the parameters in the **Marker Visualizations**, **Whole Body Visualizations** or **LED Visualizations** section of the Tracking tab
2. Set the parameters in the Scenes tab
3. Set the parameters in the Sequences tab
4. Set the parameters in the Events tab
5. Set the parameters in the Behav Combo 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.

#	Drug	Dosage	Hours since dosage	Cam #	Date	Time	Duration	Analyze
1	D-47-A	200	1.50	1	06.16.2015	13:22:42	00:00:18	☑
2	D-47-A	210	1.50	2	06.16.2015	13:22:42	00:00:18	☑
3	D-47-A	211	1.50	1	06.16.2015	13:24:27	00:00:21	☑
4	D-47-B	2	0.00	2	06.16.2015	13:24:27	00:00:24	☑
5	D-47-B	2	0.00	1	06.16.2015	13:32:16	00:00:21	☑
6	D-47-B	3	4.90	2	06.16.2015	13:32:16	00:00:21	☑

10.10.2 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.

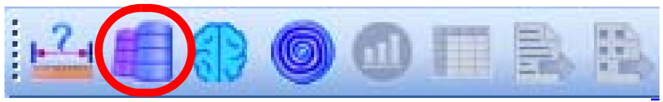
- **Object for Computation** parameters (available for the LED and Color Markers tracking modes but not for Object Contour mode)
- **Global Parameters** (**Session Duration**, **Total Track Length** and **Average Speed**)
- **Zone 1/2/3/... Parameters**. Zone-specific parameters become available after 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 trigger an external device (for example, turning on an LED or activating a pellet dispenser). To configure a zone-specific event, see Section 7.10, “Adding Zones (Static and Dynamic)” on page 149 and Section 7.13.1, “Events Based on a Zone” on page 161.

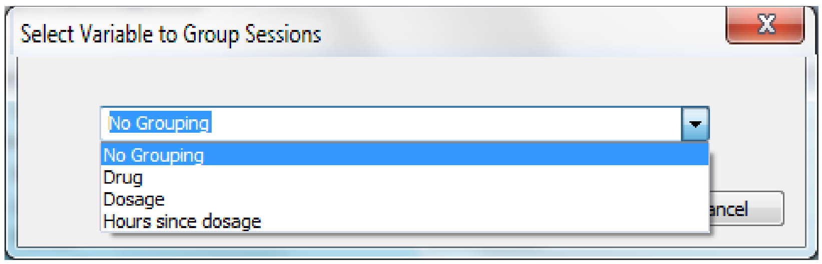
10.10.3 Perform an Analysis and Group Event Results by Session Number

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.

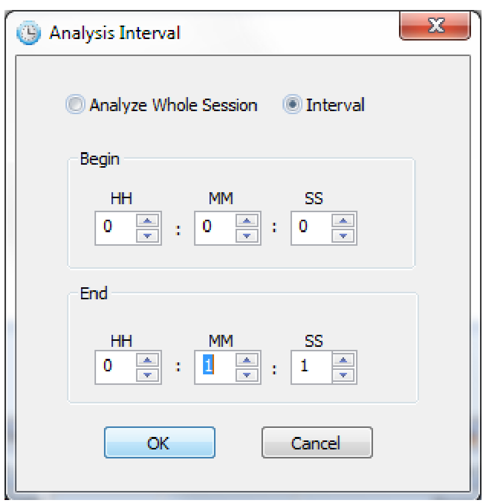


2. The **Select Variable to Group Sessions** dialog box opens.

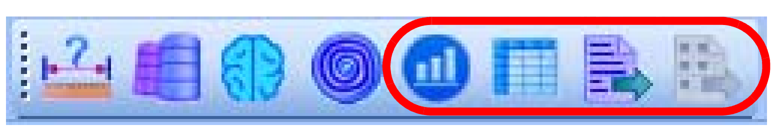


3. Ensure that the choice in the dropdown list is **No Grouping**, which is the default value. Selecting this option (**No Grouping**) instructs the system to sort results according to Session number.

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**.

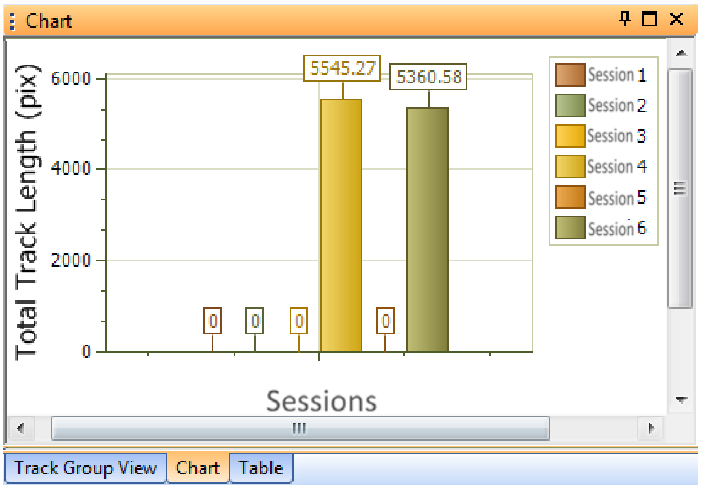


After you set the Analysis Interval, the **View chart with analysis results**, **View table with analysis results** and **Export analysis results per single sessions** icons become active. (The **Export analysis statistics** icon remains inactive because no meaningful statistics can be generated for individual sessions for this type of data.)

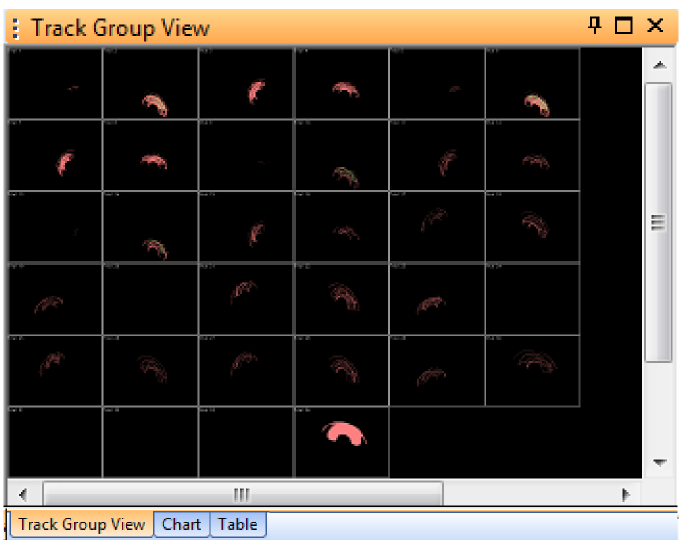


5 Click the **Compute parameters for groups or single sessions** icon to instruct the system to perform the calculations that will be used for behavioral analysis.

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.

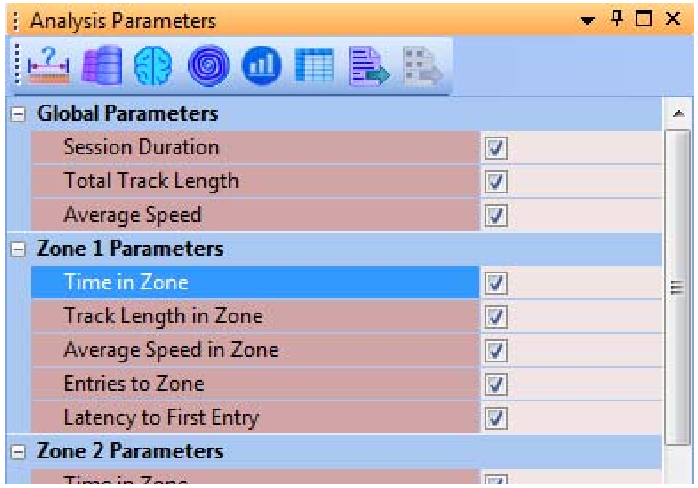


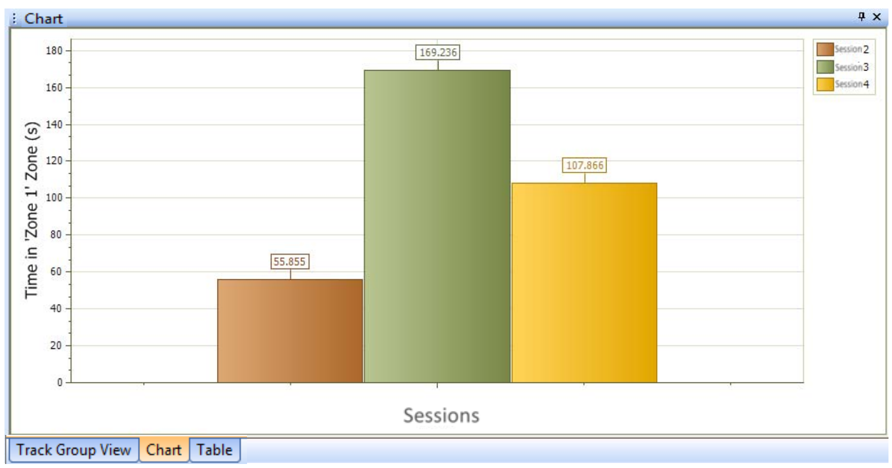
7 Click on the **Track Group View** tab to see a record of the animal's movements during the session.



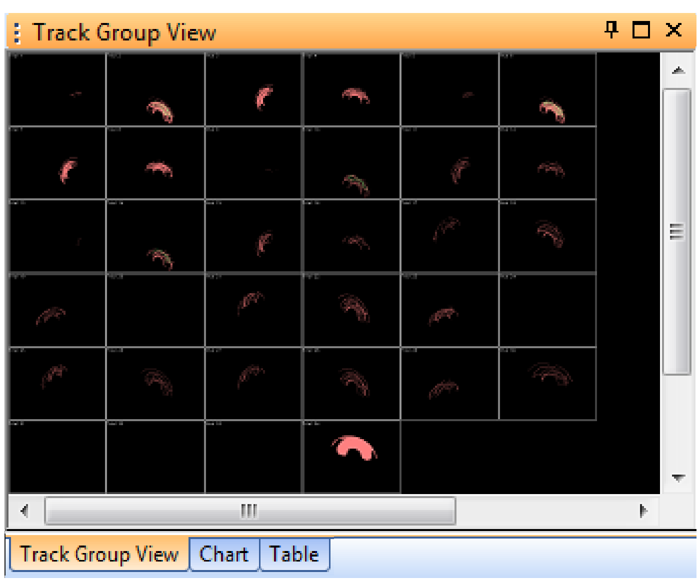
8 You can visualize (display) various sets of data in the **Chart** tab by highlighting an item in the Analysis Parameter list. In the example below,

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 triggered behavioral events 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

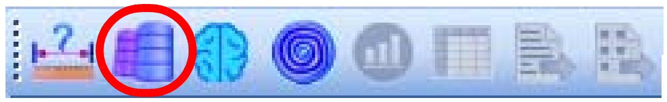
No.	Event Name	Target		Object	Output	Count
		Type	Name			
EV1.1	Event 1.1	Zone ZD1.2	Zone 1.2	ACG	N/A	0
EV1.2	Event 1.2	Seq SQ1.2	Sequence 1.2	Mrk 3	N/A	0
EV1.3	Event 1.3	10.0 degrees	N/A	Mrk 3	N/A	0
EV1.4	Event 1.4	29.2 cm/s	N/A	Mrk 4	N/A	0
EV1.5	Event 1.5	± 24.0 degrees	N/A	Mrk 7	N/A	0
EV1.6	Event 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

10.10.4 Perform an Analysis and Group Event Results by Variables

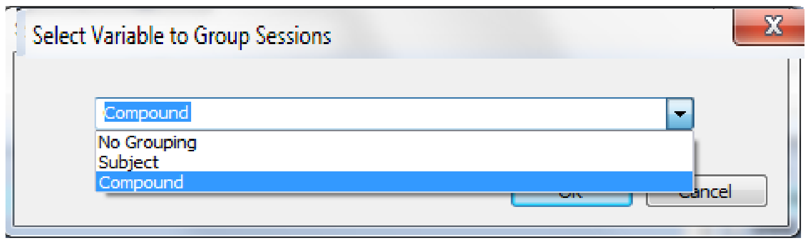
This section explains how to set the options for data analysis based on the variables associated with the sessions in an experiment.

1 In the **Analysis** toolbar, click the **Select one of the user-defined variables to group sessions** icon.

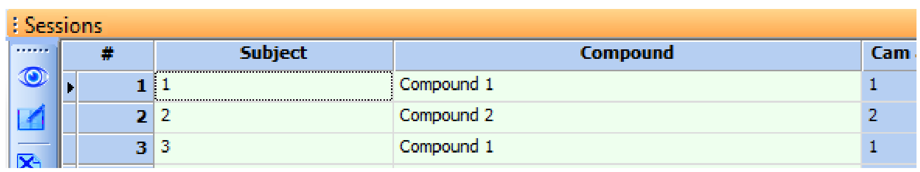


2 The **Select Variable to Group Sessions** dialog box opens.

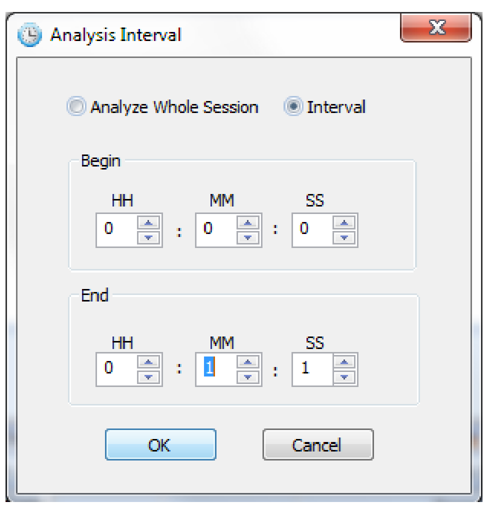
3 In the dropdown list, select one of the variables for the analysis.



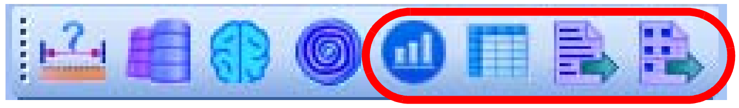
Note that the variable options in the dropdown list are the same as the headings of the variable columns in the Sessions window.

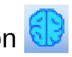


4 (Optional) Adjust the time interval by clicking the **Set Interval for Analysis** icon and adjusting **Begin** and **End** times to apply to all sessions in the experiment. The default value is **Analyze Whole Session**.



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.

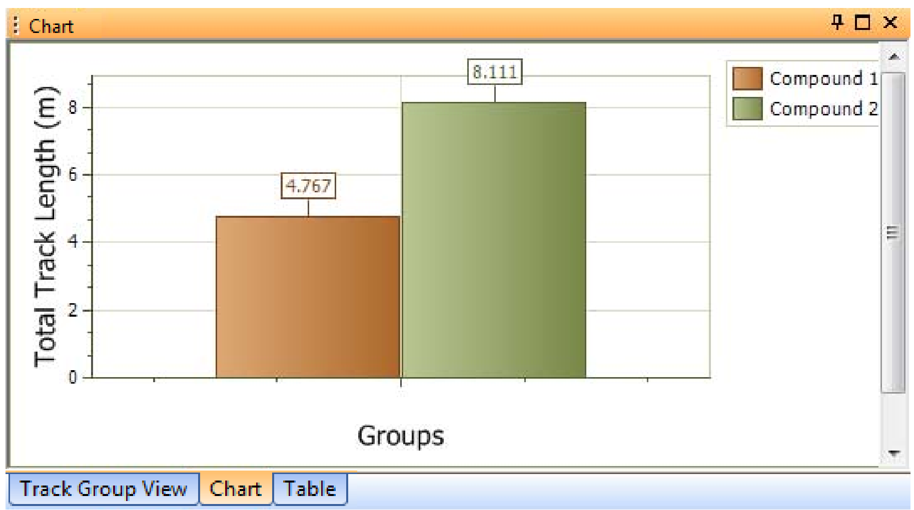


5 Click the **Compute parameters for groups or single sessions** icon  to instruct the system to perform the calculations that will be used for behavioral analysis.

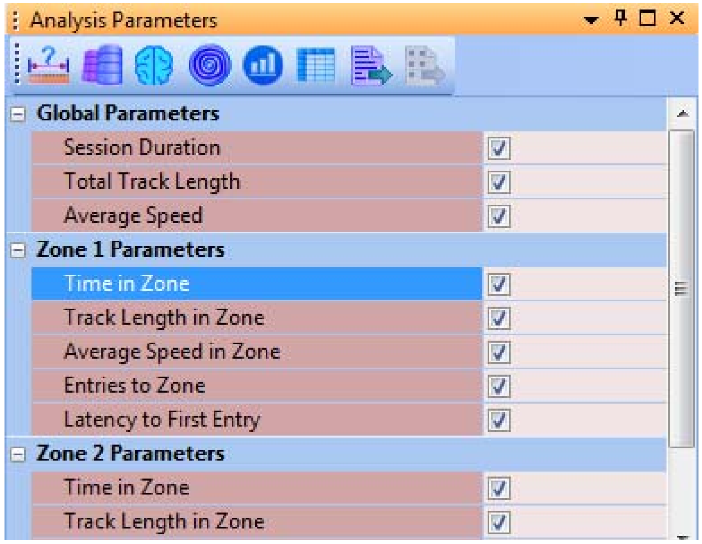
6 View the results.

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.

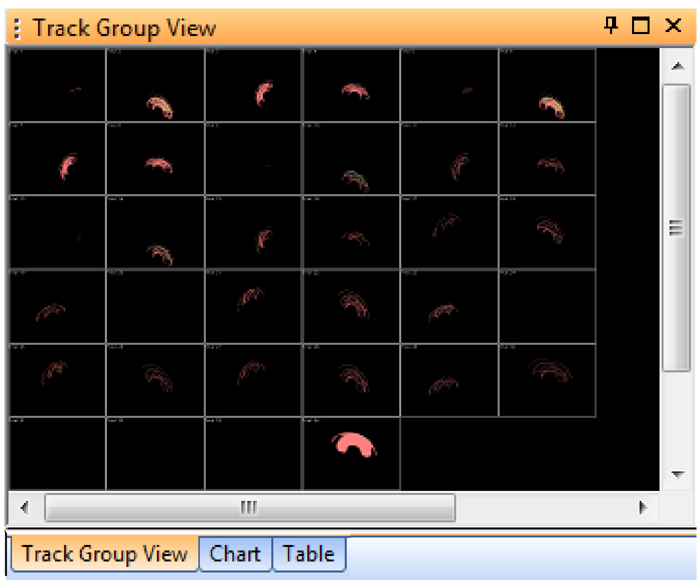
In general, the chart shows the average value for all sessions in each Group.



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 triggered behavioral events 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

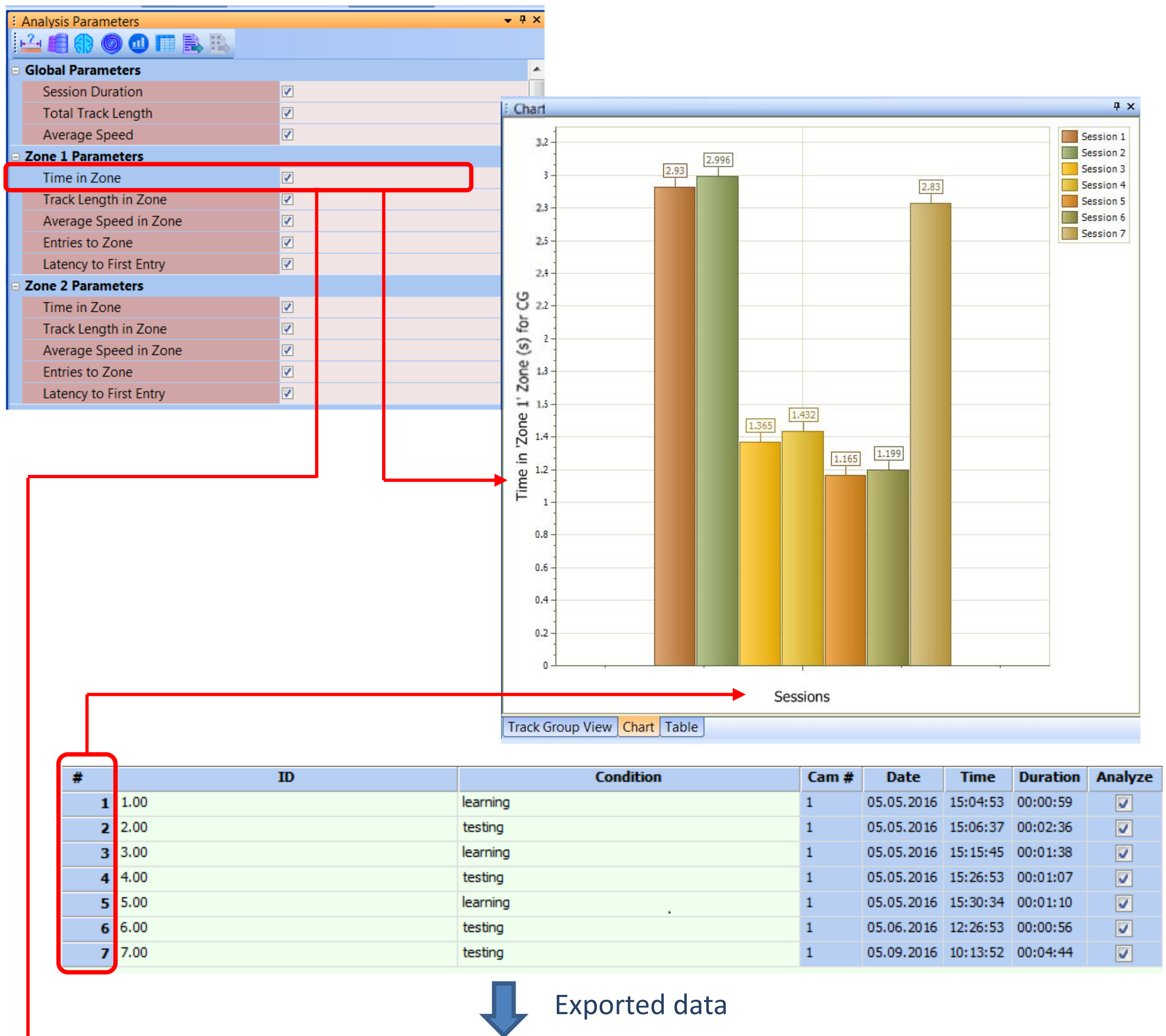
Time, s		Track Length, cm	
Last	Cumulative	Last	Cumulativ
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0

10.11 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.

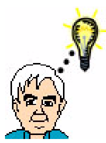
As shown in the example below, the exported data includes global parameters (session duration, total track length and average speed) and zone-related parameters (time in zone(s) and zone sequence(s), track length, average speed, entries to zone, and latency to first entry). The data are the same as what is shown in the **Chart/Table** tabs and **Sessions** tabs. See the example below.



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 7.13.4, "Events Based on Speed" on page 168 for the method the system uses to calculate average speed.

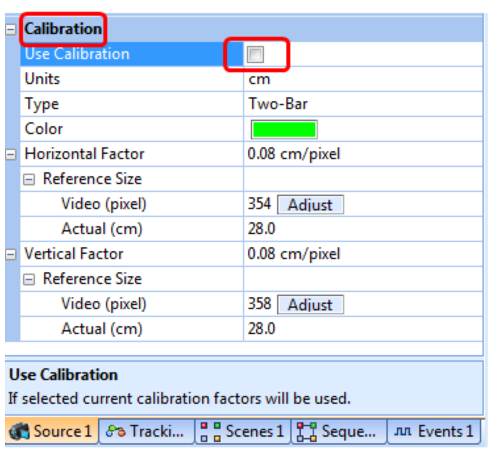


TIP

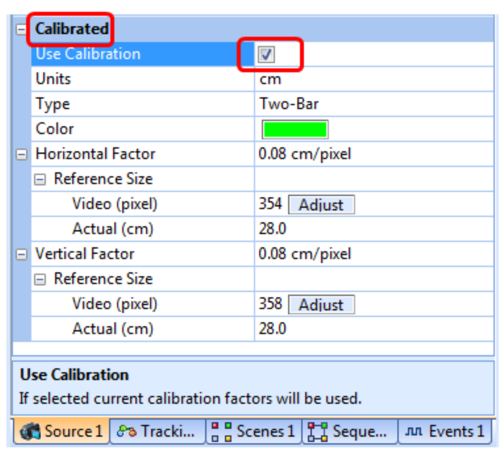
Select the "Use Calibration" checkbox for calibrated data

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 4, 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.

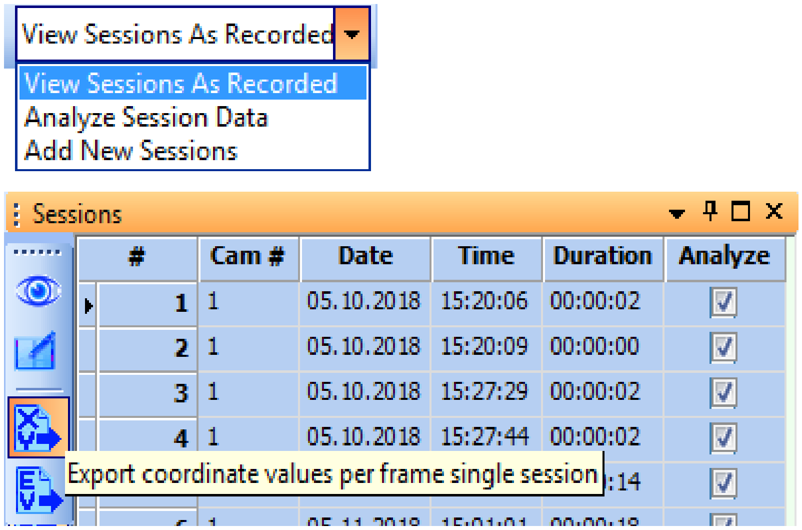
- **Average Speed in Zone** (pixels, cm or inches per second)—The average speed of the animal during the time(s) the event was true in the session. See Section 7.13.4, “Events Based on Speed” on page 168 for the method the system uses to calculate average speed.
- **Entries to Zone**—The number of times 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.

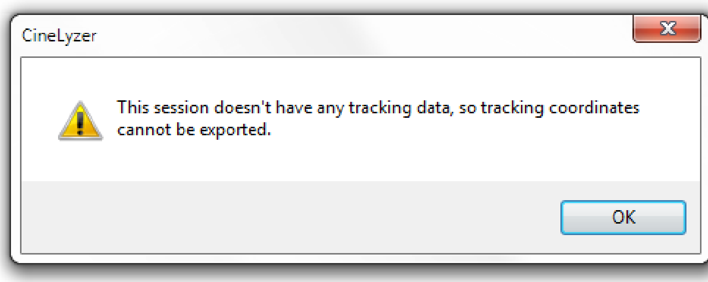
10.12 Exporting Coordinate Values and Motion Measure per Frame

This section describes the coordinate values you can export to a comma separated values (CSV) file. You must be in **Files** mode and **View Sessions as Recorded** to export.



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 file displays the frame number, time (seconds from start of the recording), X coordinate, Y coordinate and Motion Measure. Motion Measure is applicable when **Object Contour** tracking is done, and it is explained below.

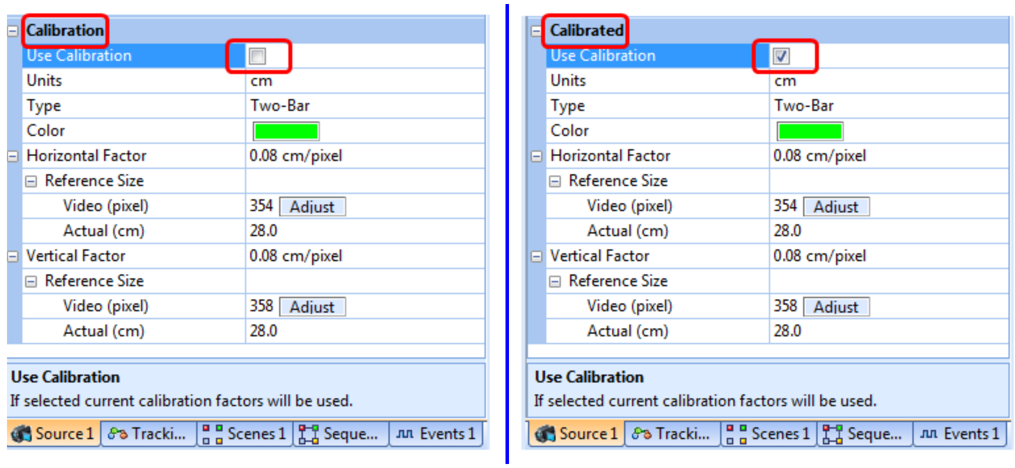
Frame,	time,	X,	Y,	MotionMeasure
71,	2.33057,	262.382,	216.407,	1
72,	2.36387,	260.231,	218.248,	20
73,	2.39716,	266.992,	259.734,	354
74,	2.43046,	264.150,	228.365,	337

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 is checked prior to recording the session, the exported values will be displayed in the calibration units (cm or inches). If the **Use Calibration** box is unchecked prior to recording, the values are displayed in pixels.



Note: If you repeatedly check and uncheck the **Use Calibration** box (which is not recommended), the system applies calibration according to logical rules. These rules are explained in Appendix E, Modifying Arenas and Zones.

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

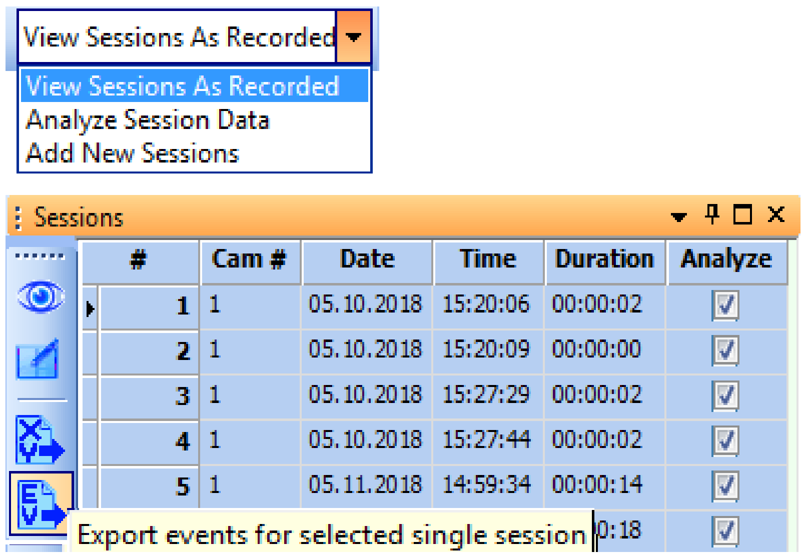
If the animal travels outside the arena, or if it is obscured from the view of the camera (for example, if it travels under an object inside the arena), the system inserts a value of "-10000" in the exported data for that frame. Frames that contain a "-10000" should be excluded from your analysis, or in some cases you may want to consider manually interpolating data in those frames.

10.13 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.

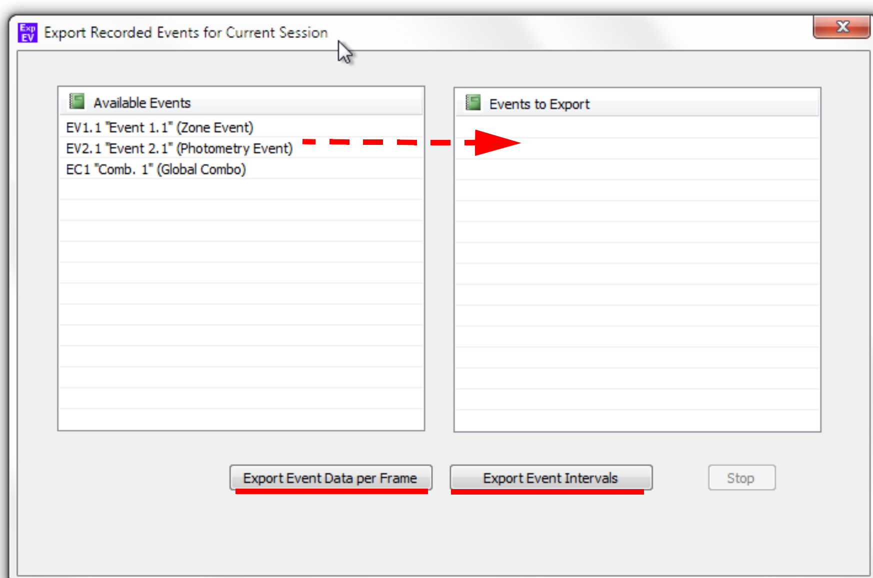
Use the following procedure to export event data.

1 In **Files/View Sessions As Recorded** mode, click the **Export events for selected single session** button.



The system opens the export dialog.

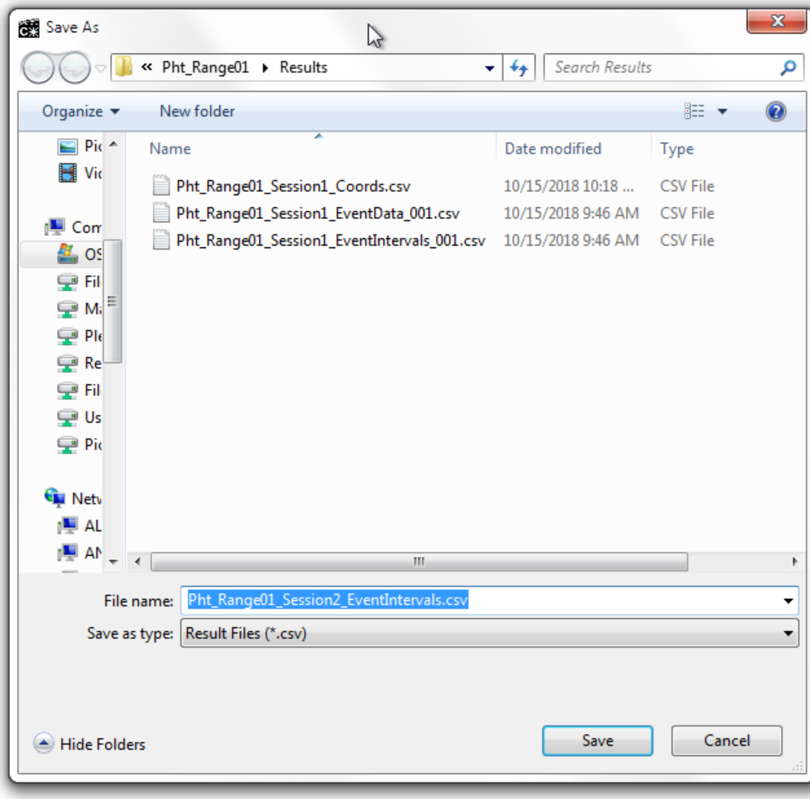
2 In the export dialog, drag the data types you want from the **Available Events** column and drop them in the **Events to Export** column.



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 Click either the of the Export buttons at the bottom of the dialog (see the image above).

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 file displays the frame number, time (seconds from start of the recording), indicator for the first event being true (1) or false (0), and the track length the animal covered during this time frame. Similar data is presented for the additional events that occurred during the session. The example below displays the typical format of the CSV file. (Spaces have been added here for clarity.)

#,	Timestamp,	"Event 1.1",	"Track Length (pix) for Event 1.1",	"Event 2.1",	"Comb. 1"
1203,	0.000000,	0,	0.000,	0,	0
1204,	0.033333,	0,	0.000,	0,	0
1205,	0.066666,	0,	0.000,	0,	0
1206,	0.099999,	0,	0.000,	0,	0
1207,	0.133332,	0,	0.000,	0,	0

.....

....

Data Format—Export Event Intervals

The file displays the start and end times for each of the events that occurred during the session. The example below displays the typical format of the CSV file. (Spaces have been added here for clarity.)

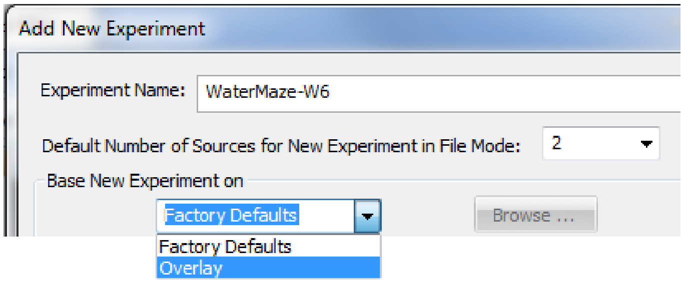
Event,	"Start Time (s)",	"End Time (s)"
"Event 1.1",	0.022005,	1.283333
“Event 3.2",	0.000110,	0.487378
"Comb 2.1",	1.604720,	2.089742
"Comb 2",	0.789423,	1.890942

.....

....

10.14 Using the Overlay Feature during Analysis

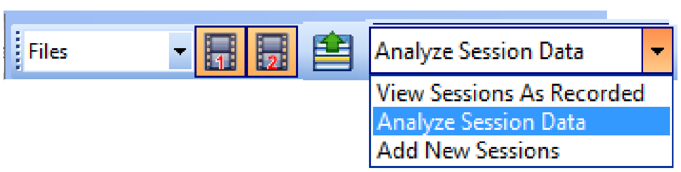
As described in Section 3.5, "Creating a New Experiment" on page 37, when you create a new experiment, the system gives you the option of basing its geometry and video parameter values on [1] **Factory Defaults** or [2] an **Overlay** (a set of geometry and video parameter values that was previously 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.

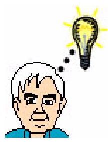
Modifying the overlay when analyzing sessions

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

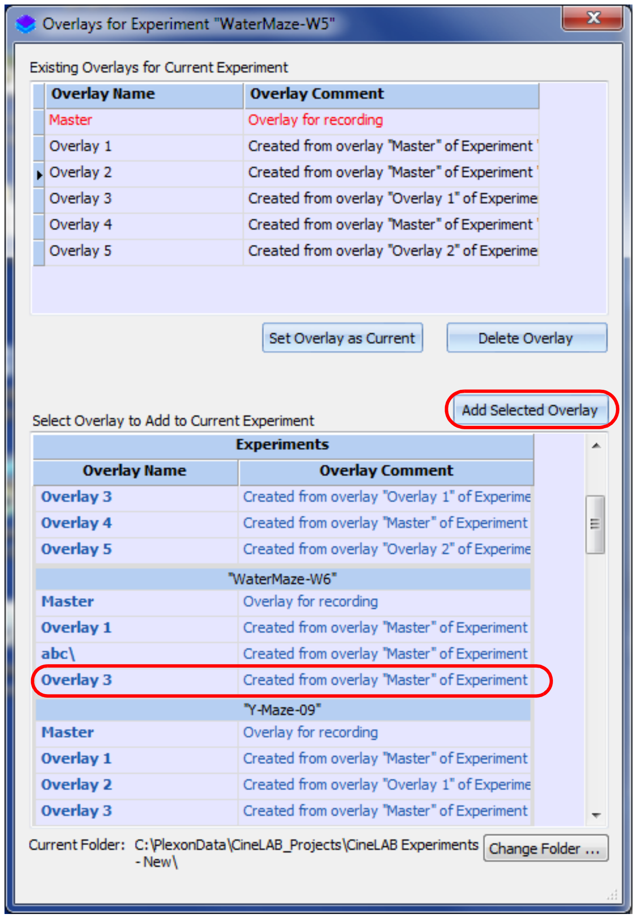
— Record a session in the new experiment (the data is not important in this case)

— In the future, you can reload that new Master overlay whenever needed

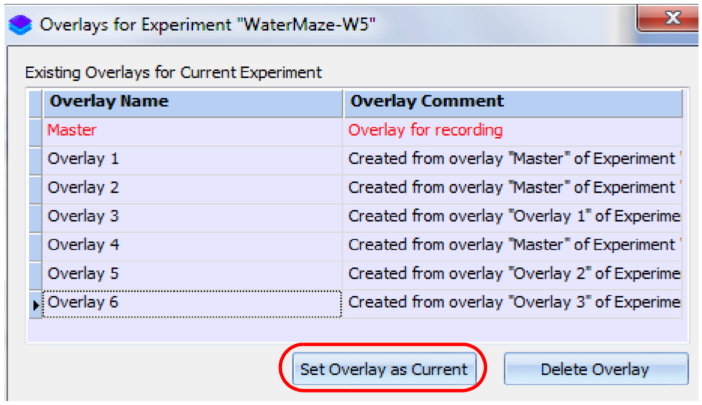
— Return to your original experiment and continue recording sessions, modifying the Master overlay if needed

Procedure

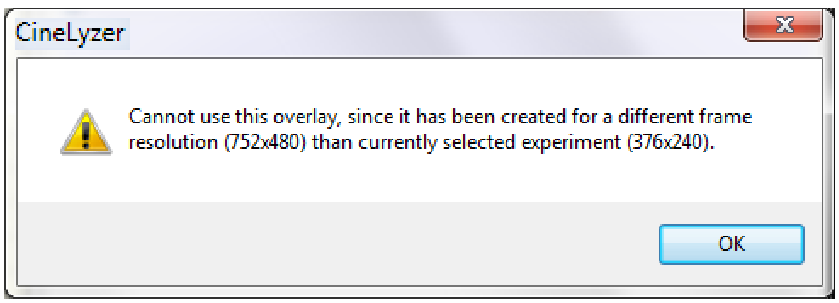
1. Ensure that the system is in **Files/Analyze Session Data** mode.
2. To apply a different overlay to an existing session, click on the **Edit and select overlays** icon .
3. Select the desired overlay from the list in the dialog box. For example, in the list below, select Overlay 3 of WaterMaze-W6 to be added to the available overlays for WaterMaze-W5. Then click the **Add Selected Overlay** button.

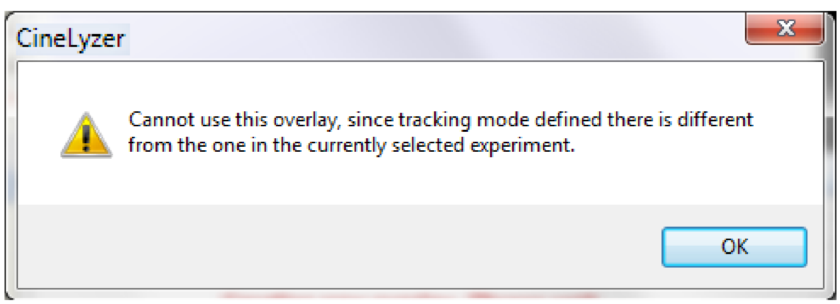


4 Select the appropriate overlay (in this example, Overlay 6, which you previously added to the list of available overlays), then click the **Set Overlay as Current** button.

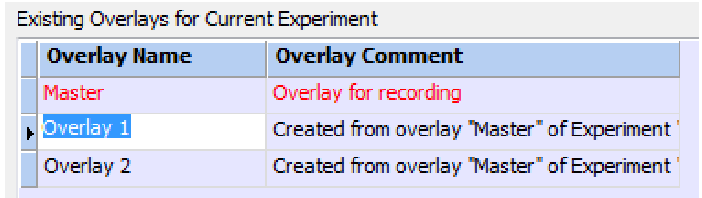


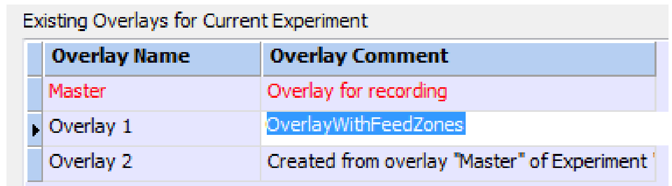
Note: The system does not allow you to assign an overlay to an experiment if the frame resolution or tracking mode of the overlay is different from the frame resolution or tracking mode of the currently selected experiment. Instead, the system displays the applicable dialog box; see below.





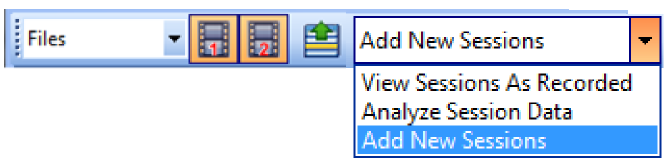
5 If desired, you can modify the name or comments by double clicking in the field and entering new text.

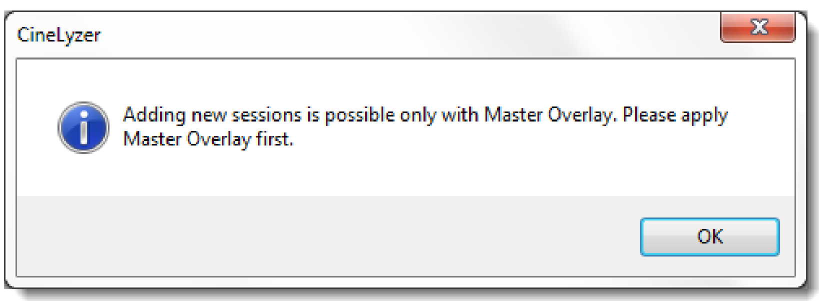




Adding New Sessions

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.





10.15 Caveats—Understanding Tracking Data in System Computations

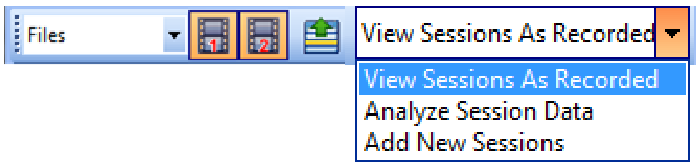
In any experiment, once a session has been recorded with tracking data enabled (that is, a session for which tracking data has been recorded), the tracking data for that session cannot be changed. However, you can still analyze the existing data and use the existing recorded video in several ways.

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 6.18, "Modifying Arenas and Calibration During an Experiment" on page 137.

Chapter 11
Coordinating Behavior and Optical Stimulation

11.1 Introduction

The CineLyzer® System can be connected through the USB Digital Input/Output Interface (DIO Interface) to the Plexon® PlexBright® 4 Channel Controller (PlexBright Controller) to provide coordination between an animal's behavior and optogenetic stimulation. Typically, this capability is used to start, stop, pause or unpause stimulation when a specific event is detected by the CineLyzer System, for example, when an animal is in a certain location in the experiment chamber or when the animal exhibits a particular behavior.

For details about the DIO Interface, see Appendix C, USB Digital Input/Output Interface. The DIO Interface is included in the CineLyzer System.

The status bar at the bottom of the CineLyzer GUI displays an I/O icon that is red 

if the USB Digital I/O unit is not connected, and green 

if the unit is connected.

Note: The PlexBright Controller and the associated Radiant™ software and cables are sold separately.

See the applicable user guide, *PlexBright 4 Channel Optogenetic Controller with Radiant Software* (available on the Plexon website), for operation of the controller and the associated software.

These procedure requires CineLyzer software Version 4.1 or later and Radiant software Version 2.1 or later. (If you have different versions, please contact your Plexon representative to review the software compatibility.)

11.2 Connecting CineLyzer System to the PlexBright Controller

The connection between the two systems is made through the Opto Controller DIO Bare Wire cable. These input/output functions require the appropriate PlexBright Controller hardware, software and license.

The cable is shown in the image below. The standard length is 15 ft. The pinouts, wire colors and input/output functions are listed later in this section.

11.3 Sending CineLyzer Output Signals to the PlexBright Controller

The CineLyzer System can be configured to generate digital output signals when a behavioral event takes place. You can use the digital output signals from the CineLyzer System as input into the PlexBright Controller to trigger the stimulation to start, stop, pause or unpause. The procedure for configuring

CineLyzer events and digital outputs is provided in Section 7.17, “Specifying Digital Outputs” on page 190. (Instructions for configuring digital inputs on the PlexBright Controller are in the applicable user guide. User guides are available on the Plexon website.)

11.4 Example—Continuous Stimulation When Animal Is In a Zone

In this experiment, the PlexBright Controller delivers continuous stimulation at 20Hz when the animal is present in a specified zone. Sample results are presented for a real-time place preference/avoidance scenario.

In this example, one side of a two-sided chamber is paired with optogenetic stimulation. The animal expressing light-sensitive ion channels within the brain has a fiber-optic stub implanted within a brain region hypothesized to be involved in reinforcing/aversive states. If the animal moves into the stimulated zone, it receives 20 Hz LED stimulation delivered to that brain region; when the animal leaves the zone, the stimulation is paused. The presence of the animal in both sides is monitored to see if a preference (or avoidance) is developed for the LED-paired zone.

11.4.1 Configure the CineLyzer Parameters and Connect Wires

1 Draw at least one zone in your arena. Give the name “Stimulated” to one of the zones.

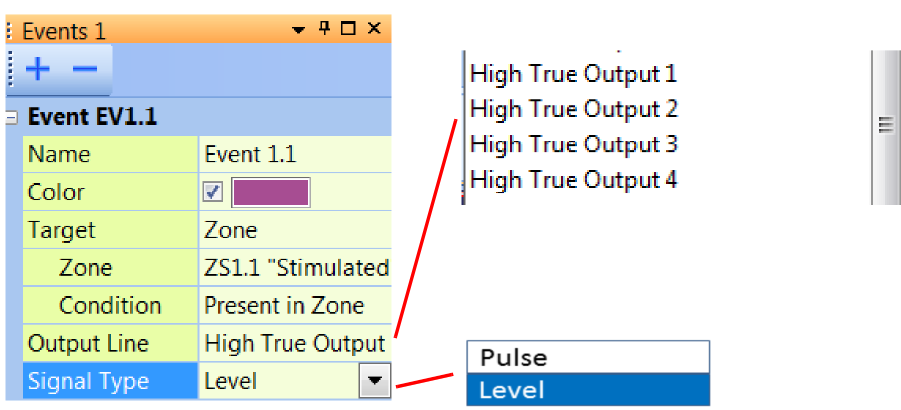
2 Set up an event for the “Stimulated” zone as follows:

a In the **Condition** row, select **Present in Zone**.

b In the **Output Line** row, select an available **High True Output** line for Channel 1, 2, 3 or 4.

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

c In the **Signal Type** row, select **Level**.



Note: A pulse signal from the CineLyzer System (**Signal Type** set to **Pulse**) corresponds to a discrete stimulation pattern in the PlexBright Controller. A continuous signal from the CineLyzer System (**Signal Type** set to **Level**) corresponds to a continuous stimulation level for a set period of time in the PlexBright Controller.

For further explanation of the signaling logic for **Pulse** and **Level,** see "Input and Output Logic" on page C-3.

3 Connect the appropriate wire on your Opto Controller DIO Bare Wire cable to your selected output on the DIO Interface—Brown for HT1, Green for HT2, etc. Refer to the pinout list, below.

4 Make sure your connector is grounded on both the input and output sides as shown in the pinout list, below.

Note: All Ground wires (GND) are a common ground at the PlexBright Controller, so you can use Pin 17, 18 or 19 for grounding.

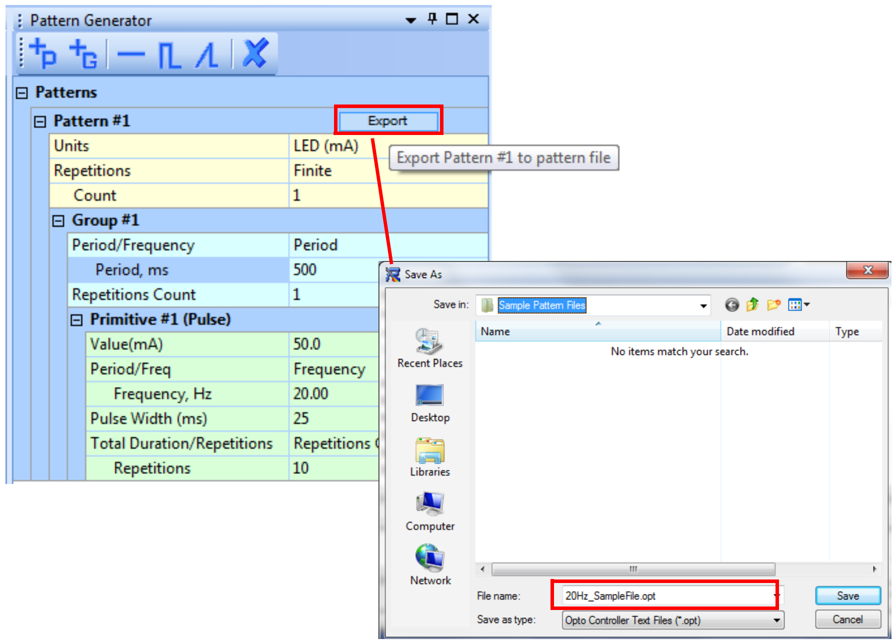
CineLyzer Digital Output (High True)	Wire Color ------------------ Bare wire side — Connect to CineLyzer DIO Interface	PlexBright Controller Digital Input Pin — Plug into **Digital In** on the PlexBright Controller
HT1	Brown	1 (Start/Pause Ch 1)
HT2	Green	5 (Start/Pause Ch 2)
HT3	White	9 (Start/Pause Ch 3)
HT4	White/Orange	13 (Start/Pause Ch 4)
GND	White/Violet	17 (GND)

11.4.2 Configure the Radiant Parameters

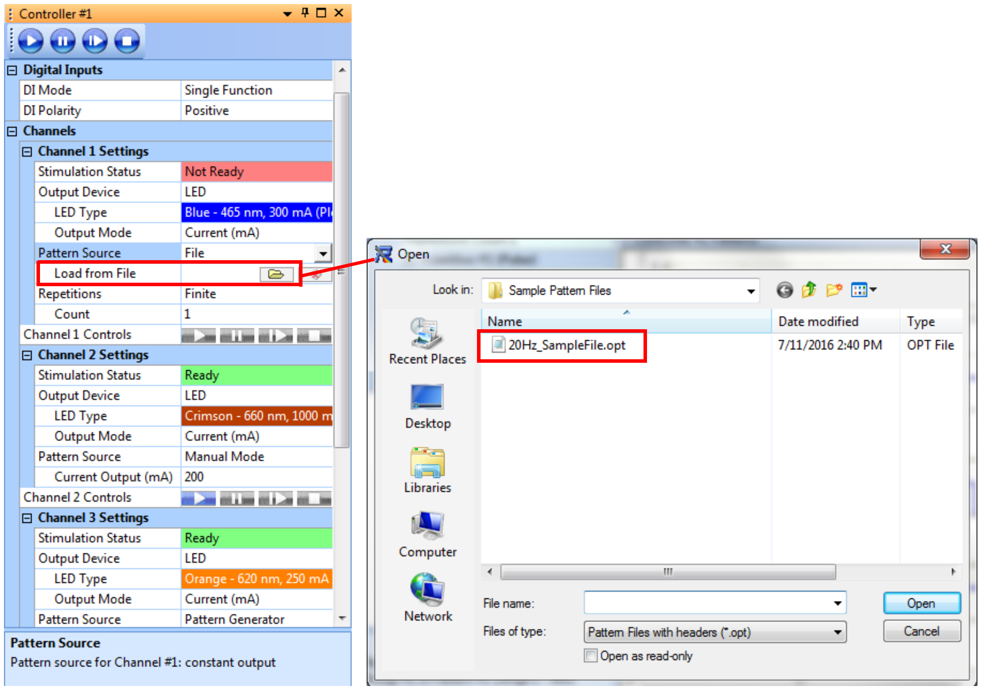
Note: Images of the Radiant software GUI may change over time. Updated images and instructions for configuring digital outputs on the PlexBright Controller are in the applicable user guide, available on the Plexon website.

1 Set up a 20Hz stimulation pattern in the pattern generator.

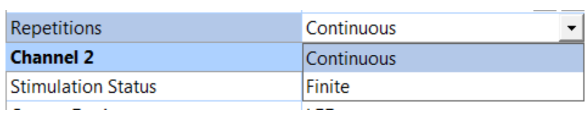
2 Export the pattern and name it.



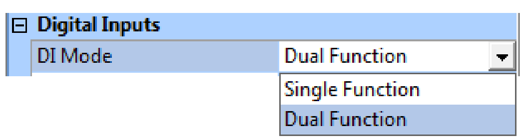
3 Load the pattern from the file into the channel you will be using for stimulation in the PlexBright Controller.



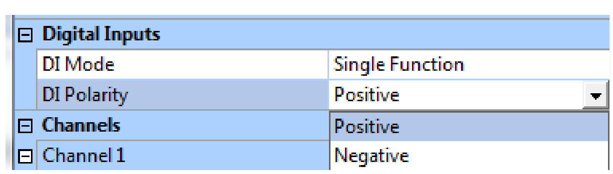
4 In the **Repetitions** row for the channel, select **Continuous**.



5 In the **DI Mode** row (digital input), select **Dual Function**. This will ensure that the channel plays the stimulation pattern for as long as your digital input is delivering a signal, and pauses when the digital input is not delivering a signal.

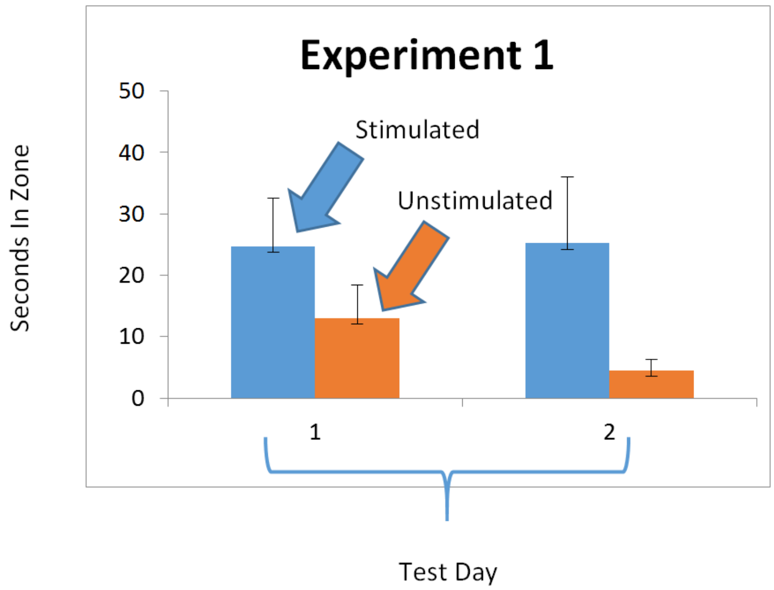


6 In the **DI Polarity** row, select **Positive** (the default value) if you are interfacing with a High True input; select **Negative** if you are interfacing with a Low True input. The current example uses Positive. The details regarding Positive and Negative are provided in the PlexBright Controller user guide.



11.4.3 View the Results

The data is used to determine whether a preference (or avoidance) is developed for the LED-paired zone. Results might be similar to those shown in the diagram below:



The systematic approach to analyzing data with the CineLyzer System tools is described in Chapter 10, Analyzing Data and Adding Sessions.

11.5 Transmitting PlexBright Output Signals to the CineLyzer System

The PlexBright Controller can be configured to generate digital output signals based on the stimulation activities. You can use the digital output signals from the PlexBright Controller as input into the CineLyzer System to trigger the starting or stopping of video recording. The procedure for configuring CineLyzer Input Events is provided in Section 5.6, “Configuring the Input Events Parameters” on page 74. (Instructions for configuring digital outputs on the PlexBright Controller are in the applicable user guide, available on the Plexon website.)

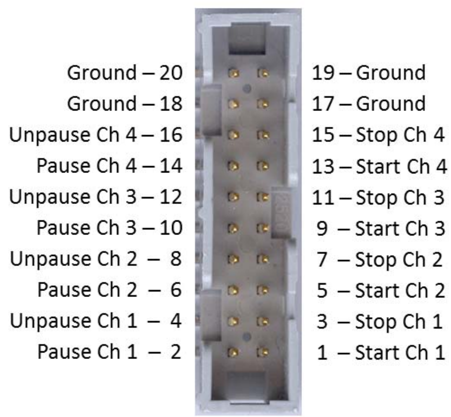
Example:
You can connect the Opto Controller DIO Bare Wire cable from the **Ch 1 Running – Ch 4 Running** digital outputs on the PlexBright Controller to the **HT1 – HT4** digital inputs on the CineLyzer DIO Interface. These digital inputs can be used to start and stop recording in CineLyzer, and would be commonly used to generate video files that are tied to optogenetic stimulation periods.

If the PlexBright Controller channel has been appropriately configured using the Plexon Radiant™ software, when the PlexBright Controller generates a Ch 1 Running, Ch 2 Running, Ch 3 Running or Ch 4 Running output signal, the corresponding input pin on the CineLyzer DIO Interface will receive an input signal. If the Input Event has been properly configured on the CineLyzer System, it will trigger the start or stop of recording. The connections for this example are shown in the table below.

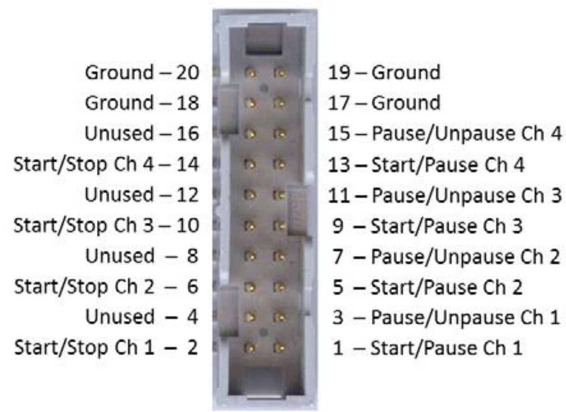
PlexBright Controller Digital Output Pin	Wire Color	CineLyzer System Digital Input
1 (Ch 1 Running)	Brown	HT1
5 (Ch 2 Running)	Green	HT2
9 (Ch 3 Running)	White	HT3
13 (Ch 4 Running)	White/Orange	HT4
17 (GND)	White/Violet	GND

11.6 Opto Controller DIO Bare Wire Cable Specifications

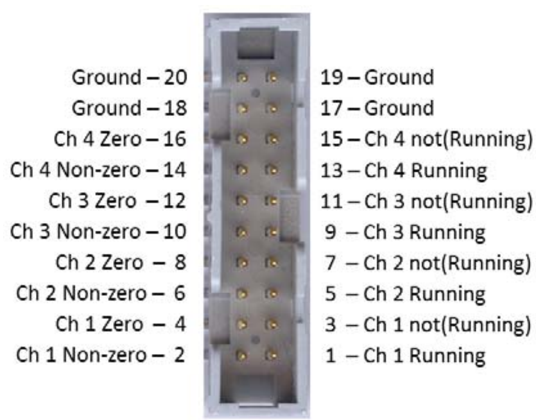
The Opto Controller DIO Bare Wire cable can be used to connect to either the Digital In or Digital Out port of the PlexBright Controller. The functionality of the wires depends on whether the cable is used with the digital input (DI) port or the digital output (DO) port of the Controller. The pinout diagrams below give the functionality for each case.



PlexBright Controller
Digital Input (Single Function)



PlexBright Controller
Digital Input (Dual Function)



PlexBright Controller
Digital Output Functions

The pinout and wire details are listed below. “DI Function” and “DO Function” refer to the digital input and output functions (respectively) on the PlexBright Controller. See the applicable user guide, *PlexBright 4 Channel Optogenetic*

Controller with Radiant Software (available on the Plexon website), for details about using these digital controls and for all other information about the operation of the controller and the associated software.

Pin	Wire Color	DI Mode — Single Function	DI Mode — Dual Function	DO Function
1	Brown	Start Ch 1	Start/Pause Ch 1	Ch 1 Running
2	Red	Pause Ch 1	Start/Stop Ch 1	Ch 1 Nonzero
3	Orange	Stop Ch 1	Pause/Unpause Ch 1	Ch1 Not(Running)
4	Yellow	Unpause Ch 1	Unused	Ch 1 Zero
5	Green	Start Ch 2	Start/Pause Ch 2	Ch 2 Running
6	Blue	Pause Ch 2	Start/Stop Ch 2	Ch 2 Nonzero
7	Violet	Stop Ch 2	Pause/Unpause Ch 2	Ch 2 Not(Running)
8	Gray	Unpause Ch 2	Unused	Ch 2 Zero
9	White	Start Ch 3	Start/Pause Ch 3	Ch 3 Running
10	Black	Pause Ch 3	Start/Stop Ch 3	Ch 3 Nonzero
11	White/Brown	Stop Ch 3	Pause/Unpause Ch 3	Ch 3 Not(Running)
12	White/Red	Unpause Ch 3	Unused	Ch 3 Zero
13	White/Orange	Start Ch 4	Start/Pause Ch 4	Ch 4 Running
14	White/Yellow	Pause Ch 4	Start/Stop Ch 4	Ch 4 Nonzero
15	White/Green	Stop Ch 4	Pause/Unpause Ch 4	Ch 4 Not(Running)
16	White/Blue	Unpause Ch 4	Unused	Ch 4 Zero
17	White/Violet	Ground	Ground	Ground
18	White/Gray	Ground	Ground	Ground
19	White/Black	Ground	Ground	Ground
20	NC	NC	NC	NC

Appendix A
Optimizing Camera Positioning for the Experiment

A.1 Overview

This appendix provides details of camera setup and usage. It is intended to assist the scientist in preparing the cameras 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 camera(s) securely and ensure that each camera can view the experimental arena fully.
- Ensure that the experimental arena is well lit (and uniformly lit).
- Consider displaying the timestamp and frame number on each video frame, as described in Section 5.7, “Configuring the Source Parameters” on page 77.
- Become familiar with the many parameters that are configurable in the CineLyzer 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 cameras with calibration in mind. Calibration of linear dimensions (in inches or centimeters) works most accurately when the camera is orthogonal to the arena.
Each of the arenas (that is, the arenas for Cameras 1, 2, 3 and/or 4) can be calibrated independently.

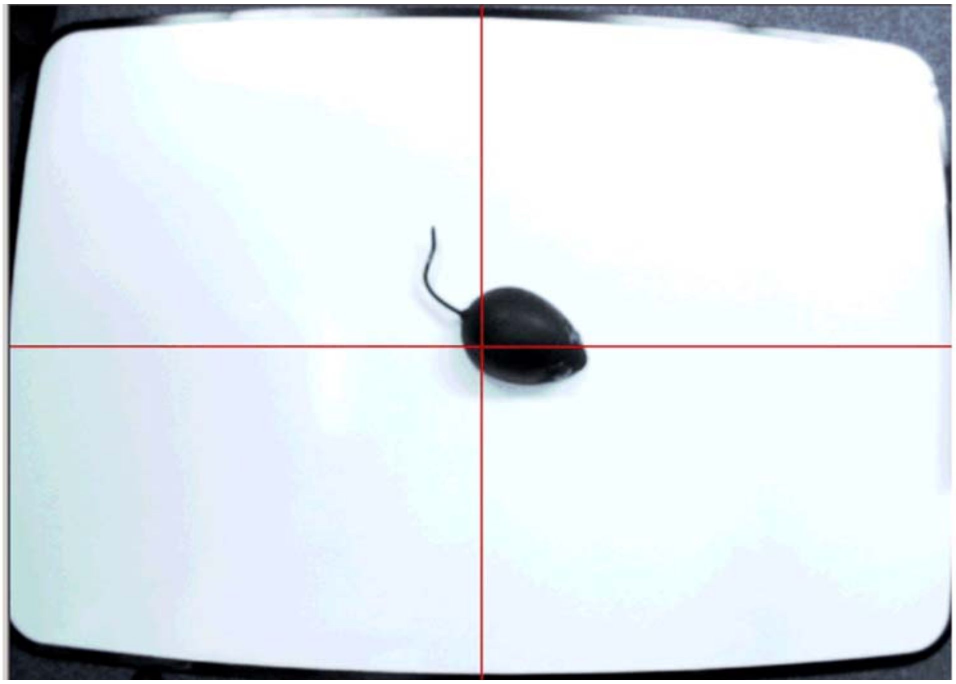
The calibration procedure is explained in Chapter 4, Calibrating the Arena Dimensions.

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 cameras supplied by Plexon with the standard CineLyzer® System generate screen images that are 752 x 480 pixels or 376 x 240 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

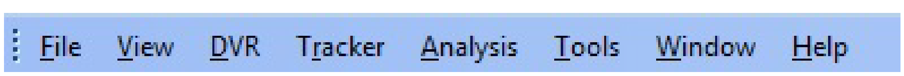
Reset to Default Layout

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**.

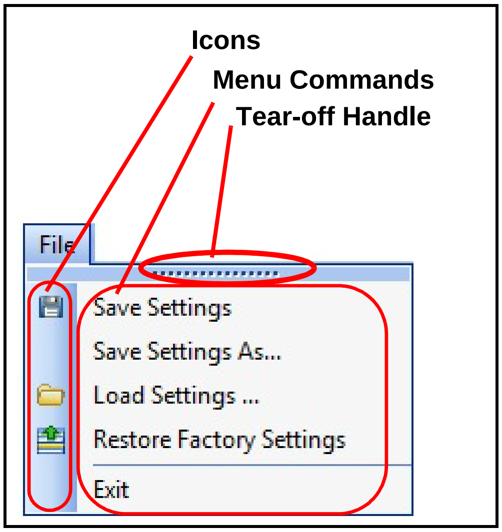
B.2 Screen Elements

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.

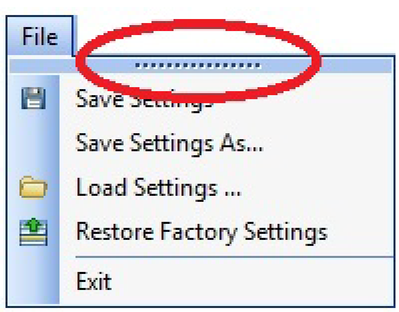


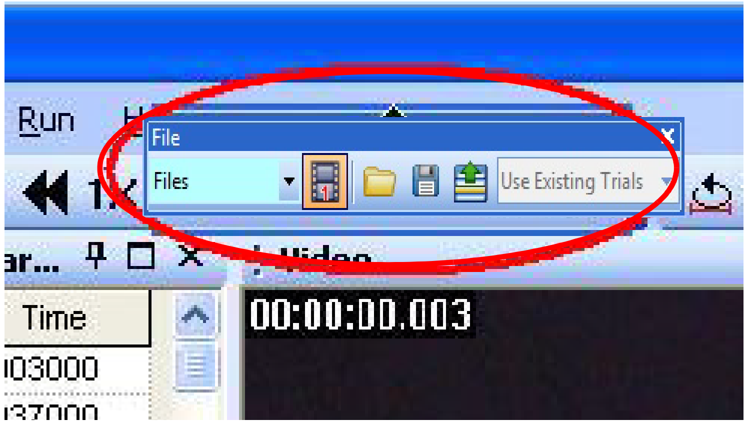
Each menu of the Plexon User Interface contains menu commands and may also contain Icons, Hot Keys and a Tear-off Menu Handle as shown by the illustration below.

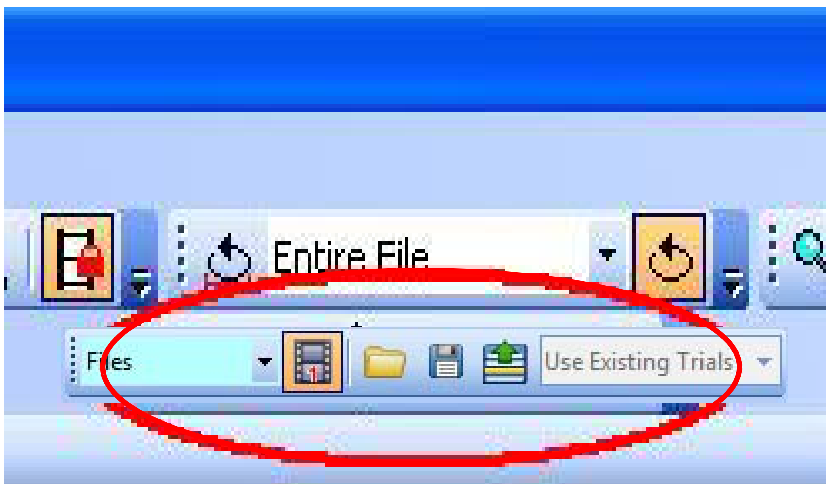


While all Plexon applications are shipped with their available menu commands organized into a set of menus, the contents of the menus can be customized and new menus can be created.

- **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.

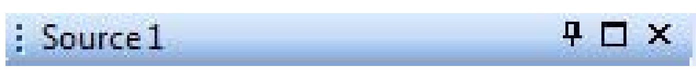




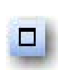


B.2.2 Windows

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.



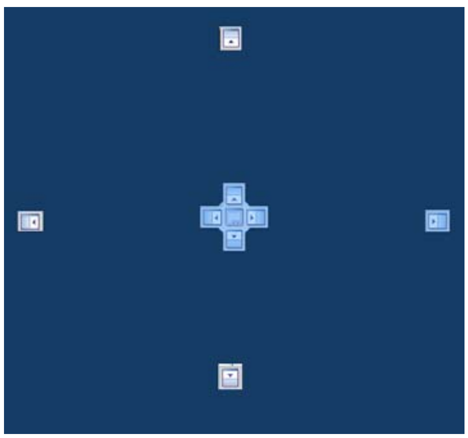
- **Auto Hide Button**  - The **Auto Hide** button "pins" a window to the screen to keep it visible or "rolls up" a visible window into a tab. When the window is pinned, the **Auto Hide** button points in a vertical direction. If the window is rolled up, the **Auto Hide** button points in a horizontal 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.

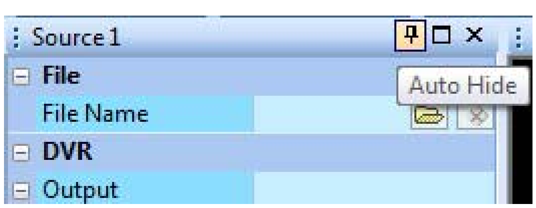
- **Close Button**  - The **Close** button closes the window.

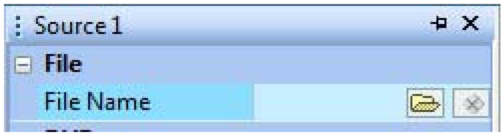
Plexon software applications often display several windows simultaneously. These windows may be resized by using standard resizing methods and may also be repositioned by dragging and dropping and by using **Docking Stickers**. The image below shows the repositioning of a window (denoted by the blue transparent rectangle) and various **Docking Stickers**. These **Docking Stickers** allow you to dock the window being moved in one of several ways described below.



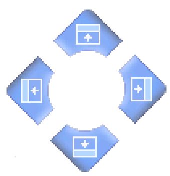
You can position a window by floating it, docking it at the desired docking sticker at a window edge or in a tab.

- **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.



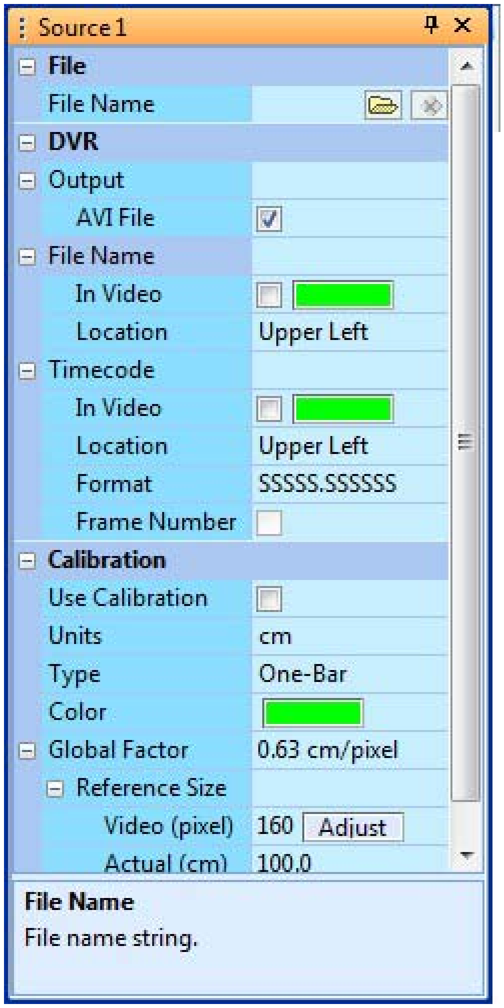


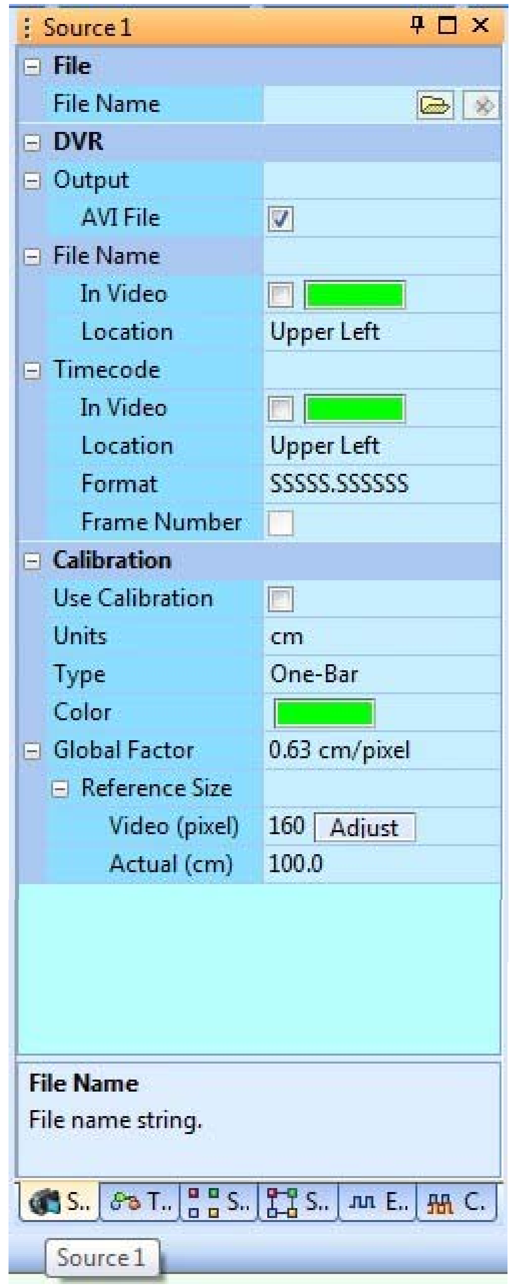
- **Docking a Window at the Application Frame** - When you begin to drag a window, a transparent blue rectangle appears to indicate the position of the window and the four **Docking Stickers** appear individually at each edge of the application frame to allow you to dock the window to the respective 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**.



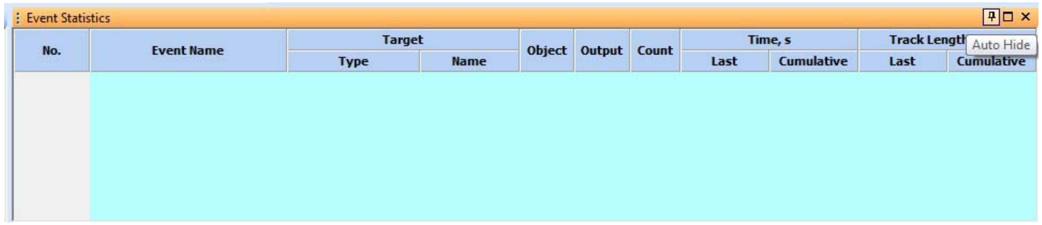
- **Docking a Window at a Window Edge** - When you move the window inside another window, the window **Docking Stickers** appear inside the window grouped together near the center of the window. Releasing the mouse button while it is over one of these stickers (except the center one) will dock the moving 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.

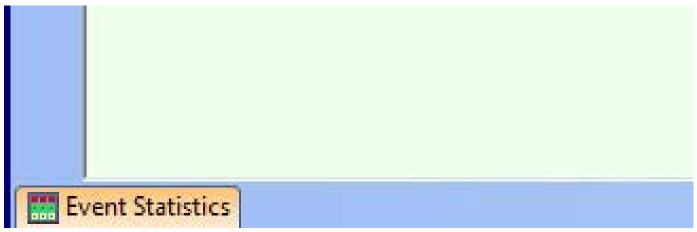




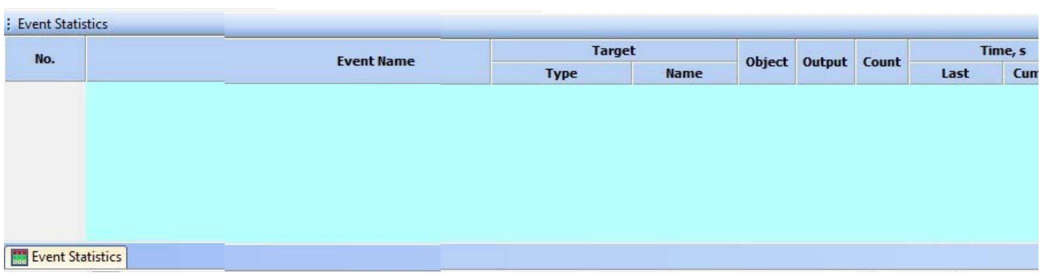
— **Hidden Windows** - Docked windows whose access is not needed often can be hidden or “rolled up” by pressing the **Auto Hide** button. When hidden, the window is represented by a sidebar tab. Sidebar tabs may be located at the left, right, or bottom of the screen and indicate hidden windows. To show 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:



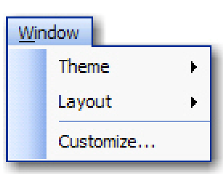
If you click the **Auto Hide** button again, the window will remain open after you move the mouse away from the sidebar.

B.3 Standard Menu Items and Dialogs

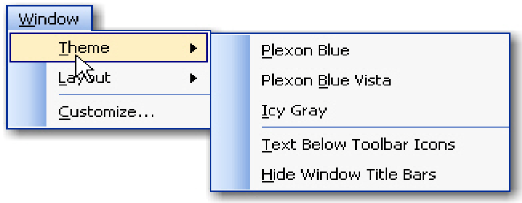
In Plexon software applications, some menu items may have the same functionality across several applications. These items are standard menu items and consist of the **Window** menu, the **Run** menu, and the **Help** menu.

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.

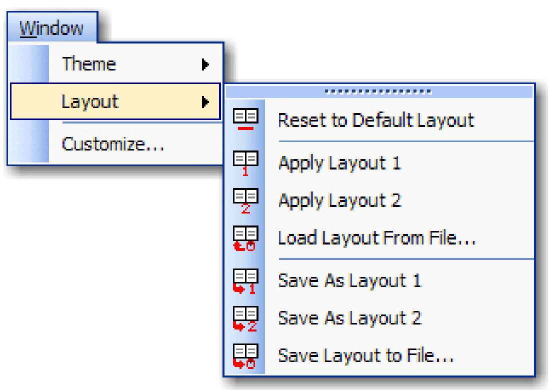


  — **Theme Group** - The top group of items are theme toggle items that may be selected to apply to the user interface look-and-feel. Only one of the themes may be selected at a time.

  — **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.

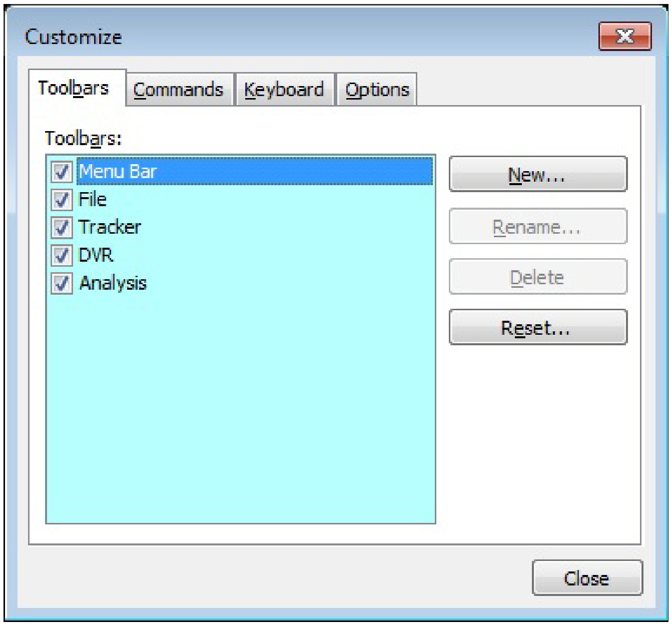


  — **Reset to Default Layout** - Clicking on this item resets the layout to the factory default.

  — **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.

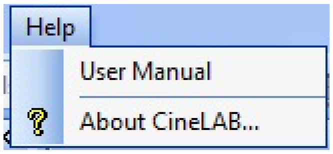
- **Customize** - Clicking **Customize** displays the **Customize** dialog box as shown below



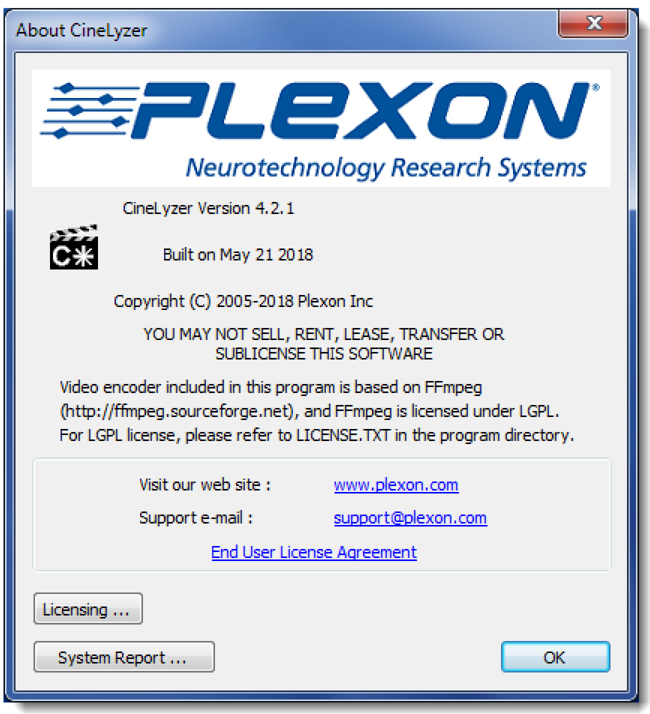
For details on using the **Customize** dialog box, see "Customization" on page B-15.

B.3.2 Help Menu

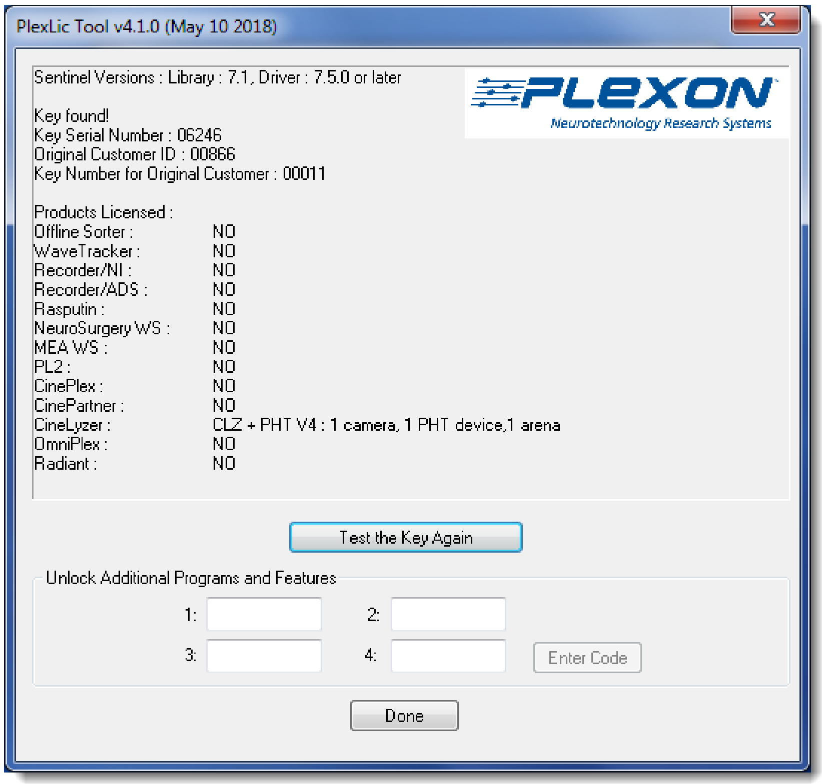
The **Help** menu contains four items: **Help**, **Quick Reference**, **Web Update**, and **About XXXXX** where **XXXXX** is the name of the application.



- **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.



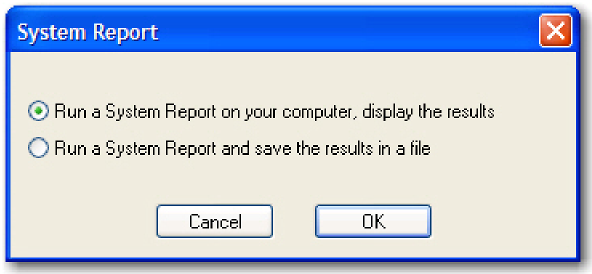
– The information area includes information on license keys and a list of all the Plexon products and their licensed status on this computer.

– 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.

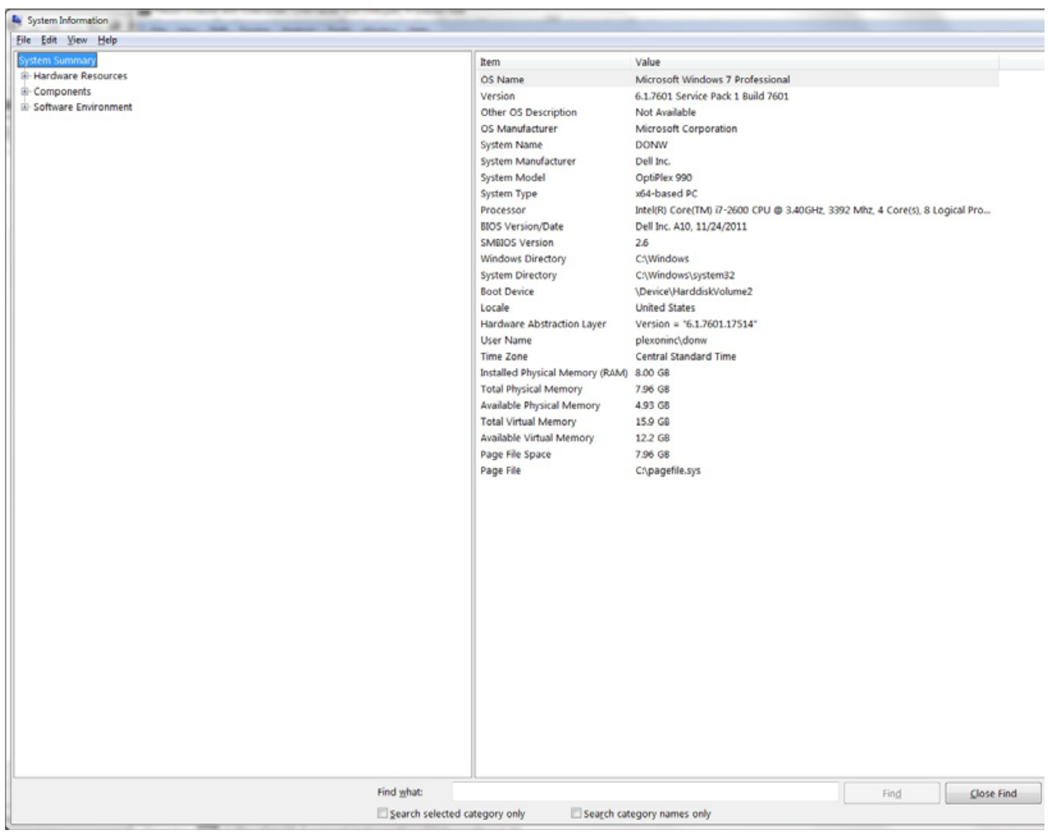
– If you have more than one key installed, the **Next Key>>** and **<<Prev Key** buttons appear. You may use these buttons to cycle through and test all keys.

– 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.



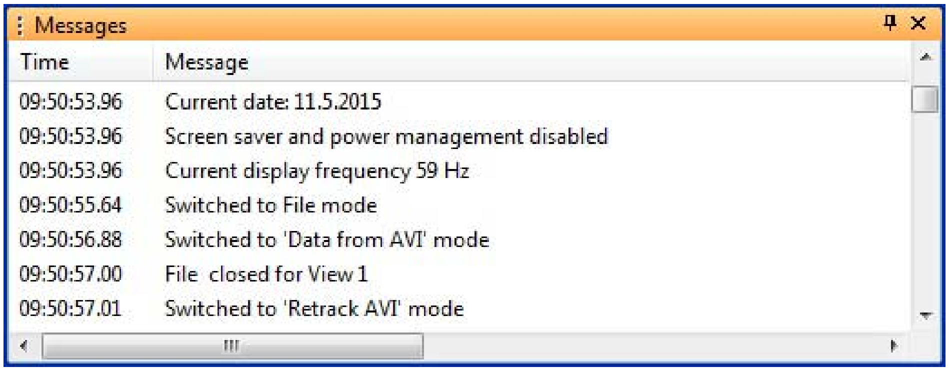
– After selecting a choice, clicking **OK** will launch the standard Microsoft® System Information tool. The image below shows the System Information report displayed on the monitor.



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.

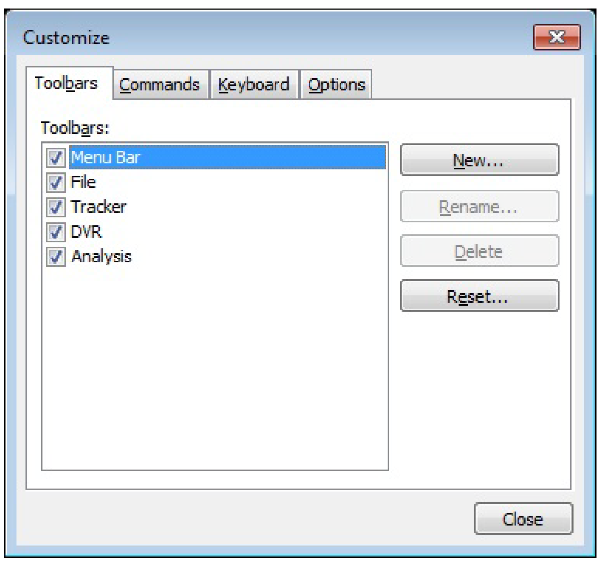
B.4 Customization

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:

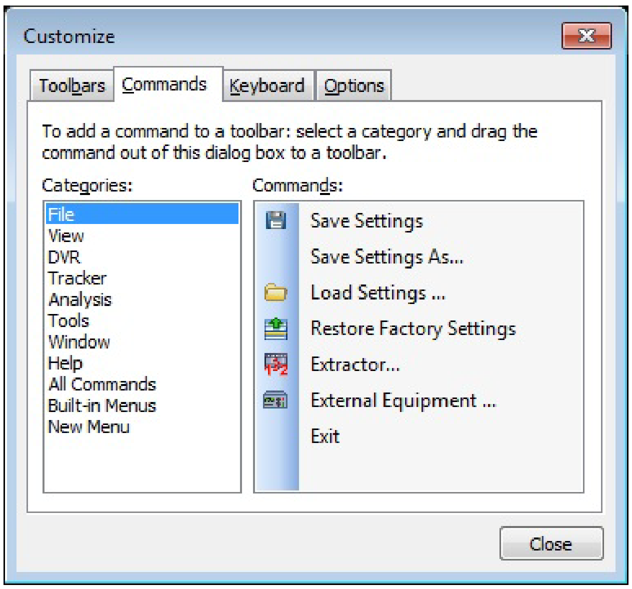


- **Toolbars** - This box contains a list of the toolbars for the application. Click a toolbar checkbox to have it appear in the main application window.
- **New** - This button opens the **New Toolbar** dialog box. You can use this feature to create a custom toolbar for commands frequently used. Enter 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

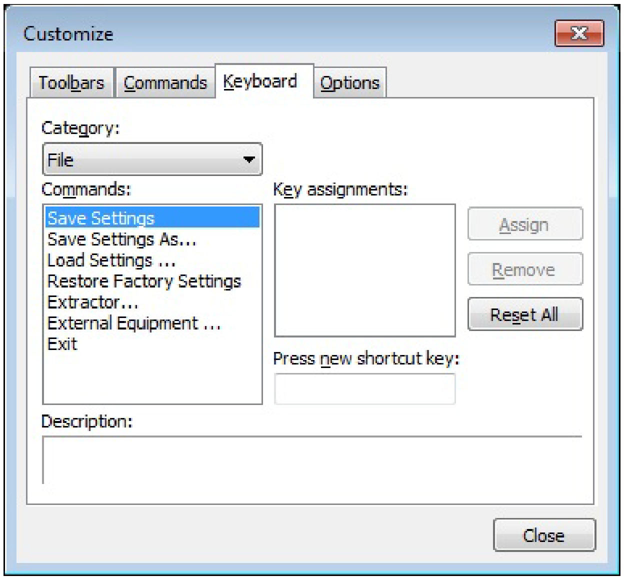
The **Commands** tab is used to customize which commands are available in toolbars. An image of the **Commands** tab follows:



- **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:



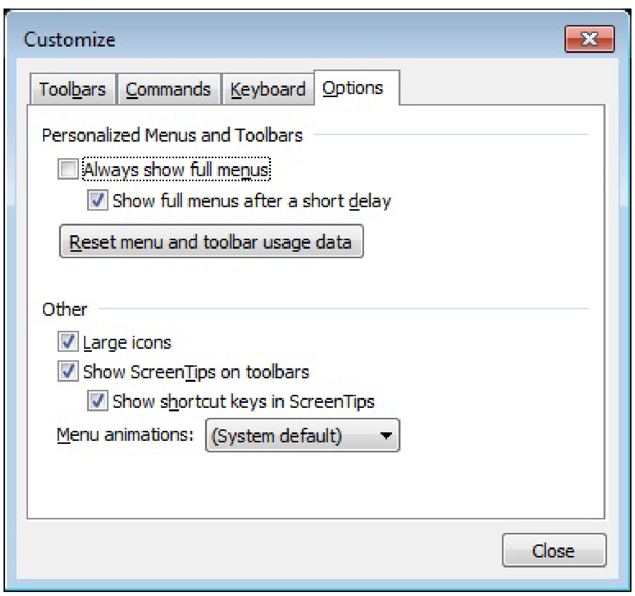
- **Category** - This is a list of all the main menu headings. Select a menu heading to category to see the associated commands in the **Commands** area.
- **Commands** - This is a list of all the commands associated with the selected main menu heading in the **Category** area.
- **Key assignments** - 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
USB Digital Input/Output Interface

C.1 CineLyzer USB Digital Input/Output Interface—Description

The Plexon® CineLyzer® USB Digital Input/Output Interface (DIO Interface) is included in each CineLyzer System. It provides lines for 12 different input events (six high true and six low true inputs) and 12 different output events (six high true and six low true outputs). The allowed voltage range is 0V to 5V.

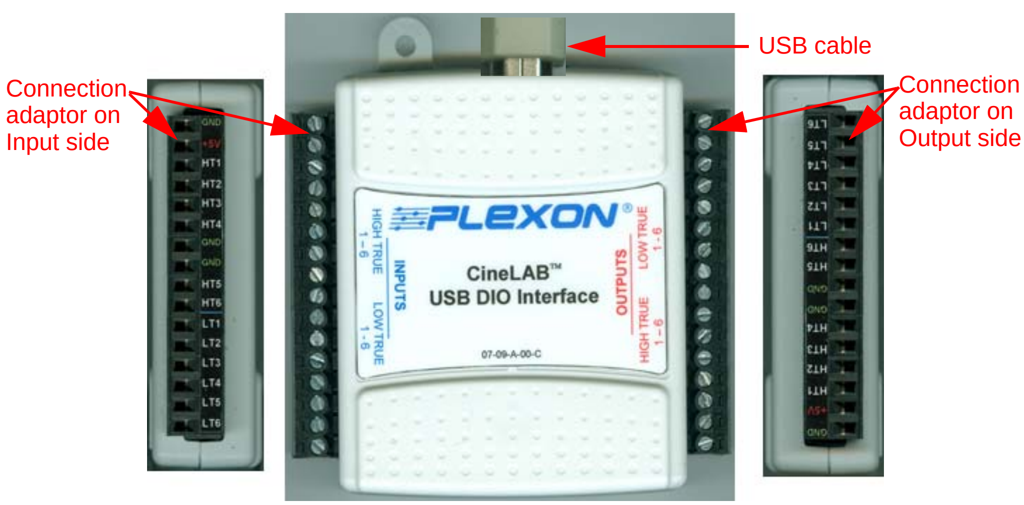
CAUTION

Limit voltage to 0–5V at the DIO Interface pins

Do not apply more than 5V to any of the input/output pins on the DIO Interface. Doing so could damage the device.

DIO Interface and Connection Adaptors

DIO Interface with USB Cable and Connection Adaptors Attached (top view and side views)



C.2 Input and Output Logic

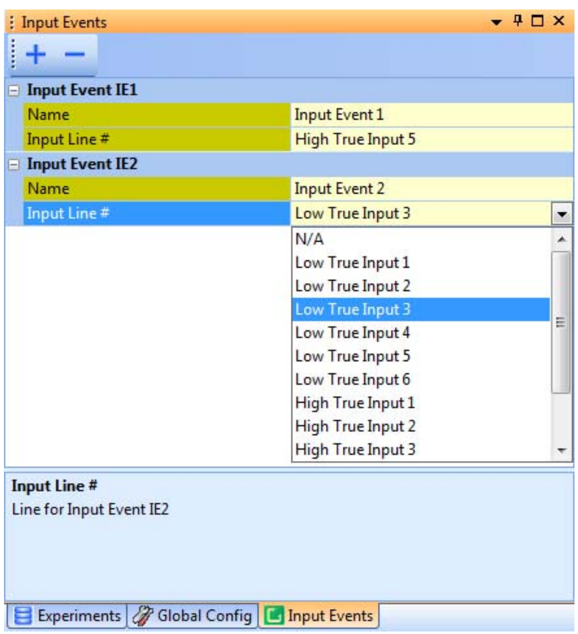
DIO Interface Input Logic

Input signal voltages can vary from 0V to 5V. The DIO Interface 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 specified camera frame rate (the setting for the **Frame Rate, fps** parameter in the Global Config tab).

To assign a specific input line to an event, use the **Input Events** tab.

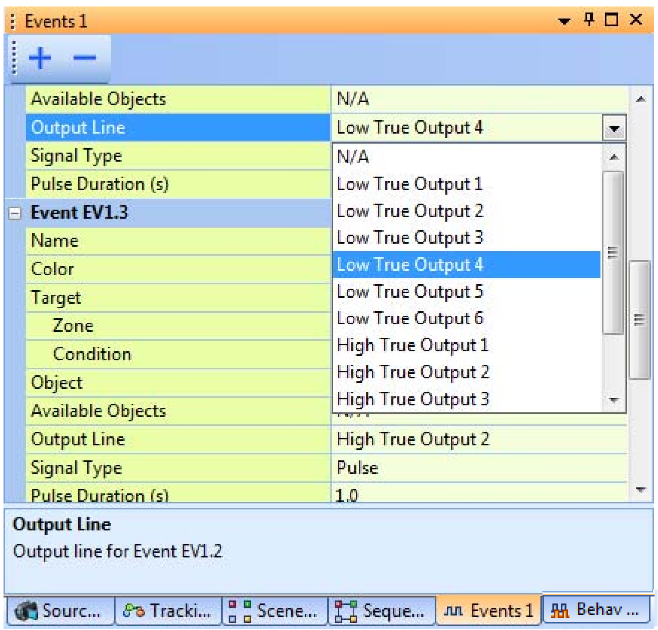


DIO Interface Output Logic

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, 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, 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 5.0V (not asserted) and 0V (asserted).

To assign a specific output line to an event, use the **Events** tab (shown below).



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

C.3 Hardware

The DIO Interface is shipped with two Input/Output (IO) Status Indicators, an Input Generator, two Connection Adaptors and a USB cable. These parts are shown in the images below.

CAUTION

Push the adaptors and connectors straight in

To avoid bending or damaging any pins, always use a straight push to attach or detach DIO Interface adaptors and connectors.

C.3.1 LED Indicator Lights

In the images that follow, some of the LED lights are on. These lights are interpreted as follows:

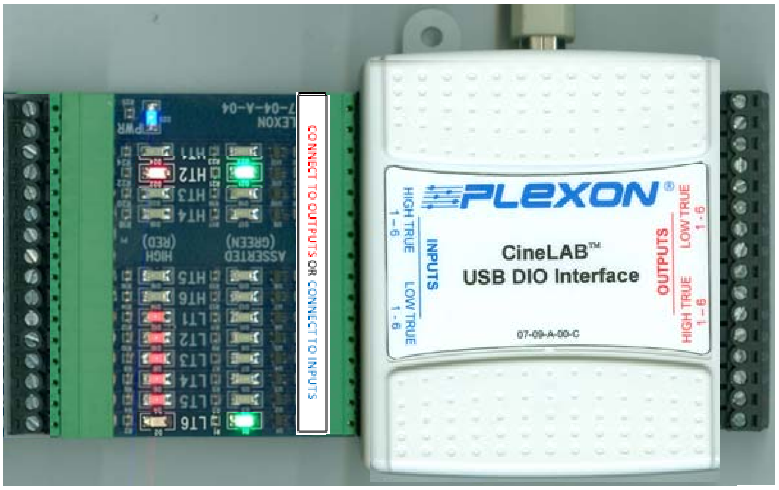
- The blue power (PWR) lights on the Input Generator and IO Status Indicator are on whenever they are plugged into the USB Interface unit (individually or in series), the USB Interface unit is connected through the USB cable to a USB port on the PC, and the PC is turned on.
- The green lights on the Input Generator turn green when the corresponding buttons are pushed.
- The LOW TRUE lights on the IO Status Indicator are red when the IO Status Indicator is plugged into the DIO Interface unit. When an input or output is present on a circuit, the red light turns off and the green light (ASSERTED) turns green.
- The HIGH TRUE lights on the IO Status Indicator remain off when the IO Status Indicator is plugged into the DIO Interface unit. When an input or output is present on a circuit, the red light and the green light (ASSERTED) both go on.

C.3.2 Configurations

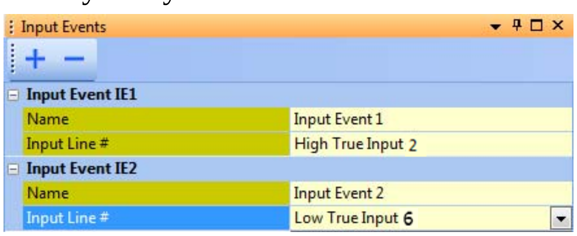
The IO Status Indicator board(s) can be attached at either the Input or Output side of the DIO Interface, or on both sides. The Input Generator should be attached only to the Input side of the DIO Interface.

DIO Interface (right) and IO Status Indicator (left)

The LED lights on the IO Status Indicator give you a visual indication of the signals that are asserted. In the image below, for example, input signals are asserted on Channel HT2 and Channel LT6.

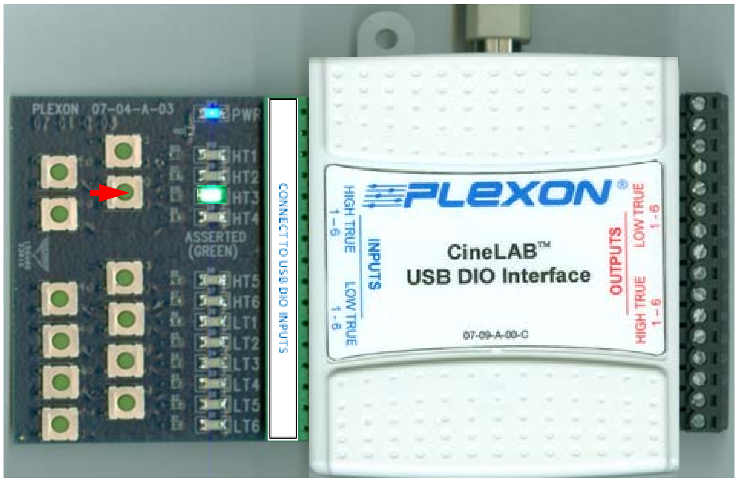


If you have configured Events for these two channels (as shown below), your CineLyzer System should show that these Events have been detected.

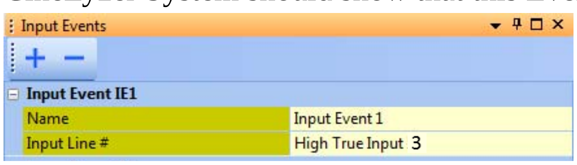


DIO Interface (right) with Input Generator (left) attached to Input side

The configuration shown below is useful when you want to verify that your hardware and software are properly detecting inputs and your software is configured correctly. For example, in the image below, the button for Channel HT3 is being pushed, so Channel HT3 is asserted (green) on the Input Generator.



If you have configured an Input Event for this channel (as shown below), your CineLyzer System should show that this Event has been detected.

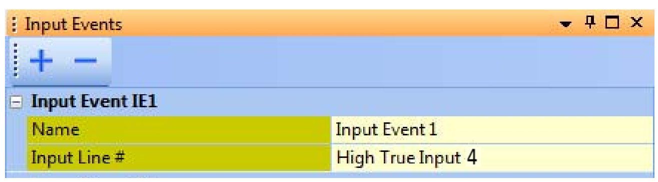


DIO Interface with IO Status Indicators on Input and Output Sides

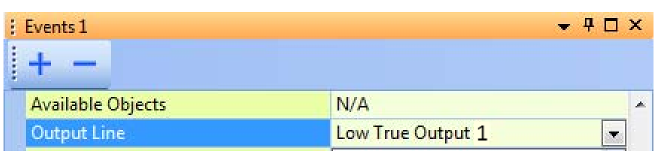
In the configuration shown below, IO Status Indicators are attached to both the Input and Output sides of the DIO Interface. The IO Status Indicators are not required for operation of the DIO Interface; however, they are extremely useful while setting up an experiment, and can be left in place if desired.

In this example, there is an input signal asserted on Channel HT4 and an output signal asserted on Channel LT1.

If you have configured an Input Event for Channel HT4 (as shown below), your CineLyzer System should show that this Event has been detected.



The LT1 LED on the Output side is lit, which shows that you have configured an Output Event for Channel LT1 (as shown below), and the event has actually occurred.



C.4 Voltage Limit

The DIO interface pins are rated at 5V maximum. The allowed voltage range is 0–5V. If you will be connecting external devices to the DIO Interface, ensure that appropriate voltage adaptors are in the circuits. For example, if your external device inputs and outputs are up to 28V, use adaptors that reduce the voltage to 5V or lower before the attachment to the DIO Interface. In many cases, adaptors are available from the suppliers of the original equipment.

CAUTION

Limit voltage to 0–5V at the DIO Interface pins

Do not apply more than 5V to any of the input/output pins on the DIO Interface. Doing so could damage the device.

C.5 Connecting CineLyzer System to Optogenetic Stimulation Device

The CineLyzer System can be connected through the DIO Interface to the Plexon PlexBright® 4 Channel Controller (PlexBright Controller) to provide coordination between an animal's behavior and optogenetic stimulation. For details of the connections and procedure, see Chapter 11, Coordinating Behavior and Optical Stimulation.

Note: The PlexBright Controller and the associated Radiant™ software and cables are sold separately.

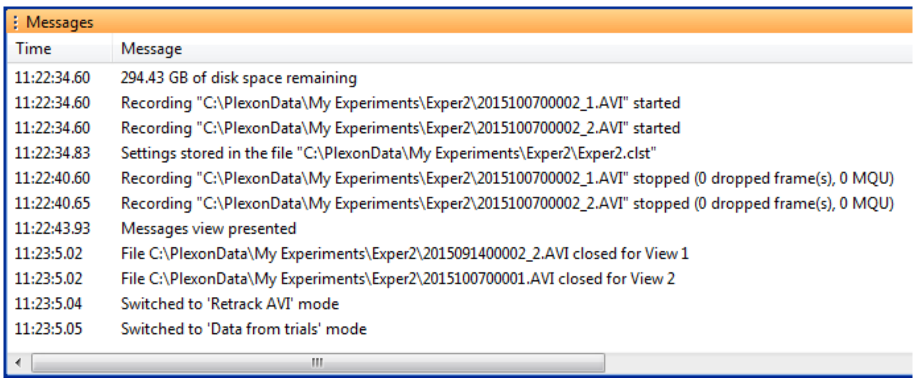
Appendix D
CineLyzer System Messages

D.1 Types of Messages

This appendix lists the system messages that the CineLyzer® System software displays in reaction to current conditions and certain user actions. Messages are displayed in the Messages window of the CineLyzer System user interface.

Informational messages are called *simple messages*, and the more urgent messages are called *critical messages*.

To display the Messages window, from the main menu select **View** > **Messages**. The window displays information similar to the example below. In this example, the system checks for remaining disk space and creates new sessions (new recordings) for Video1 and Video2.



The Messages window can be closed if not needed.

D.2 How to Use Messages

Messages provide a convenient flow of information to the user, and alert you to problems occurring within the CineLyzer System. Therefore, messages can be useful for monitoring and troubleshooting the system.

D.3 Simple Messages

The table below lists the simple messages. In most cases, it is not necessary for you to take action when a simple message is displayed.

Note: In this table, [...] and <N> refer to text and numeric strings, respectively. These strings can differ for each instance of the message.

Table 1: CineLyzer System Simple Messages

Message #	Message Text
C100	CineLyzer version [...] Starting...
C101	Current date:
C102	Date changed. Current date: [...]

Table 1: CineLyzer System Simple Messages (Continued)

Message #	Message Text
C103	Current display frequency
C104	Display frequency successfully changed to <N> Hz
C105	External equipment configured as [...]
C106	External equipment now configured as [...]
C107	Factory default settings loaded
C108	File opened for View
C109	File closed for View
C110	Cameras reset
C111	Frame resolution changed
C112	Loaded settings were created for a different resolution. Arena, zones and calibration (if present) are properly resized.
C113	Startup settings loaded from [...]
C114	Settings loaded from [...]
C115	Background image loaded from [...]
C116	Settings stored in the file [...]
C117	Background image stored in the file [...]
C118	Play file started
C119	Event Combinations view presented
C120	Video view presented
C121	Recorder Properties view presented
C122	Area Properties view presented
C123	Messages view presented
C124	Previous video configuration is not applicable due to hardware changes or absence of Rasputin or OmniPlex key
C125	Video configuration has been automatically reset to One File
C126	The layout loaded contains panels incompatible with the current license. These panels are removed.
C127	Remote start received and discarded
C128	Remote stop received and discarded
C129	Remote pause received and discarded

Table 1: CineLyzer System Simple Messages (Continued)

Message #	Message Text
C130	Video configuration has been automatically set to connect all available cameras according to the license
C132	Video configuration has been automatically set to One File
C133	Video configuration has been automatically reset to Camera 1
C135	Armed for remote control - DVR control by Plexon NDAQ is now enabled
C136	Remote control disabled - DVR control by Plexon NDAQ is now disabled
C137	Recording [...] started by Plexon NDAQ
C138	Recording [...] paused by Plexon NDAQ
C139	Recording [...] resumed by Plexon NDAQ
C140	Recording [...] stopped by Plexon NDAQ (<N> dropped frame(s), <N> MQU) **Note:** MQU = maximum queued units. For a description of MQU, see “Monitoring the Video During Recording” on page 9-253.
C141	Recording [...] started locally
C142	Recording [...] stopped locally (<N> dropped frame(s), <N> MQU)
C143	Recording [...] paused locally
C144	Recording [...] resumed locally
C145	Tracking switched to Object Contour mode
C146	Object Contour tracking stopped
C147	No LEDs are active. The objects for dynamic zones will be switched to N/A.
C148	Tracking switched to LED mode
C149	LED tracking stopped
C150	Can not switch to LED mode, since tracking license is not detected
C151	Tracking switched to Reflective Colors mode
C152	Reflective Colors tracking stopped
C153	Can not switch to Reflective Colors mode, since tracking license is not detected
C154	Recording [...] stopped due to missing license key
C155	Tracking stopped due to missing license
C156	No markers are active. The objects used in dynamic zones will be switched to N/A.
C157	Recording folder changed to [...]
C158	Recording [...] stopped since file size on FAT32 drive cannot exceed 4GB (<N> dropped frame(s), <N> MQU)

Table 1: CineLyzer System Simple Messages (Continued)

Message #	Message Text
C159	Recording [...] stopped due to low disk space (<N> dropped frame(s), <N> MQU)
C160	End of File. Can not start recording
C161	End of File. Can not start playing
C162	Camera framerate changed to <N>
C163	Wizard opened
C164	Wizard finished
C165	CineLyzer window size reduced by Shrink button
C166	CineLyzer window size restored by Expand button
C167	Date has changed since the last file was recorded. The file number reset to 001
C168	Can not add sequence due to missing license key
C169	Tracking settings ignored since missing license
C171	Tracker Properties view presented
C172	New shape added to Arena
C173	New shape added to Zone
C174	Shape #<N> deleted from Arena
C175	Shape #<N> deleted from Zone
C176	Tracking Events view presented
C177	Event Statistics view presented
C178	Output lines for the loaded tracking events ignored since no digital output device found
C179	All shapes deleted from Arena
C180	All shapes deleted from Zone
C181	Temporary AVI file [...] renamed to [...]
C182	Renaming temporary AVI file [...] failed
C183	Temporary DVT file [...] renamed to [...]
C184	Renaming temporary DVT file [...] failed
C185	Standalone recording [...] started
C186	Standalone recording [...] stopped (<N> dropped frame(s), <N> MQU)
C187	Recording time zero [...] received from OmniPlex Server
C188	File N activated

Table 1: CineLyzer System Simple Messages (Continued)

Message #	Message Text
C189	File N closed
C190	Zone Sequences view presented
C191	Standalone recording [...] paused
C192	Standalone recording [...] resumed
C193	Video Properties view presented
C194	Previous video configuration was [...]
C195	Switched to Camera mode
C196	Camera N started
C197	Camera N stopped
C198	Switched to File mode
C199	Camera settings for camera <N> have been applied to all available cameras
C207	Video Edit view presented
C212	Initializing cameras at start
C216	Extended Color Markers tracking stopped
C217	Tracking switched to Extended Color Markers mode
C222	No calibration data present
C223	Tracking settings ignored since they are incompatible with the current license
C224	Waiting to start recording
C225	Zone copied
C226	Experiments view presented
C227	Sessions view presented
C228	Single track view presented
C229	Group track view presented
C230	Combination Events properties for source [#] presented
C231	Input events view presented
C232	Source settings for Source <N> have been applied to all available sources
C233	Tracking settings for Source <N> have been applied to all available sources
C234	Area settings for Source <N> have been applied to all available sources
C235	Sequences settings for Source <N> have been applied to all available sources

Table 1: CineLyzer System Simple Messages (Continued)

Message #	Message Text
C236	Tracking events settings for Source <N> have been applied to all available sources
C237	Combination events settings for Source <N> have been applied to all available sources
C238	Combination events settings for Source <N> have been applied to all available sources
C239	Switched to 'Data from sessions' mode
C240	Switched to 'Add tracking data' mode

D.4 Critical Messages

The table below lists the CineLyzer System critical messages. In most cases, it is important for you to take action (or revise your previous action) when a critical message is displayed.

Note: In this table, [...] and <N> refer to text and numeric strings, respectively. These strings can differ for each instance of the message.

Table 2: CineLyzer System Critical Messages

Message #	Message Text
C800	Left and right point of head direction vector should be different
C801	Neck and nose point of head direction vector should be different
C802	Cannot load this video file, since its frame parameters don't match frame size for the selected experiment
C803	Cannot load this video file, since its frame rate doesn't match frame rate for the selected experiment.
C804	The number of event combinations can not exceed 100.
C805	Can not add event combination due to missing license key.
C806	Can't associate selected Marker with event since this marker is associated with dynamic zone used for generation of this event. Select another Marker.
C807	The number of input events can not exceed 100.
C808	Cannot add input event due to missing license key.
C904	Current CineLyzer does not support this version of settings file. Try to read another file.
C906	Apparently Adobe Acrobat Reader is not installed on this system This is required to display the help file. Please go to http:/www.adobe.com, download the lastest version, and install it.
C907	Cannot find the manual at: [...]
C908	Error code <N> has occurred while trying to open [...].
C999	Polygon can not have less than 3 points.
C910	Can not create self-intersecting polygons.
C911	Recording folder doesn’t exist. Please choose another one.
C912	Saved layout does not exist
C913	Unable to load the requested layout! The default layout applied
C914	Can not be armed for remote recording until Wizard is exited.
C915	Can not start recording until Wizard is exited.
C916	Can not add new zone: number of zones of interest can not exceed 100.

Table 2: CineLyzer System Critical Messages (Continued)

Message #	Message Text
C917	Can not add new zone: the boundary of the last existing zone is not defined.
C918	The number of shapes in arena can not exceed 100.
C919	The number of shapes in zone can not exceed 100.
C920	Overlapped zones are not allowed in sequence
C921	Can't delete selected zone since it is used for generation of tracking event(s). Modify the event(s) first.
C922	Can't delete selected zone since it is used in sequence(s). Modify sequence(s) first.
C923	The number of events can not exceed 100.
C924	Can't delete the last remaining shape from the selected zone since it is used for generation of tracking event(s). Modify the event(s) first.
C925	Can not be armed for remote recording due to missing license key
C926	Can not add event since LED or Marker tracking mode is not selected.
C927	Can not add event since no LEDs selected.
C928	Can not add event since no markers selected.
C929	Can't delete selected sequence since it is used for generation of tracking event(s). Modify the event(s) first
C930	Unable to save current layout
C931	Can not add event combination since tracking mode is not selected.
C932	Can not add event combination since no single events are defined.
C933	Can not add new event combination since not all events for the last combination are defined.
C935	Can't uncheck selected LED since it is used for generation of tracking event(s). Modify the event(s) first.
C936	Can't uncheck selected marker since it is used for generation of tracking event(s). Modify the event(s) first.
C937	Can not add event since tracking mode is not selected.
C938	Can not add event since no LEDs selected
C939	Can not add event since no markers selected.
C948	Can not add new sequence since the previously added sequence contains no zones.
C949	Can not add new sequence since the last zone in the previously added sequence is undefined.
C950	The number of sequences can not exceed 100.

Table 2: CineLyzer System Critical Messages (Continued)

Message #	Message Text
C951	Can't delete selected sequence since it is used for generation of tracking event(s). Modify the event(s) first.
C952	Can not add new zone to the current sequence since the last zone in the sequence is undefined.
C953	Sequence can not contain more than 100 zones.
C954	Can not add new zone to the current sequence since no zones is defined.
C955	Files opened have different compression. Record, Play, Pause and Stop will be disabled.
C956	Files opened have different frame rate. Record, Play, Pause and Stop will be disabled.
C957	Can not open the same file twice.
C958	Start and end point of head direction vector should be different.
C959	Start point of head direction vector and end point of reference vector should be different.
C960	Can't associate selected LED with event since this LED is associated with dynamic zone used for generation of this event. Select another LED.
C961	Loading startup settings from [ ] failed because this version of settings file is not supported by Current version of CineLyzer
C962	Current CineLyzer does not support this version of settings file. Try to read another file.
C963	Loading last saved settings file [...] failed because the file is missing. Factory default settings have been applied
C964	Settings loading failed because file [...] is missing. Factory default settings have been applied
C965	No settings file to load. Factory default settings have been applied
C966	Can not start recording due to missing license key
C967	Can not start recording since disk information can not be retrieved
C968	Can not start recording. File number can not exceed 999. Delete or rename video (*.AVI and *.DVT) files in the recording folder and try again
C969	Can not start recording due to low disk space.
C970	Can not add arena due combination to missing license key
C971	Can't associate dynamic zone with selected LED since this LED is used for generation of event associated with this zone. Select another LED.
C972	Can't associate dynamic zone with selected Marker since this Marker is used for generation of event associated with this zone. Select another Marker.

Table 2: CineLyzer System Critical Messages (Continued)

Message #	Message Text
C973	Only Motion JPEG RGB files are accepted. Record, Play, Pause and Stop will be disabled.
C974	Files opened have different frame resolution. Record, Play, Pause and Stop will be disabled.
C975	Only resolution 640x480 or 320x240 is supported. Record, Play, Pause and Stop will be disabled.
C976	CineLyzer only supports MJPEG or Plexon MPEG4 AVI files. Try another file.
C977	Video frame width must be a multiple of 16. Record, Play, Pause and Stop will be disabled.
C978	Video frame height must be a multiple of 8. Record, Play, Pause and Stop will be disabled.
C982	Display resolution is not enough to run all available cameras even in 320x240 resolution. Number of cameras to use is reduced to fit the display resolution.
C983	Connections should be different.
C984	Connection points must be different.
C985	Open socket failed
C986	No network connection found
C987	Cannot connect to selected node.
C988	Cannot apply calibration: Number of cameras in the calibration file less than number of active cameras. Please try different calibration file or deactivate cameras.
C993	Video signal not detected
C995	Cannot write to the file. The file might be opened by other application. Please close the file first

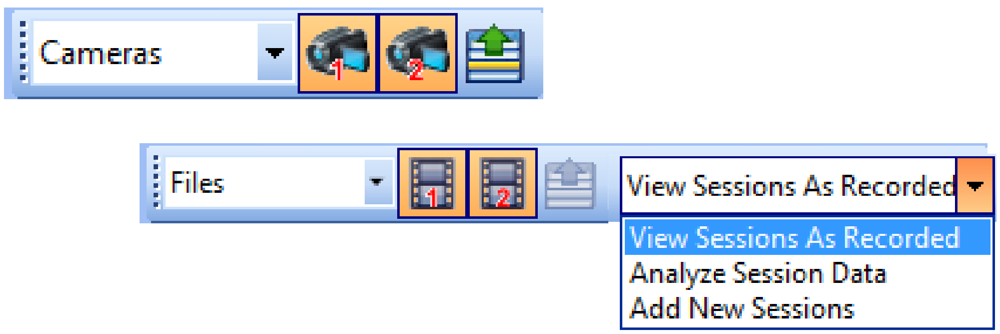
Appendix E
Modifying Arenas and Zones

E.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 recordings made in these modes, except as noted:

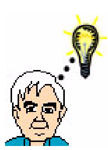
- **Cameras** mode
- **Files/Analyze Session Data** mode
- **Files/Add New Sessions** mode

Note: No changes to configuration data can be made in **Files/View Sessions As Recorded** mode.



E.2 Recalibrating the Arena and Recalculating Tracking Data

You can recalibrate the arena dimensions 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 impact of recalibrating on previously recorded sessions is explained in this section.



TIP

The system preserves tracking data in pixels

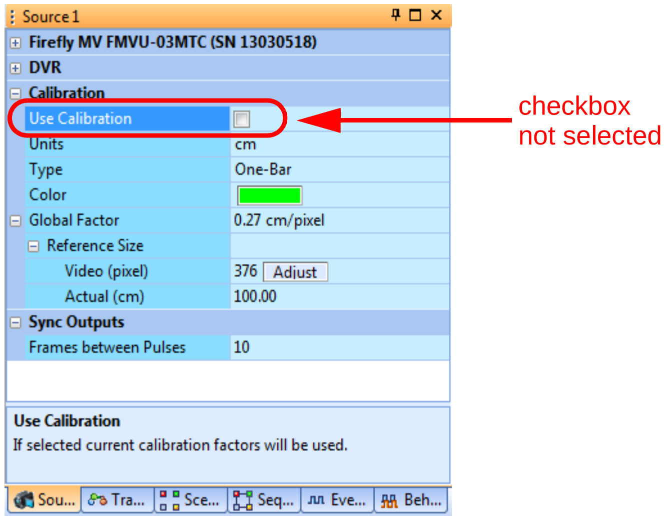
Even if you recalibrate the arena after one or more sessions are recorded, the system preserves the uncalibrated data for all sessions. That is, the track length and speed of the subject, in pixels and pixels per second, are not affected by future calibration actions. You can think of the uncalibrated data (in pixels) as the original raw data, and the calibration factor (cm/pixel or inches/pixel) as an optional convenience for the researcher.

The procedure in this section will explain how to view the original uncalibrated data, even after you have applied a calibration factor.

Here are examples that illustrate the rules the system follows for calibration.

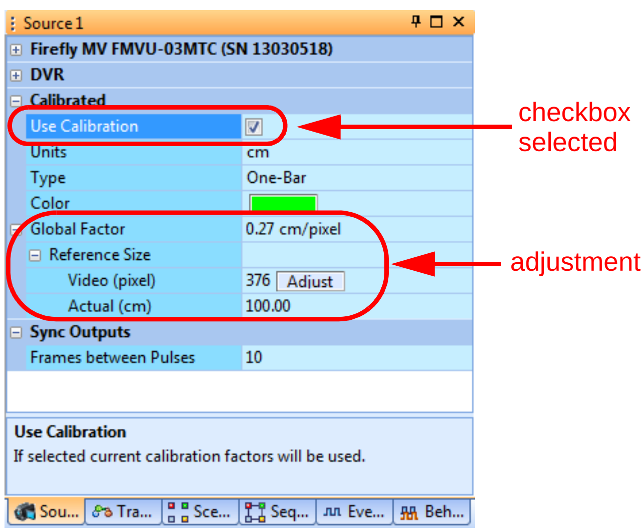
Example A
Uncalibrated sessions followed by calibration and recomputation

1 Record several sessions without any calibration, that is, with the **Use Calibration** checkbox deselected.

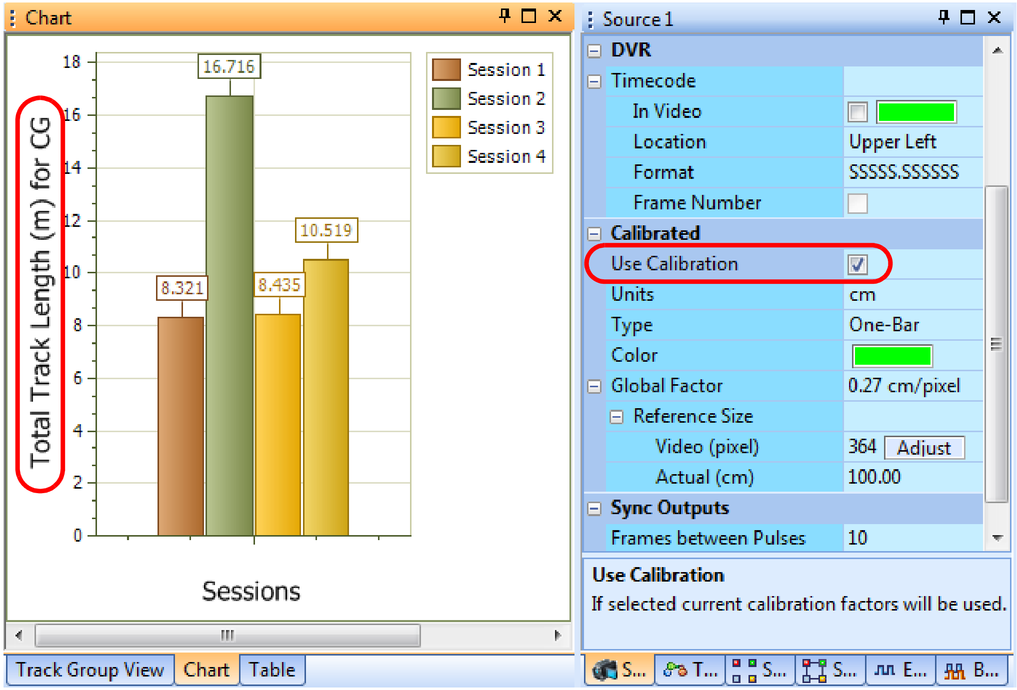


2 Press the **Compute** button. Notice that the track lengths for all the previously recorded sessions are displayed in pixels.

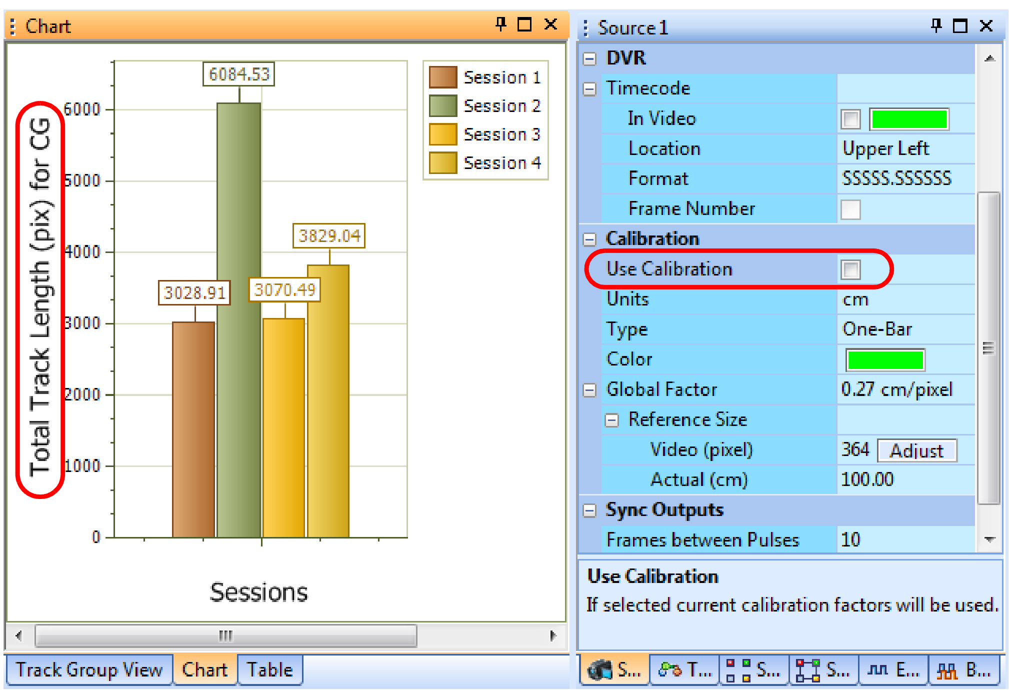
3 Calibrate the arena (adjust the **Global Factor** in the Scenes tab as needed and select the **Use Calibration** checkbox), then press the **Compute** button.



4 Observe the track length data. Notice that the system applies the **Global Factor** to all previous sessions. It will also apply the same factor to all future sessions.



5 If you want to view the original raw (uncalibrated) data again, deselect the **Use Calibration** checkbox and click the **Compute** button. You will see the original track length data displayed in pixels.



Example B
Calibrated sessions followed by removal of calibration and recomputation

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. Deselect the **Use Calibration** checkbox, then press the **Compute** button,
4. Observe the track length data. Notice that the system displays the raw (uncalibrated) data for all sessions. Future sessions will also be uncalibrated.

Example C
Calibrated sessions followed by recalibration and recomputation

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 the track length data. Notice that the system does not apply the new calibration factor (the **Global Factor**) to any previously calibrated sessions. However it will apply the new factor to future sessions.
5. If you want to view the raw (uncalibrated) data, deselect the **Use Calibration** checkbox and click the **Compute** button. You will see the raw track length data displayed in pixels.

Example D
Resetting the calibration

1. Record several sessions some without calibration and some with calibration.
2. Deselect the **Use Calibration** checkbox.
3. Adjust the **Global Factor** in the Scenes tab.
4. Select the **Use Calibration** checkbox again.

   Note: Step 2 through Step 4 have the effect of resetting the calibration factor that will be applied to all previous and future sessions.

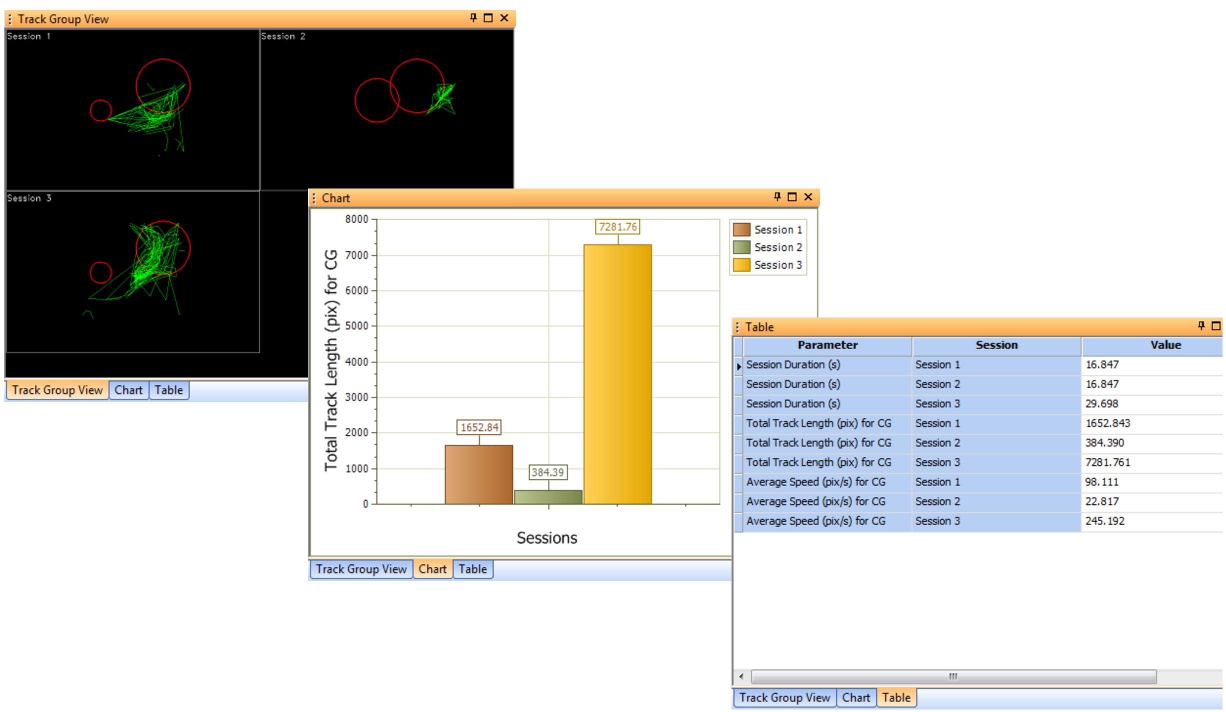
5. Press the **Compute** button.
6. Observe the track length data. Notice that the system applies the new calibration factor to all previously recorded sessions, even to sessions that were previously calibrated with a different factor. it will also apply the new factor to future sessions.
7. If you want to view the original raw (uncalibrated) data again, deselect the **Use Calibration** checkbox and click the **Compute** button. You will see the original track length data displayed in pixels. This original raw (uncalibrated) data is preserved by the system.

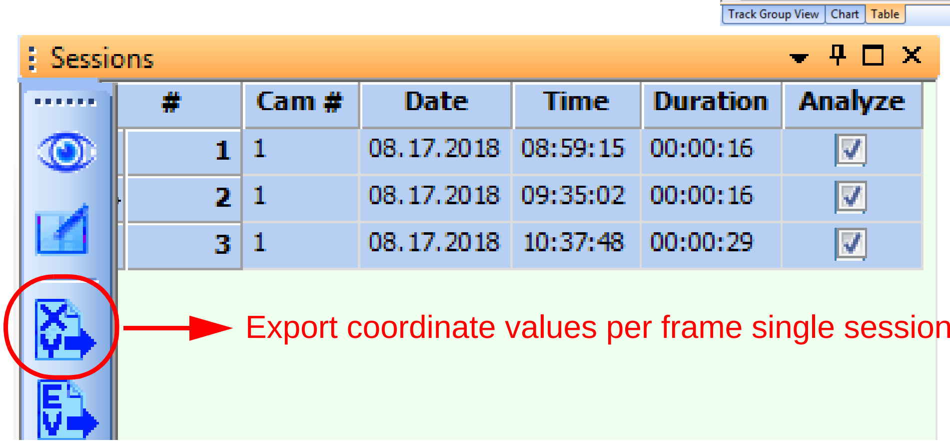
E.3 Modifying Arena Shapes in Cameras Mode and Files/Add New Sessions Mode

In **Cameras** mode and **Files/Add New Sessions** mode, when you move an arena shape or resize an arena shape, the changes apply only to future sessions, not to any of the previously recorded sessions.

E.4 Modifying Arena Shapes in Files/Analyze Session Data Mode

In **Files/Analyze Session Data** mode, if you modify the shape or position of an arena during an experiment (that is, after session(s) have already been recorded), it will typically affect the data that the system includes or excludes from the data reports for that session. The data reports include tracking data that appear in the **Track Group View / Chart / Table** window, and tracking data that you export. These views are shown below.





The arena modification will *not* alter the arena positions or dimensions for the other previously-recorded sessions. This feature allows you to alter the arena positions and shapes for one session to minimize tracking errors due to any shifting of the camera positions, or to focus more precisely on certain regions of the test apparatus or the subject's behavior in a certain location. You can also recalibrate the modified arena if necessary.

Note: If you have a list of arena settings from a previous experiment, you can apply that complete list of settings to your current experiment. See Section 10.14, "Using the Overlay Feature during Analysis" on page 305.

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 are recorded when you switch to **Files/Add New Sessions** mode (until you modify the position or dimensions again).

E.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, including the sessions that were already recorded. This will affect the data for the previous sessions.

- 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.

In all cases, the revised arena will be used for future sessions, until it is modified again.

CAUTION

Deleting an arena shape can exclude past data

If you delete an arena shape from an experiment, the system applies the deletion to all past and future sessions. This means that any tracking data that occurred within this arena shape is excluded from data reports. Therefore, you should generally *not* delete shapes. Instead, create a *new* experiment and delete the shape in that new experiment; this preserves the data taken in all sessions of the previous experiment.

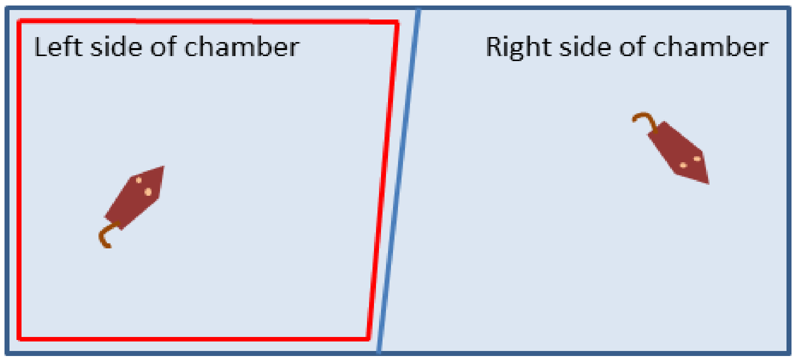
The system preserves the raw data for all sessions. That is, if you add or delete arena shapes, it will typically affect the existing session data that is included in the data reports. However, if you delete *all* arena shapes from the video window, the system will include and report all data that was previously recorded for each session. Think of the arena as a limiting process:

- When you create an arena, you are directing the system to record tracking data only inside the limits of that arena.
- When you reduce the size of an arena (by deleting one of the shapes or reducing the size of one of the shapes), you are directing the system to track within smaller limits.
- When you delete the arena completely, the system does not apply any limits, and includes all of the tracking data that was previously recorded.

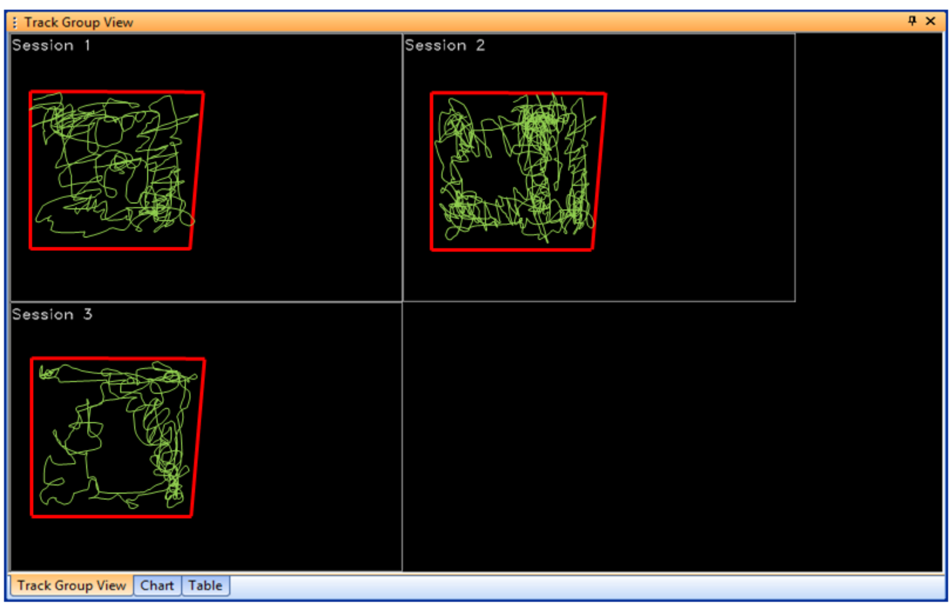
E.6 Examples of Adding, Deleting and Modifying Arena Shapes

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.

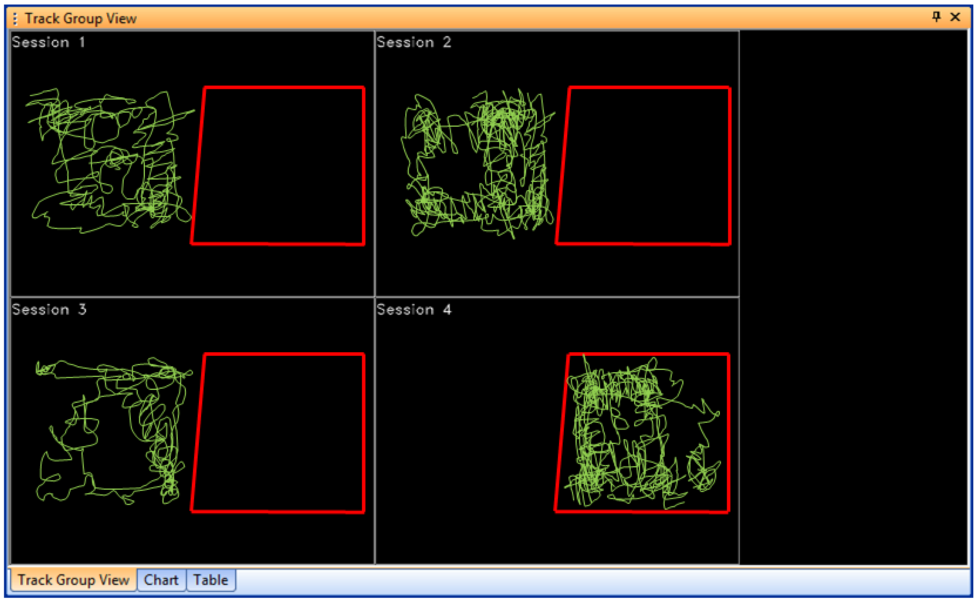


The researcher records three sessions in the left side of the chamber. The **Track Group View** looks like this.



Next, the researcher wants to record an animal in the right side of the chamber.

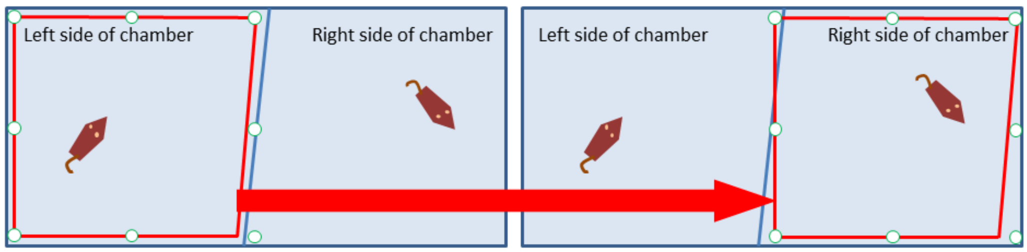
If the researcher simply deletes the existing arena shape and adds a new shape in the right side of the chamber, the **Track Group View** would look like this after recording Session 4.



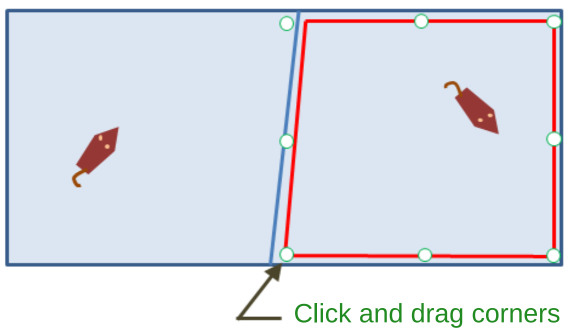
The problem with this procedure, as seen in the above view, is that deleting an arena shape deletes it for all previous sessions as well as all future sessions, and adding a shape adds it for all sessions. In general, the system keeps the same basic configuration of shapes added or deleted for all sessions, but you can move or resize shapes on a per-session basis.

> **IMPORTANT:** Deleting the existing shape and then drawing a new shape for Session 4 would also exclude all of the tracking data previously recorded in Sessions 1 through 3.
>
> Therefore, in this example, the researcher should move and resize the arena shape prior to recording Session 4, thus preserving the data recorded in Sessions 1 through 3.

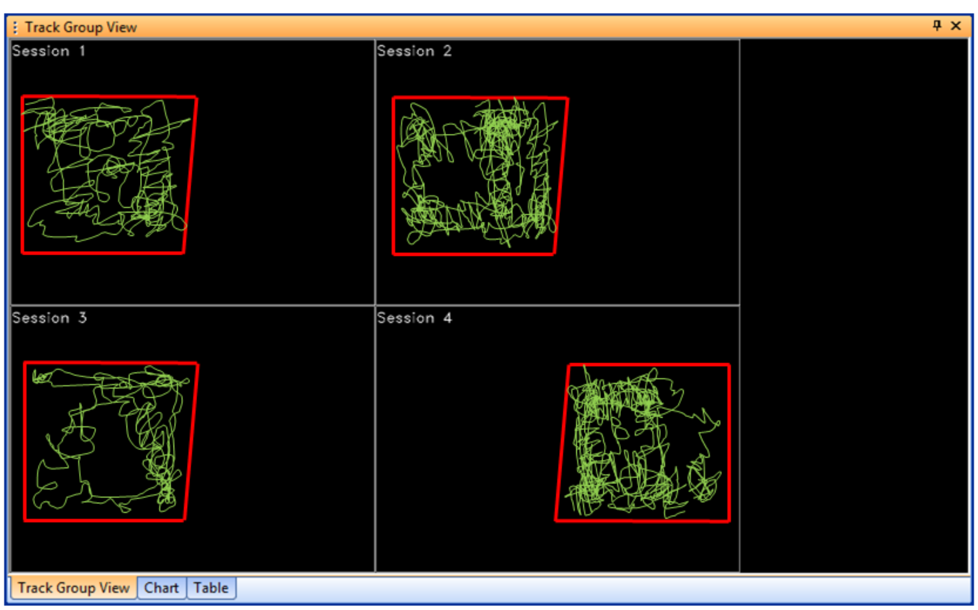
To move an arena shape, left-click somewhere inside the shape to select it. It will get selected on the video with little white dots (similar to how you move shapes in Microsoft® Word, for example). Then drag and drop it to the required position.



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.

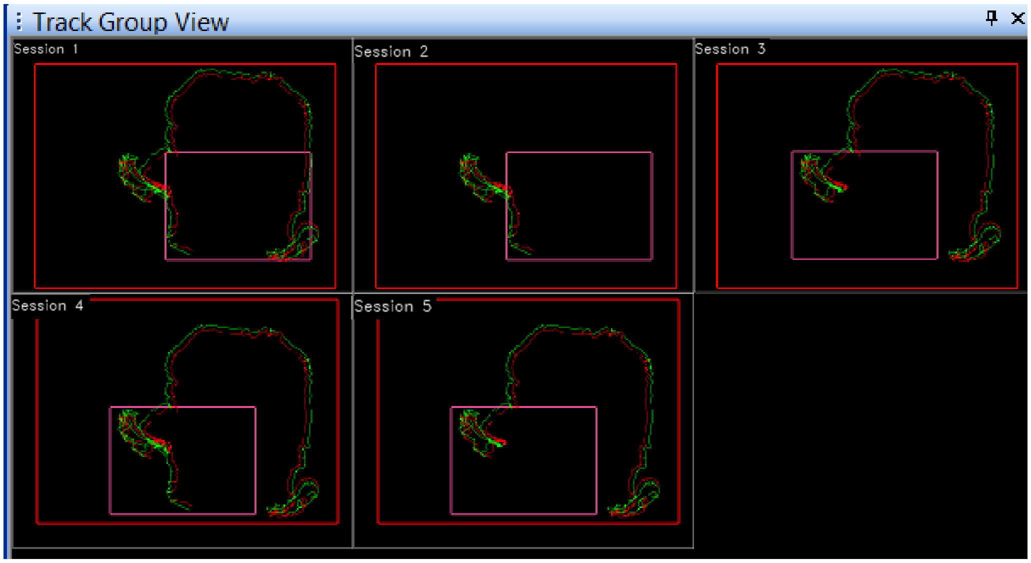


Moving and resizing the arena shape (before recording Session 4) has no effect on the data in Sessions 1 through 3—the position of the arena in those sessions is unchanged. After recording Session 4, the **Track Group View** looks like this.



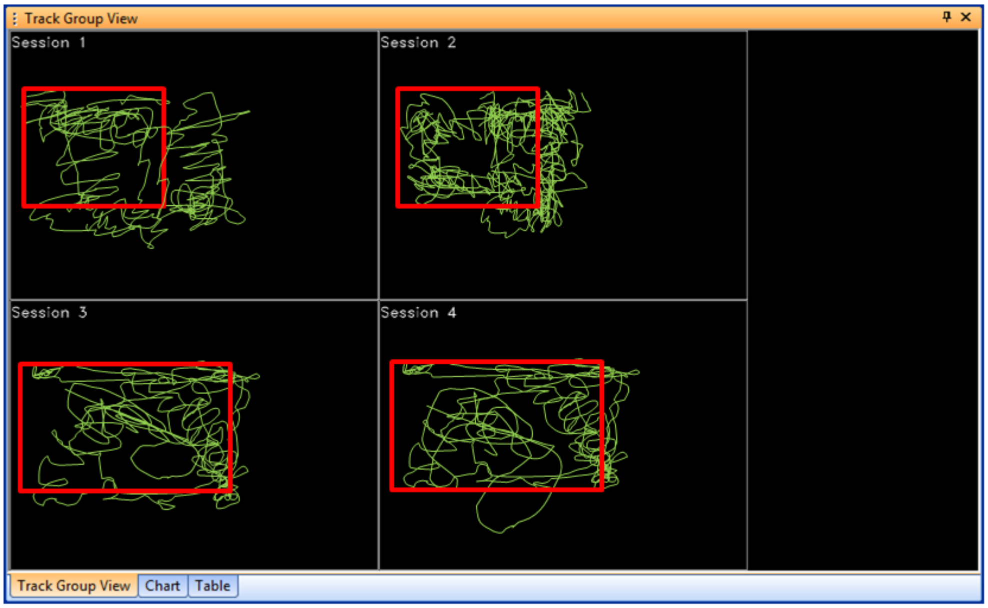
Example 2 - Arena shape (rectangle) moved after Session 2

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 (magenta colored rectangle) excluded an area of the experiment chamber in which the subject was spending a great deal of time. Therefore, prior to Session 3, the researcher moved the arena to include the area in which the subject was more active. The system continues to use the new location in Sessions 4 and 5, but does not change the arena location in the previously recorded sessions (Sessions 1 and 2).



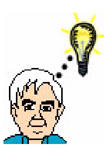
Example 3 - Arena shape (rectangle) resized after Session 2

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) failed to cover a portion of the experiment chamber in which the subject was spending a great deal of time. Therefore, prior to Session 3, the researcher increased the size of the arena to include the area in which the subject was more active. 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 4 - Arena shape (oval) added after Session 2

Addition of a shape is reflected in the **Track Group View** for the experiment. In the example below, the researcher noticed that the original arena (red colored rectangle) failed to cover a portion of the experiment chamber in which the subject was spending a great deal of time. Therefore, prior to Session 3, the researcher added an additional arena shape (the oval) so it included the area in which the subject was more active. The system applies the new (larger) shape to all future sessions and to all previously recorded sessions (including Sessions 1 and 2). Note, however that the tracking data recorded in Sessions 1 and 2 is not altered, because data was recorded only in the arena that was defined during the initial recording session.



TIP

Re-record sessions to obtain tracking data

If you want to obtain tracking data that you did not originally capture in the earlier sessions, go into **Files** mode and re-record the file using the updated arena.

Example 5 - Arena shape (rectangle) deleted after Session 2

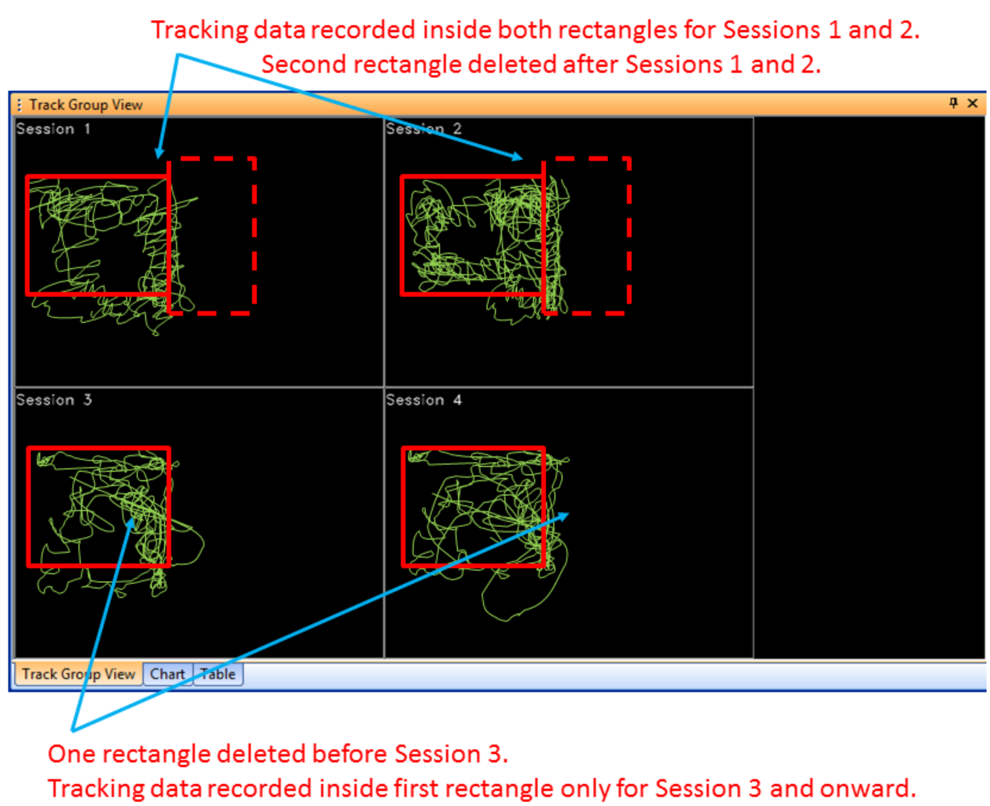
Note: We recommend you start a new experiment before deleting a shape. Failure to do so can cause data to be deleted from previous sessions. See the CAUTION message, below.

Deletion of a shape is reflected in the **Track Group View** for the experiment. In the example below, the researcher noticed that the original arena (red colored rectangle) covered more of the experiment chamber than necessary; that is, it included an area in which the subject was not spending any significant time. Therefore, prior to Session 3, the researcher deleted this arena shape. The system applies the change to all future sessions and to all previously recorded sessions (including Sessions 1 and 2). As a result, some tracking data that was previously reported in Sessions 1 and 2 is now excluded from the data reports.

CAUTION

Deleting an arena shape can exclude past data

If you delete an arena shape from an experiment, the system applies the deletion to all past and future sessions. This means that any tracking data that occurred within this arena shape is excluded from data reports. Therefore, you should generally *not* delete shapes. Instead, create a *new* experiment and delete the shape in that new experiment; this preserves the data taken in all sessions of the previous experiment.



Example 6 - Adding and resizing an arena shape in Object Contour Mode

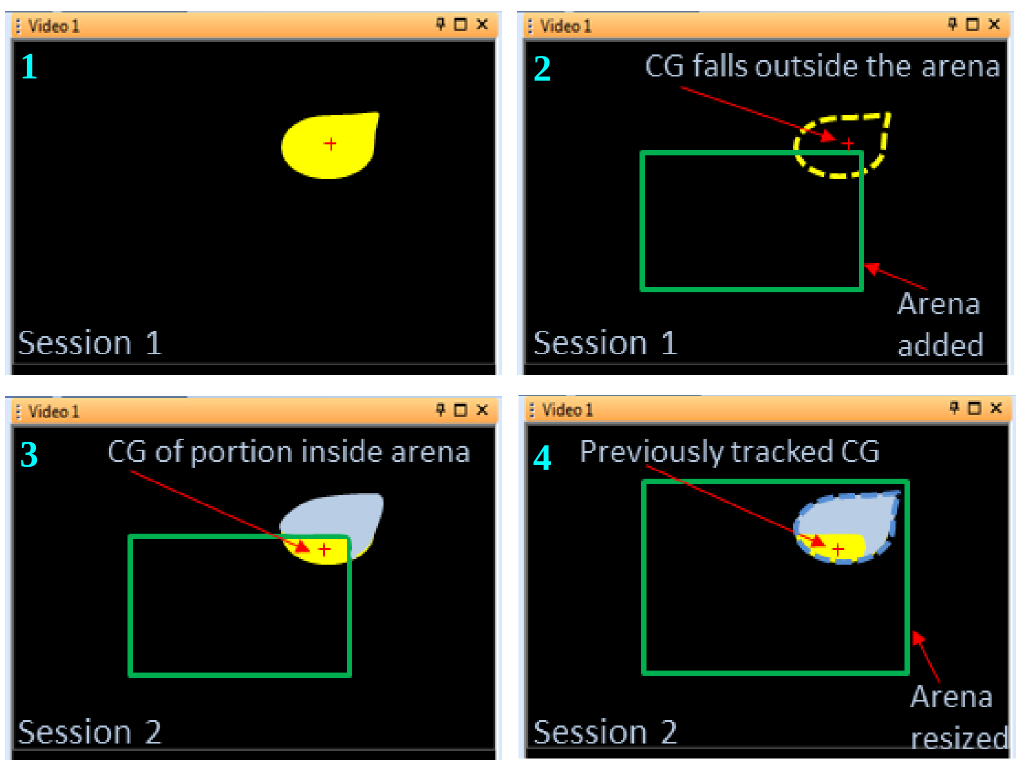
This example illustrates the behavior of the system as it tracks the center of gravity (CG) of the subject in **Object Contour** mode.

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.



E.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 a zone shape or resize a zone shape, the changes apply only to future sessions, not to any of the previously recorded sessions.

E.8 Modifying Zone Shapes in Files/Analyze Session Data Mode

In **Files/Analyze Session Data** mode, if you modify the shape or position of a zone during an experiment (that is, after session(s) have already been recorded), it will typically affect the data that the system includes or excludes from the data reports for that session. The data reports include tracking and event data that appear in the **Track Group View / Chart / Table** window, and tracking and event data that you export.

The modification will *not* alter the zone positions or dimensions for the other previously-recorded sessions. This feature allows you to alter the zone positions and shapes for one session to minimize tracking errors due to any shifting of the camera positions, or to focus more precisely on certain regions of the test apparatus or the subject's behavior in a certain location.

Note: If you have a list of arena and zone settings from a previous experiment, you can apply that complete list of settings to your current experiment. See Section 10.14, "Using the Overlay Feature during Analysis" on page 305.

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 are recorded when you switch to **Files/Add New Sessions** mode.

E.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 applies the addition or deletion to all sessions in the experiment, including the sessions that were already recorded. This will affect the data for the previous sessions.

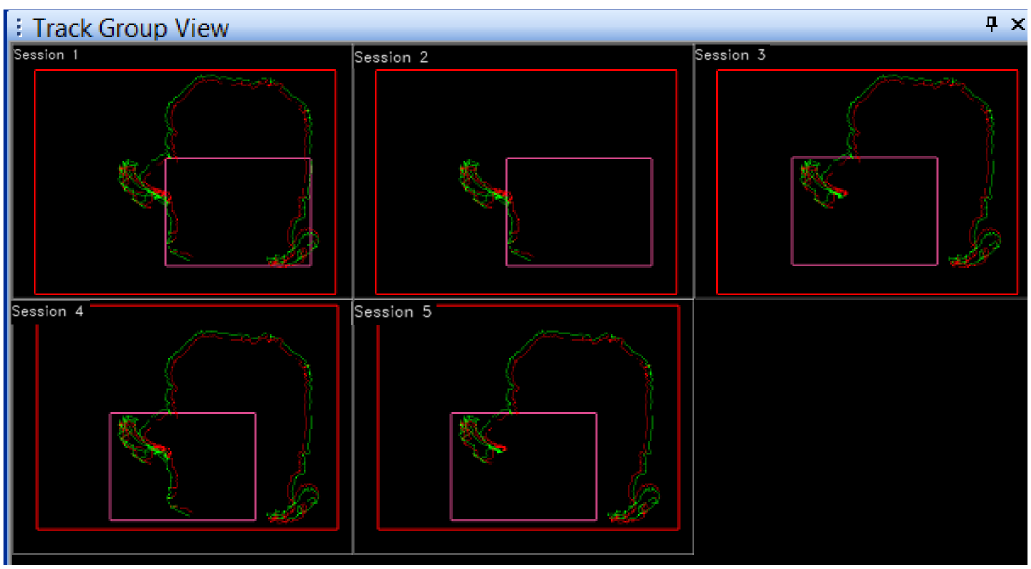
- Adding a zone shape could cause event data that was previously excluded (outside the original zone) in previously recorded sessions to be included in computations.
- Deleting a zone shape could cause event data that was previously included (inside the original zone) in previously recorded sessions to be excluded from computations.

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 are recorded when you switch to **Files/Add New Sessions** mode (until you modify the position or dimensions again).

	CAUTION **Deleting a zone shape can exclude past data** If you delete a zone shape from an experiment, the system applies the deletion to all past and future sessions. This means that any event data that occurred within this zone shape is excluded from data reports. Therefore, you should generally *not* delete shapes. Instead, create a *new* experiment and delete the shape in that new experiment; this preserves the data taken in all sessions of the previous experiment.

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 excluded an area of the arena in which the subject was spending a great deal of time. Therefore, prior to Session 3, the researcher moved the zone to include the area in which the subject was more active. 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).



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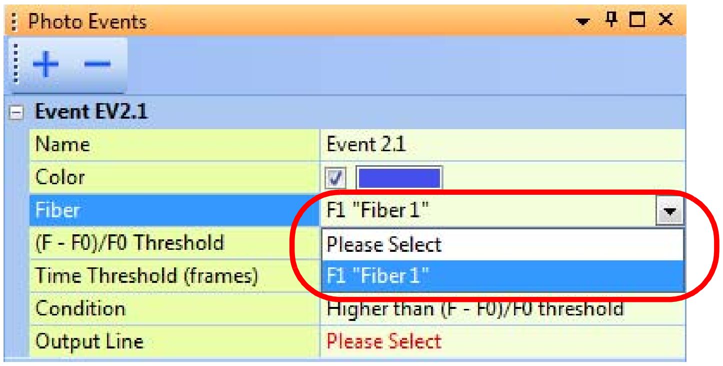
Appendix F Photometry Examples

F.1 Working with Photometry Events

This section presents examples that demonstrate the relationship of fibers to photometry events in both **Cameras** and **Files** modes.

Part 1 (in Cameras mode)

[1.1]
We defined only one fiber (**Fiber 1**), and added one event (**Event 2.1**), then associated **Fiber 1** with this event.



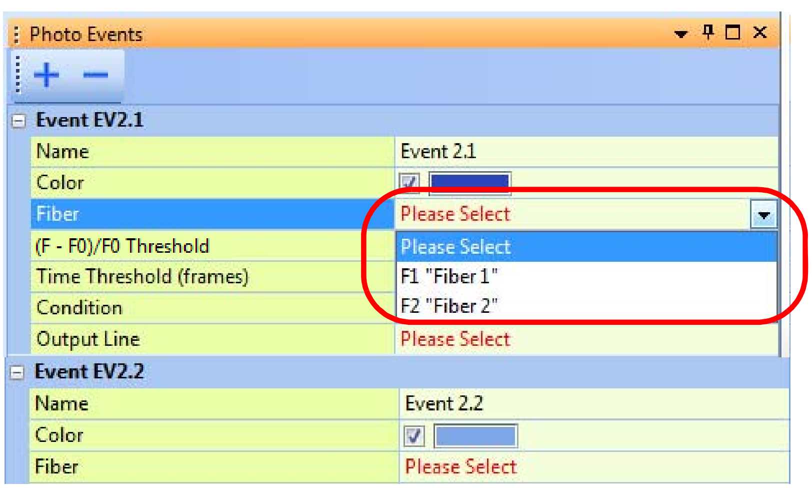
Note that the dropdown list for the **Fiber** row in the **Photo Events** tab displays all of the currently existing fibers in the experiment. As you can see in the image above, only **Fiber 1** was available in the dropdown list.

Then we record the first session, which will be displayed as Session 1 in the **Sessions** window of the GUI.

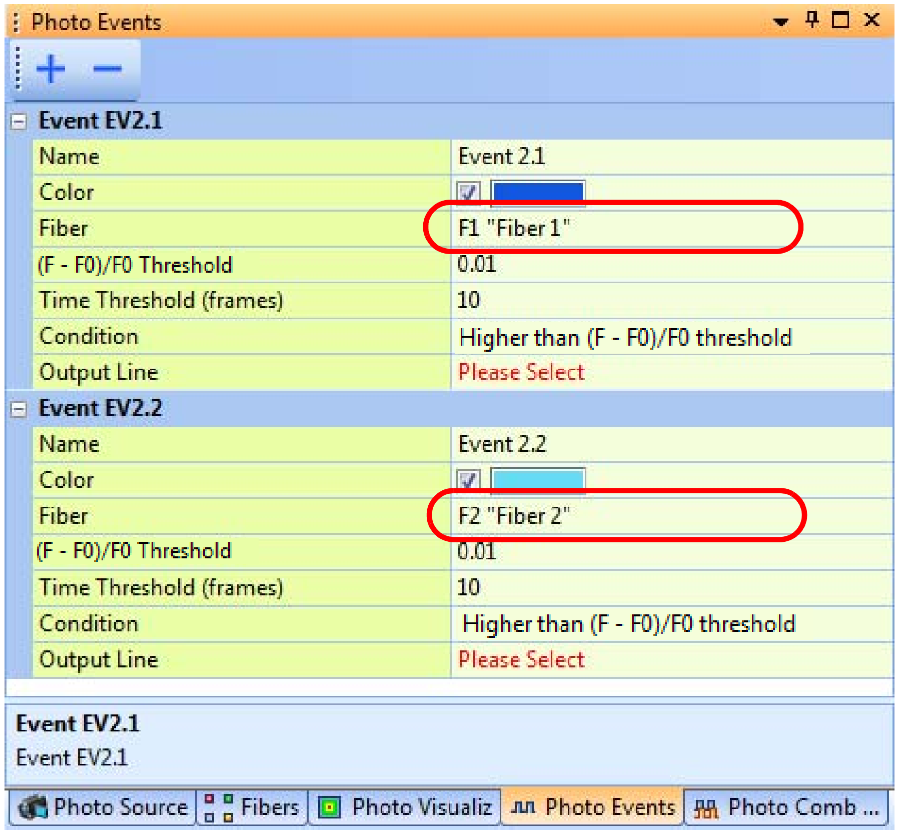
[1.2]
Later we decided to add another fiber (**Fiber 2**) and **Event 2.2**.

Now we can see that **Fiber 2** shows in the dropdown list for both events, because we have two fibers currently existing in this experiment.

If we don't change anything, these two fibers will show up in all future sessions.

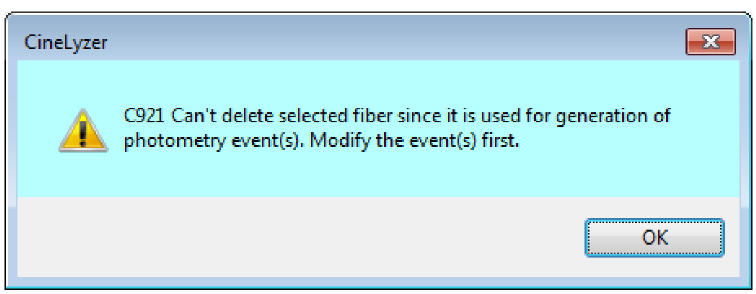


We associate **Fiber 2** with **Event 2.2**.



[1.3]

Now if we try to delete **Fiber 2,** the system denies the action and displays a message.



Now we record this session.

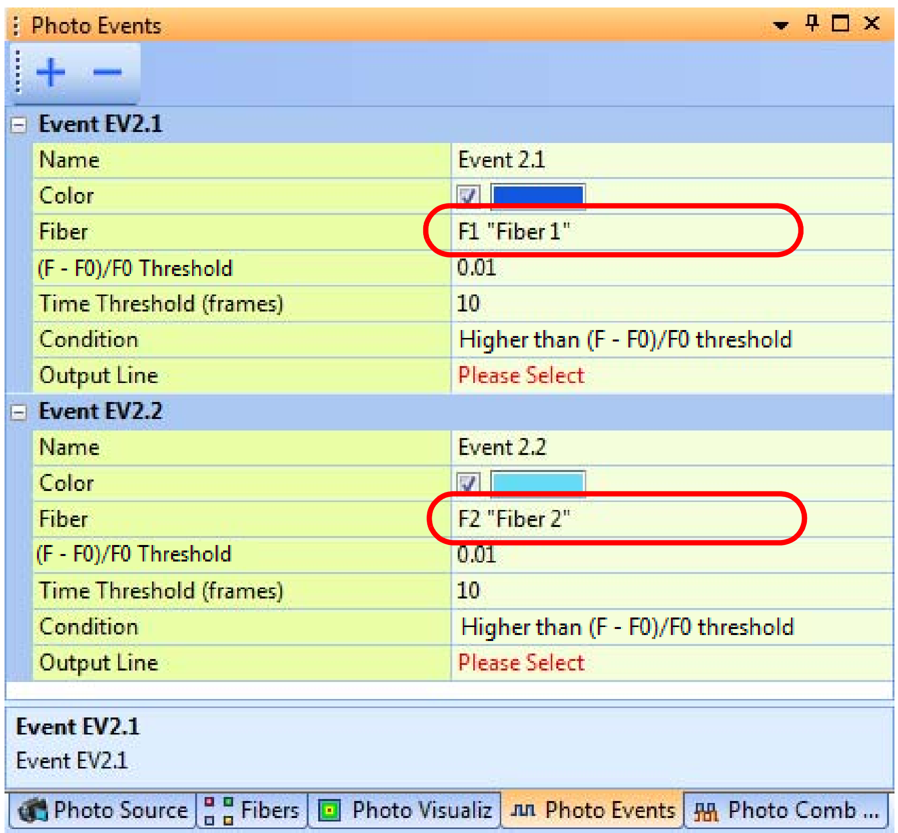
Part 2 (in Files mode / Analyze Session Data)

As described in Section F.2, “Understanding the Relationship of Fibers and Sessions” on page 8, fibers are data sources and thus belong to the sessions with which they were recorded. Their status as data sources is reflected in the photometry events also.

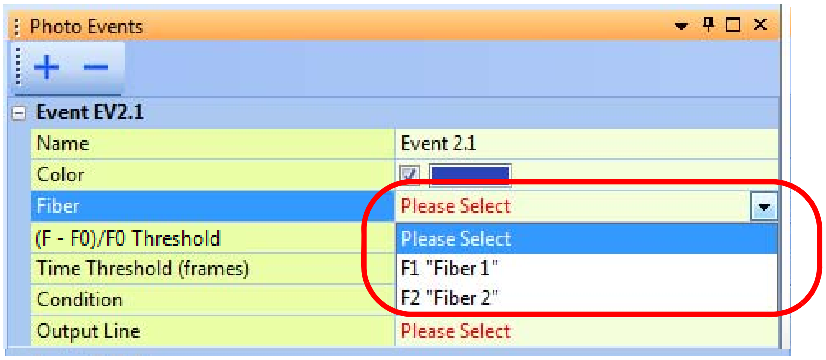
Note that in **Analyze Session Data** mode, the content of the dropdown menu in the **Fiber** row of the photometry events for each session depends on which fibers were available for that specific session.

[2.1]

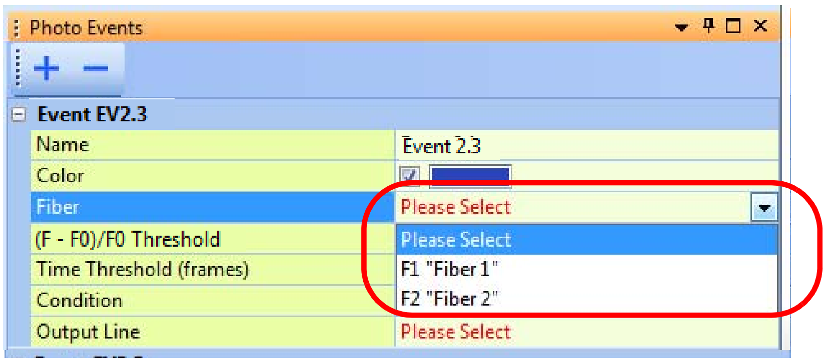
In Part 1 (above), if we select Session 2, we will see that it has two fibers, and both events will appear just like we saw them when we were recording Session 2:



And both fibers will be available in the dropdown list of each event:

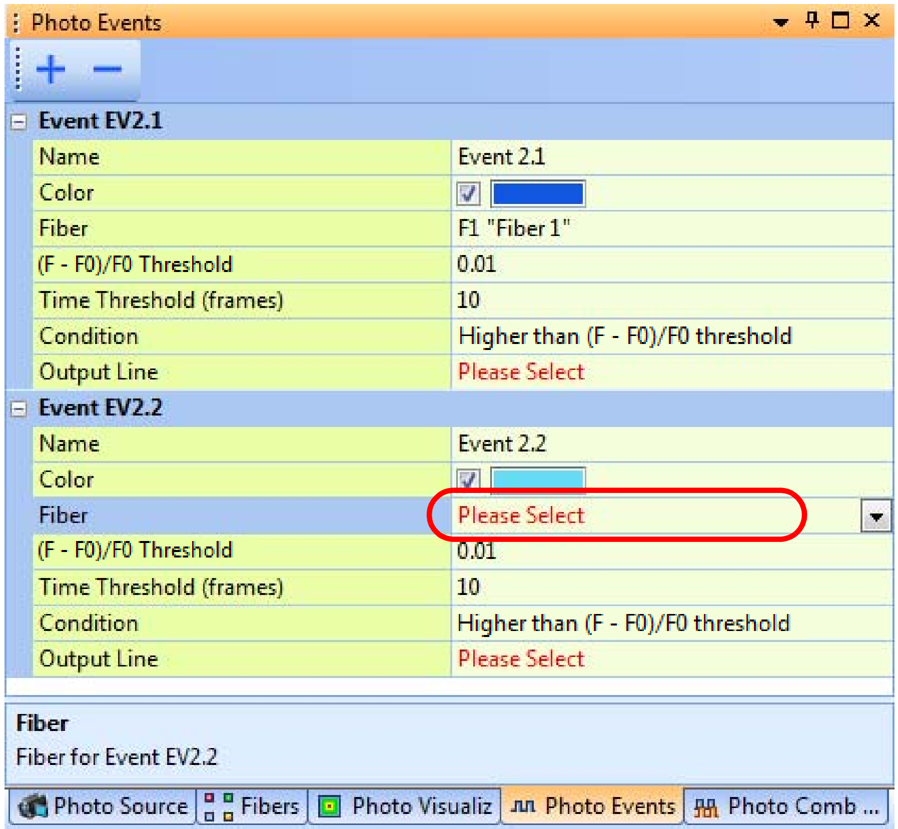


If you add a new event (**Event 2.3**), both **Fiber 1** and **Fiber 2** will be showing in its **Fiber** dropdown list as well.

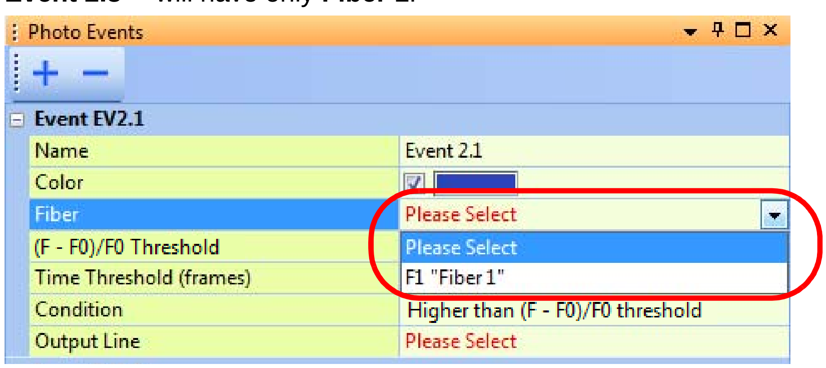


[2.2]

However, if we now select Session 1, we will see that the **Fiber** for Event 2.2 is not defined for this session.



And the dropdown list for the **Fiber** of all events—**Event 2.1**, **Event 2.2** and **Event 2.3**— will have only **Fiber 1**:



This is because Session 1 had only one fiber defined for the recording.

Part 3 (in Files mode / Add New Sessions)

If we now switch to this mode, we will get the same situation we had in **Part 1.2**, above—the latest status of the number of fibers, number of events, and their fiber content—as we had when we last edited the experiment. In this case we will see two fibers and three events, just as in **Part 1.2**, as shown below.

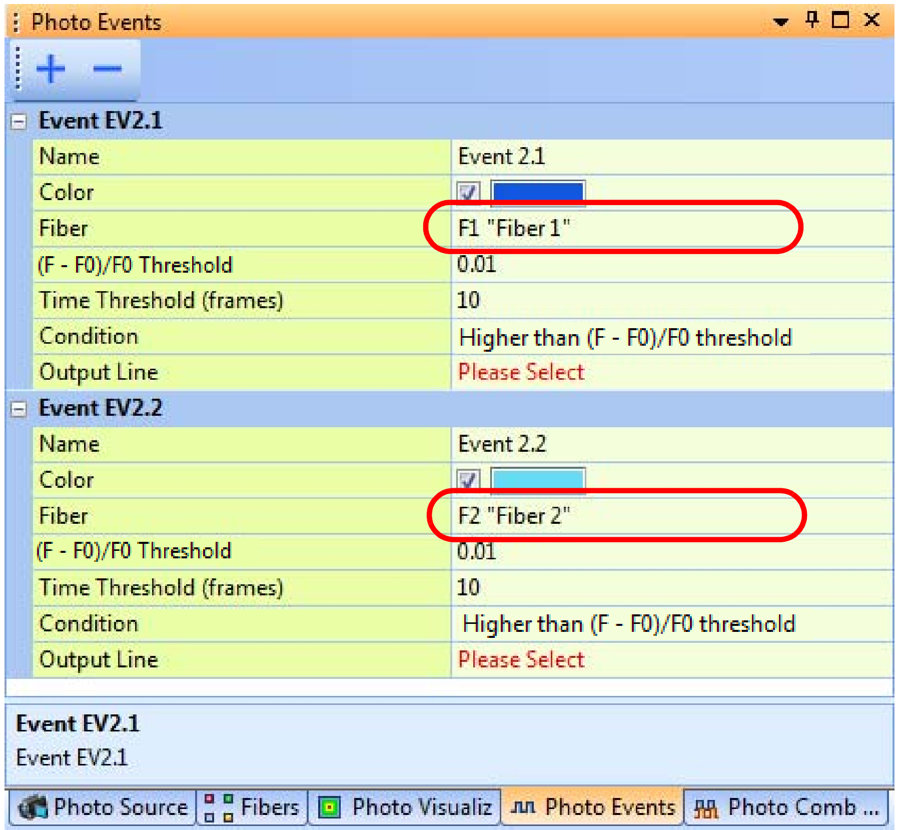


Photo Events
Event EV2.1
Name
Event 2.1
Color
Fiber
F1 "Fiber 1"
(F - F0)/F0 Threshold
0.01
Time Threshold (frames)
10
Condition
Higher than (F - F0)/F0 threshold
Output Line
Please Select
Event EV2.2
Name
Event 2.2
Color
Fiber
F2 "Fiber 2"
(F - F0)/F0 Threshold
0.01
Time Threshold (frames)
10
Condition
Higher than (F - F0)/F0 threshold
Output Line
Please Select
Event EV2.1
Event EV2.1
Photo Source
Fibers
Photo Visualiz
Photo Events
Photo Comb ...

F.2 Understanding the Relationship of Fibers and Sessions

The example presented in this section demonstrates an important aspect of the Photometry System, namely, [1] photometry fibers are data sources and [2] photometry fibers belong to the sessions in which they were recorded, therefore fiber information is stored on a per-session basis.

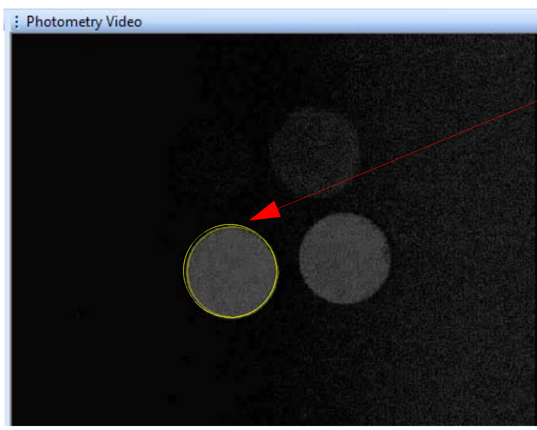
Part 1 — Create a new experiment and record sessions in Cameras Mode

1. Create a new experiment and add one photometry fiber (Fiber 1). Record Session 1.
2. Add second fiber (Fiber 2) and record Session 2.
3. If you don't change anything else in this experiment, these two fibers will show up in all future recorded sessions. You can switch to a different experiment or close the CineLyzer application. When you select this original experiment again, you will see there are two fibers available.

Part 2 — Switch to Files mode / View Sessions As Recorded

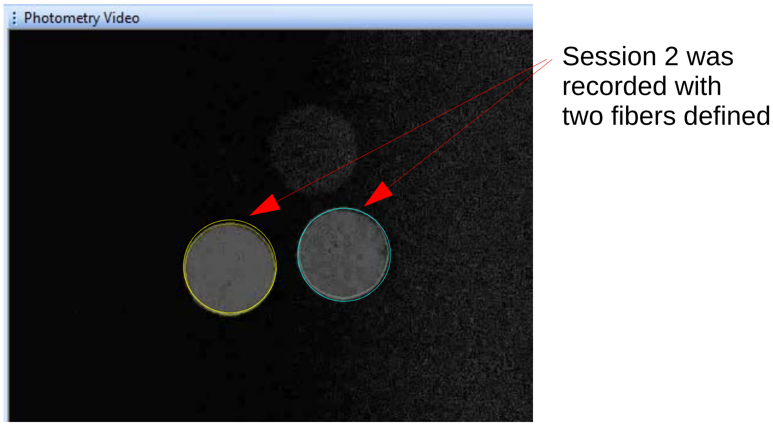
Switch to **Files** mode / **View Sessions As Recorded**. Then select the experiment we created in Part 1, above.

In our example, if we select Session 1, we'll see the **Fibers** tab has one fiber defined. Also, you will see only one fiber defined in the **Photometry Video** window:



Session 1 was recorded with one fiber defined

However, if we select Session 2, the **Fibers** tab will show two fibers defined, and you will see these two fibers in the **Photometry Video** window:



Note that in this mode (**View Sessions As Recorded**) you cannot add or remove fibers, move them or change their dimensions. They will show up the way they were when the session was recorded.

Part 3 —
Switch to Add New Sessions mode

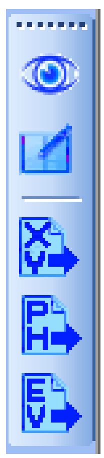
Now if you switch to the **Add New Sessions** mode, you will get to the same situation you had in Part 1 Step 2 above, that is, the latest number of fibers, their positions and dimensions you had when you last edited your experiment.

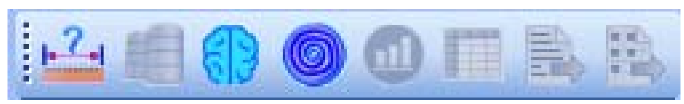
In this mode (Add New Sessions), the system allows you to add/delete fibers, move them and resize them. Any changes you make to the fibers will be recorded in the next session. This capability gives you a way to remeasure photometry data if you decided to change or adjust the fiber-defining circles in the **Photometry Video** window. Please note the following:

- You must re-record a new session from the file to remeasure photometry data.
- Photometry data remeasured from the video file will be slightly different (~10%) compared to the data computed in **Cameras** mode. This is due to video compression.

Part 4 —
Switch to Analyze Session Data

Now if you switch to the **Analyze Session Data** mode, you will get to the same situation you had in Part 1 Step 2 above, that is, the latest number of fibers, their positions and dimensions you had when you last edited your experiment. However, now you will be able to modify arenas, zones and parameter settings and perform new analyses on the existing behavioral and photometry videos. To perform new analyses and export new results (without causing any change to the original AVI file, or the data it contains), use the buttons in the calculation and export toolbars.





If you have any questions please contact Plexon at +1 214-369-4957 or support@plexon.com.

Appendix G 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.

G.1 Physical Installation Issues

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 or Intermittent

1. In Cameras mode—Be sure that all required cable connections are in place and tight. Unpredictable results may occur if they are not.
2. Be sure the lens iris is fully open
3. In Files mode—Be sure that you have selected a file in the Source tab.

Unstable Video Image

Camera mounts 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.

Video Malfunction Due to Use of Non-specified Camera

The use of non-specified cameras might give poor results. For information about the camera(s) that were tested and supplied with your system, see Section 1.5, “System Hardware, Software and Cameras” on page 10.

Poor Image Related to USB Connection to Camera

If the video image is present but scrambled, there could be a problem with the USB port or cable.

1. Use only USB ports in the expansion card on the back panel of the PC for the camera connections (video streams).
2. Try using a different USB port on the back of the PC to connect the camera.
3. If the problem persists, use another USB cable of the same type.

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. For information on focusing the cameras and setting camera parameters in the user interface, see Section 5.7, “Configuring the Source Parameters” on page 77.

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 Camera Positioning for the Experiment.

Input Lines and Output Lines Not Present in Dropdown Lists

The 12 input lines are not populated in the **Input Events** tab, or the 12 output lines are not populated in the **Events** or **Behav Combo** tabs.

To ensure that the dropdown lists with all the input lines and output lines are populated, you must connect the USB cable from the USB Digital I/O Interface (DIO Interface) to a USB port on the host PC and then start (or restart) the CineLyzer application.

Note: The status bar at the bottom of the CineLyzer GUI displays an I/O icon that is red I/O if the USB Digital I/O unit is not connected, and green I/O if the unit is connected. (Details of the USB Digital I/O unit are covered in Appendix C, USB Digital Input/Output Interface.)

G.2 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 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 CineLyzer® 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 CineLyzer System, 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 CineLyzer 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. Missing CineLyzer license key. Obtain or find the correct license key, plug it into a USB port on the computer, and start the CineLyzer software.
2. If the CineLyzer software still will not start and reports a missing license key, install the Sentinel drivers (this is done by the CineLyzer installation software or by running the file C:\Program Files (x86)\Plexon Inc\CineLyzer V4\Common Files\Sentinel System Driver Installer 7.5.8.exe).
3. If the system reports missing DLLs, reinstall the CineLyzer software. All the required DLLs are in the installation package.
4. Contact Plexon Support if the problem is not resolved.

The CineLyzer System Will Not Start In or Switch to Cameras Mode

There can be several causes for the system failing to go into **Cameras** mode. The most common causes and their solutions are shown below.

1. Missing license key. Obtain the correct license key and restart the CineLyzer software.
2. Missing or inoperative IO card or drivers.
3. Camera cables are loose or unplugged. Power all components of the system down, then check and reseat both ends of the cable(s) to the camera(s). Power the system back up, and follow the normal startup steps. Use only the ports on the USB card on the back of the PC for the camera connections (video streams).
4. Contact Plexon Support if the problem is not resolved.

G.3 Video and Recording Issues

No Video Image in the Video Window—Software

When you are working in **Cameras** mode:

1. Verify that camera (Camera 1, 2, 3 or 4 as applicable) is active. The icon for a camera turns orange if the camera is active. In the example below, Camera 1 is active and Camera 2 is inactive:

In the example below (which is applicable to the photometry system only), the behavioral camera is inactive and the photometry video stream is active:

2 If the icon for the camera is not already orange (active), do the following:

a Click on the icon to activate the camera.

b In the **Window** dropdown menu, select **Layout**, then select **Reset to Default Layout**. All normal windows for all of the connected and activated cameras should be displayed.

3 If there is still no video, contact Plexon Support.

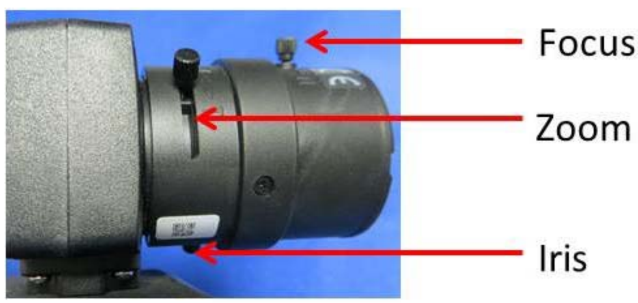
No Video Image in the Video Window—Hardware

When you are working in **Cameras** mode:

1 Camera cables are loose or unplugged. Power all components of the system down, then check and reseat both ends of the cable(s) to the camera(s). Use only USB ports in the expansion card on the back panel of the PC for the camera connections (video streams).

2 Power the system back up and follow the normal startup steps.

3 If there is still a problem ensure that the manual iris control on the lens is fully open.



4 If there is still no video, contact Plexon Support.

Cannot Focus the Video Image

The video is present, but blurry and cannot be focused at any zoom or focus setting.

1 Adjust the zoom and focus rings on the lens until a clear picture is obtained. See the figure above.

2 Ensure that an appropriate lens is on the camera. The lens that was included in the original shipment should be appropriate for many types of experiments.

However, if conditions are significantly changed, for example, you need to place the lens extremely close to a surface, a different lens might be needed.

3. Contact Plexon Support if the problem is not resolved.

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.

Live Video Images Being Corrupted

1. Use only USB ports in the expansion card on the back panel of the PC for the camera connections (video streams).
2. Try reducing monitor count, monitor resolution, or both.
3. If cards have recently been added to the PC bus, remove them to see if the problem is solved.
4. Contact Plexon Support for help in rearranging the order that existing cards are plugged into the PC bus.

The System Will Not Start Recording When the Start Icon is Pushed

1. Check that the Start icon is armed (has turned red). If it is not armed, be sure you have performed all of the steps in the applicable start up and configuration procedures
2. Check cables, especially the control cables - see the physical installation section of this document.
3. Check that the disk selected for recording video files is not full.
4. Contact Plexon Support if the problem is not resolved.

G.4 Issues with Dropped Frames and PC Overload

Dropped Frame Count Non-zero While Recording

When the computer is heavily loaded causing available video buffering to fill, video frames may be dropped. The count of dropped frames is incremented each time this happens. This is not a normal condition and probably indicates that the computer is being asked to perform more than CineLyzer processing.

1. Use only USB ports in the expansion card on the back panel of the PC for the camera connections (video streams).
2. Stop running all unnecessary or resource-consuming applications on the computer while recording, for example, Microsoft® Office® programs, Skype®, MATLAB® applications, 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 CineLyzer System 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.

If dropped frames still persist after these steps, please contact Plexon Support.

Dropped Frames Due to Unusually High Performance Demands

If your experiment involves a combination of high frame rate combined with an extremely large number of simultaneously tracked objects and simultaneous behavioral events, or other factors involving high CPU usage (for example, running browser applications or software other than Plexon software while recording), it is possible to overload the system and drop frames. If this occurs, refer to the discussion in Section 9.9, "Managing CPU Demand and Avoiding Dropped Frames" on page 255.

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CineLyzer® with Photometry

Behavioral Research System with Photometry Imaging

User Guide

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