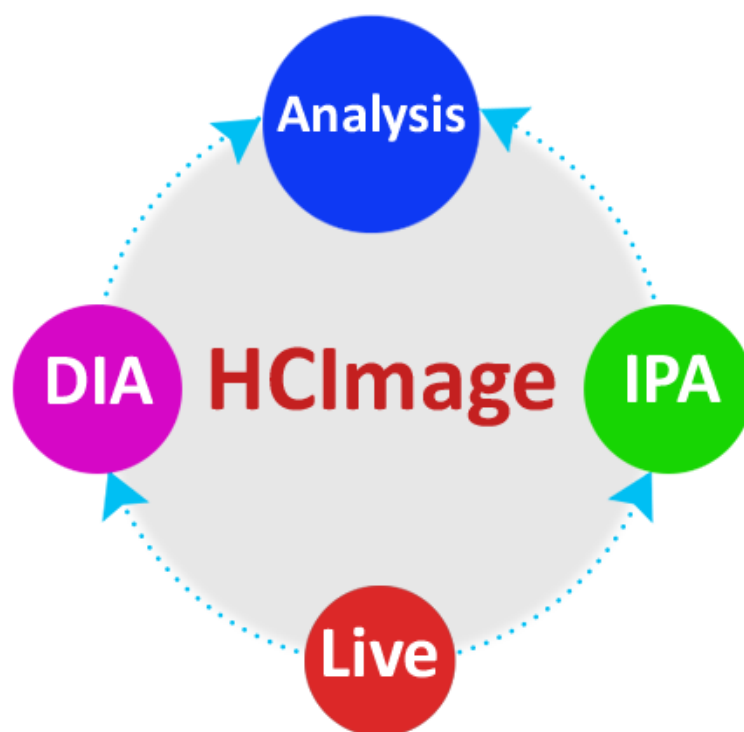


# HCIimage IPA

## Getting Started Guide



**Release 4.8**

**August 2021**

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# INSTALLATION

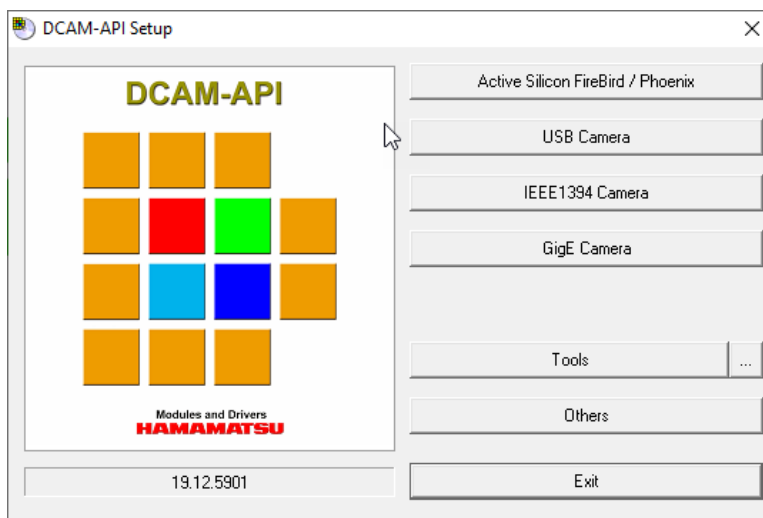
## Install HCImage

1. Insert the HCImage installation DVD into the DVD-ROM drive. If autoplay is enabled, the HCImage setup will run automatically. If autoplay fails to start, locate your DVD-ROM drive and double-click on **setup.exe**.
2. Click **Yes**, if prompted by the User Account Controls.
3. To begin the installation wizard, click **Next**.
4. Review the Software License information and click **Yes**.
5. Review the README section for up-to-date information on software compatibility and support. When you are ready, click **Yes**.
6. On the Personalize screen, enter your registration information and click **Next**.
7. Choose the Destination Folder and click **Next**. It is recommended to install the software in the default path.
8. If you are ready to proceed with the installation, click **Install**.
9. Follow the instructions on each installation page.
10. Securely connect the dongle (  ) to a USB port after the software installation has finished.
11. Install the appropriate DCAM-API drivers, see the instructions below, then turn your camera on prior to launching HCImage.
12. Click the **HCImage** icon on your Desktop to launch HCImage.
13. Register the software to receive technical support, please go to [www.hcimage.com](http://www.hcimage.com) and click **Register**.

## Install DCAM-API Drivers

Before installing the camera driver, make sure that the camera is turned off.

1. Open Windows Explorer, go to HCImage installation DVD, expand the **Drivers** folder, open the **Cameras** folder and open the **DCAM** folder. If you downloaded HCImage, please go to <http://www.dcam-api.com/> and download the DCAM-API drivers for Windows.
2. Double-click **Setup.exe** to launch the DCAM-API Setup dialog.
3. Click **Yes**, if prompted by the User Account Controls.
4. Select the appropriate driver for your Hamamatsu camera from the DCAM-API Setup dialog. If you are unsure of which driver to install, please consult the DCAM-API Compatibility Note or contact your local Hamamatsu representative. To view DCAM-API Compatibility Note, select **Others** and then click **Compatibility Note**.
5. Click **Next** to begin the installation.
6. Follow the instructions on each installation page and click **Finish** when the installation is complete.

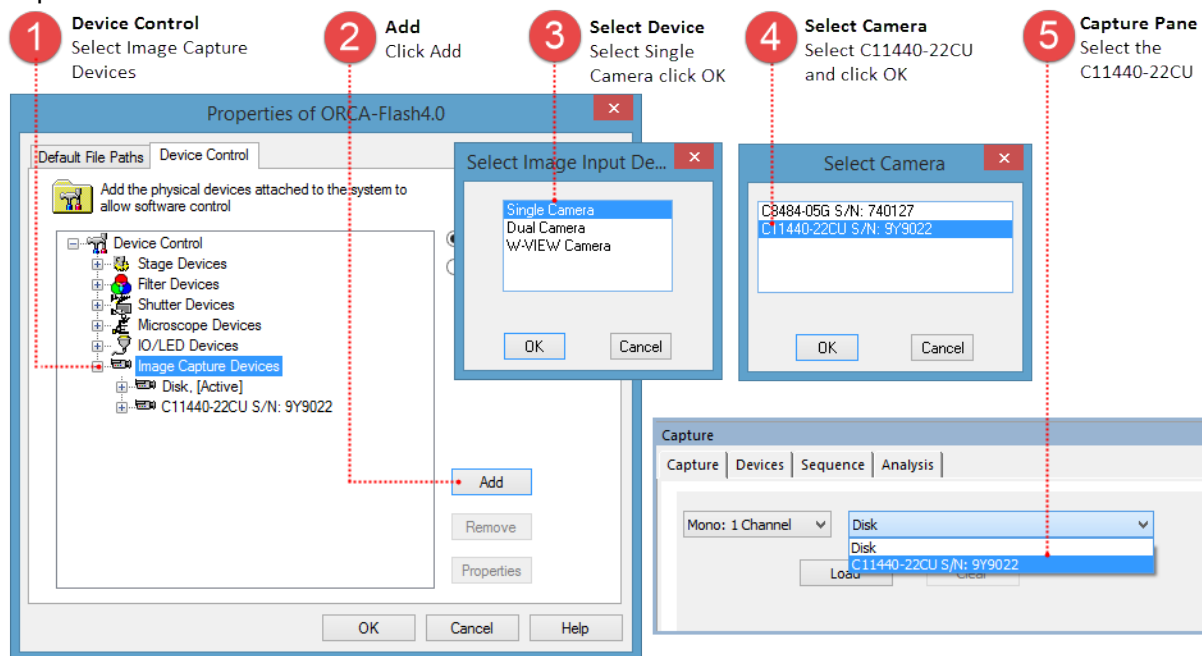


## ADD DEVICES TO A PROFILE

Add and setup peripheral hardware devices to the profile. Supported hardware includes: cameras, stages, filters, shutters, I/O and microscope devices. For a list of supported devices, please visit our website at <http://hcimage.com/support/hardware.htm>.

### Add a Camera

Launch HCImage, go to File, select Current Profile and then follow the steps below to add a camera to the profile.



### Add an Olympus IX-83 Automated Microscope

#### Olympus 64-bit Drivers from the HCImage DVD

Be advised that this application installs the Olympus Camera and Microscope drivers, as well as copying all of the necessary dlls into the HCImage directory.

1. Open the DVD contents in Windows Explorer and navigate to Drivers\Microscopes\Olympus\Olympus 3 Series\x64.
2. Double-click on **Olympus\_x64 Install.exe** and follow the installation instructions.
3. Click **Yes**, if prompted by the User Account Controls

**Note:** If using a Hamamatsu 1394 camera, this driver installation may supersede the Hamamatsu driver causing communication problems. To recover from this issue, please see "**Unable to communicate with Hamamatsu 1394 camera**" on page 30.

#### Configure with the Touch Panel Controller

The microscope drivers have been installed, time to configure it using the touch panel controller (TPC) and then add it as a device in HCImage. The first step is to turn on the IX3-CBH (microscope control box) and then the touch panel controller.

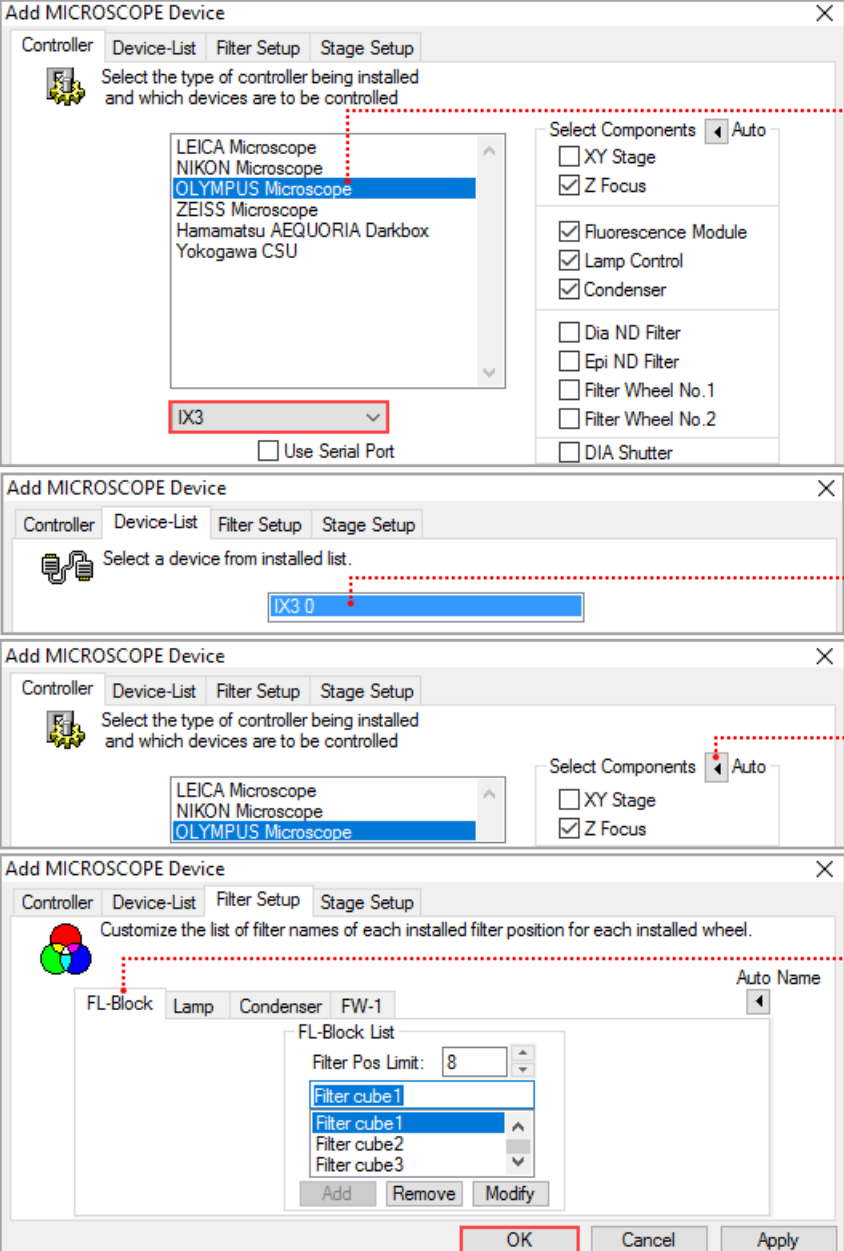
**Note:** The "Power On" sequence for turning the equipment on before use should be: Light Source > PC > Camera > IX3-CBH > Touch Panel Controller > Launch HCImage.

An initial system setup is required when using the microscope for the first time or after replacing one of the components. The microscope is setup and configured using the TPC.

1. Go to **System Setting** in the **Menu** screen.
2. Select **Unit**, enter the components connected to the IX83 for each module and tap **OK** to save the settings.
3. Select **Optical**, enter and configure the objectives, mirror units and condenser.
4. Select **Customized**, enter the focus limits and parfocality correction.
5. When the setup is complete, tap **X** to exit to the **Menu** screen.

### Add Microscope to a Profile

Once the microscope has been setup from the touch panel controller, the next step is to add the microscope to a profile and configure it in HCImage. Launch HCImage, go to File and select Current Profile. In the Device Control tab, select Microscope Devices and click Add.



The figure shows four sequential screenshots of the 'Add MICROSCOPE Device' dialog box, illustrating the steps to add a microscope device:

- 1 Device Control**: Select the microscope manufacturer and the model. The 'Controller' tab is active, showing a list of manufacturers (LEICA, NIKON, OLYMPUS, ZEISS, Hamamatsu AEUORIA, Yokogawa CSU) and a dropdown menu set to 'IX3'. The 'Select Components' section on the right has 'Auto' selected, with checkboxes for XY Stage, Z Focus, Fluorescence Module, Lamp Control, Condenser, Dia ND Filter, Epi ND Filter, Filter Wheel No. 1, Filter Wheel No. 2, and DIA Shutter.
- 2 Device List**: Select the device that is listed. The 'Device-List' tab is active, showing a list of installed devices with 'IX3 0' selected.
- 3 Device Control**: Select Auto and HCImage will update the components list. The 'Controller' tab is active, showing the same list of manufacturers and the 'IX3' dropdown. The 'Select Components' section on the right has 'Auto' selected, with checkboxes for XY Stage, Z Focus, Fluorescence Module, Lamp Control, Condenser, Dia ND Filter, Epi ND Filter, Filter Wheel No. 1, Filter Wheel No. 2, and DIA Shutter.
- 4 Filter Setup**: Verify the settings for the individual components, modify if necessary and click OK. The 'Filter Setup' tab is active, showing a list of filter names for each installed filter position for each installed wheel. The 'FL-Block' tab is active, showing a list of filter names (Filter cube1, Filter cube2, Filter cube3) and a 'Filter Pos Limit' of 8. The 'OK' button is highlighted.

## Add a Filter Wheel and a Shutter

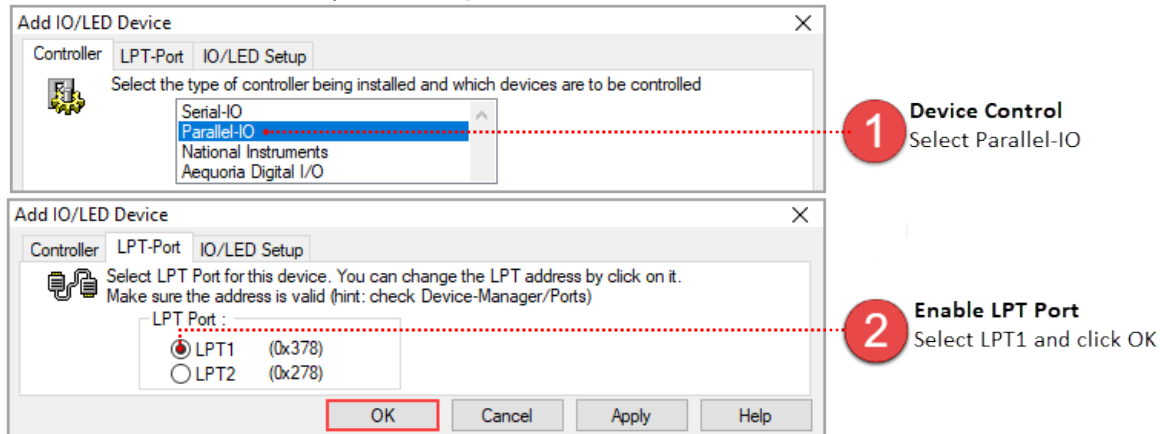
Launch HCIImage, go to File and select Current Profile. In the Device Control tab, select Filter Devices, click Add and follow the instructions below.

The image displays four sequential screenshots of the 'Add FILTER Device' dialog box, illustrating the steps to add a filter wheel and shutter. Red dotted lines and numbered callouts (1-4) highlight the specific actions required in each step.

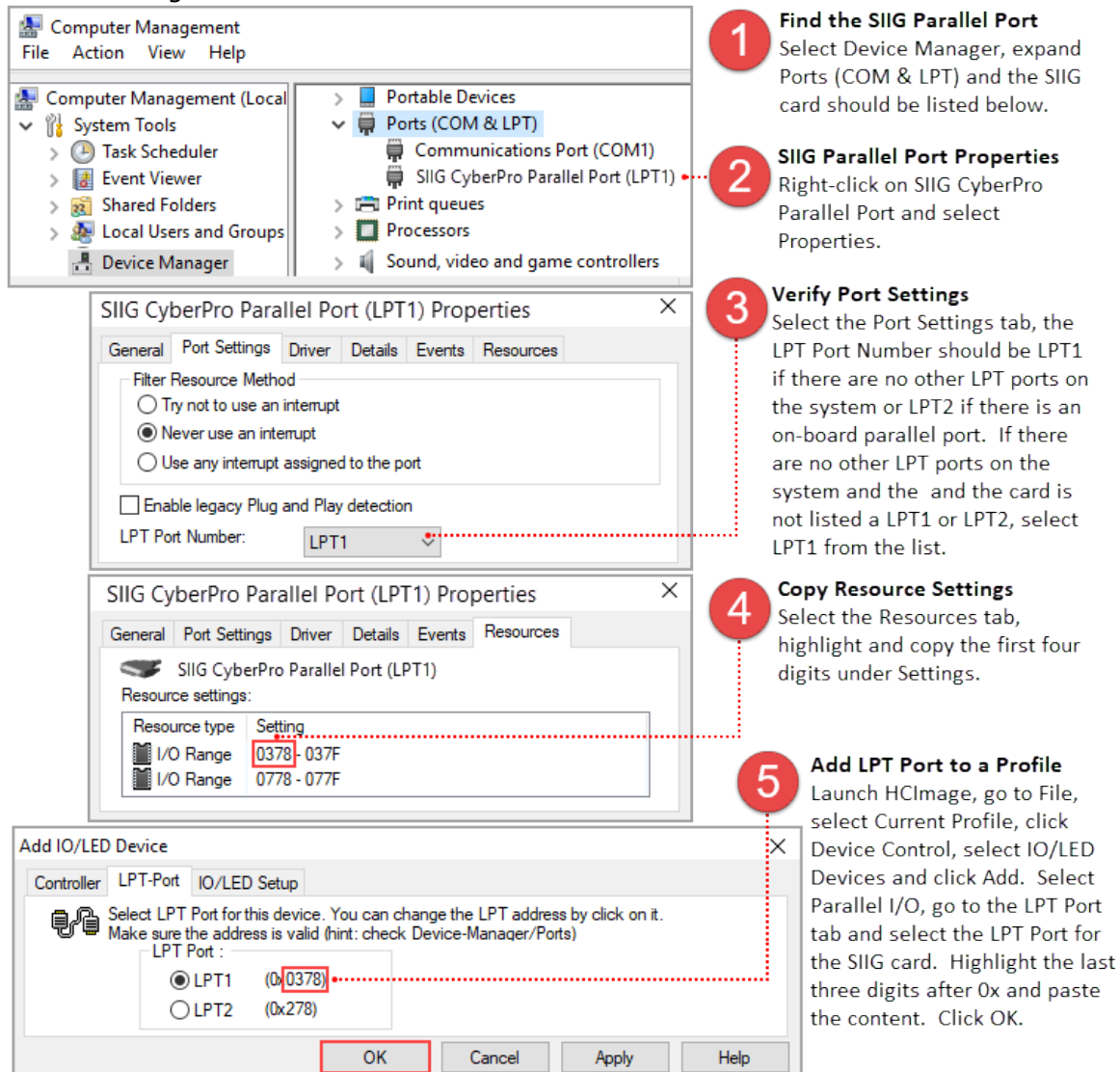
- 1 Device Control**  
Select the controller, the type of filter wheel and enable Add Shutter.  
In the first screenshot, the 'Controller' tab is active. A list of controllers is shown, with 'PRIOR Filter' selected. To the right, the 'Filter Wheel' section has 'Single' selected, and the 'Add Shutter' checkbox is checked.
- 2 Enable COM Port**  
Select the COM Port for the device.  
In the second screenshot, the 'COM-Port' tab is active. A list of COM ports is shown, with 'COM1' selected. The 'Communication settings' section shows 'Baud Rate' set to 9600, 'Data Bits' set to 8, 'Parity' set to None, and 'Stop Bits' set to 1.
- 3 Filter Setup**  
Select the filter position, enter the name and click Modify.  
Repeat for each filter position.  
In the third screenshot, the 'Filter Setup' tab is active. The 'Customize Positions' section shows a list of filter positions. The first position is '380 nm', which is highlighted. The 'Modify' button is highlighted.
- 4 Shutter Setup**  
Set the status to Active and click OK.  
In the fourth screenshot, the 'Shutter Setup' tab is active. The 'Shutter Settings' section shows three shutter positions. 'Shutter 1' is set to 'Active', 'Shutter 2' is set to 'None', and 'Shutter 3' is set to 'None'. The 'OK' button is highlighted.

## Add a Parallel Port as an IO/LED Device

In the Device Control tab, select IO/LED Devices and follow the instructions below.




If the computer doesn't have a parallel port, we recommend the SIIG CyberParallel PCIe port card (<http://www.siig.com/it-products/serial-parallel/parallel/pcie/dp-cyberparallel-pcie.html>). Install the parallel port card and driver as per the instructions provided with the card and then launch the Device Manager and follow the instructions below.



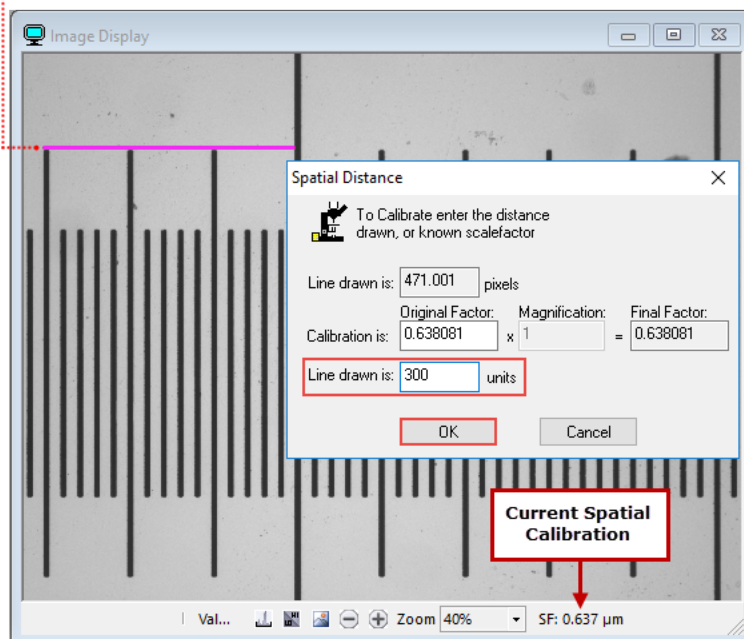
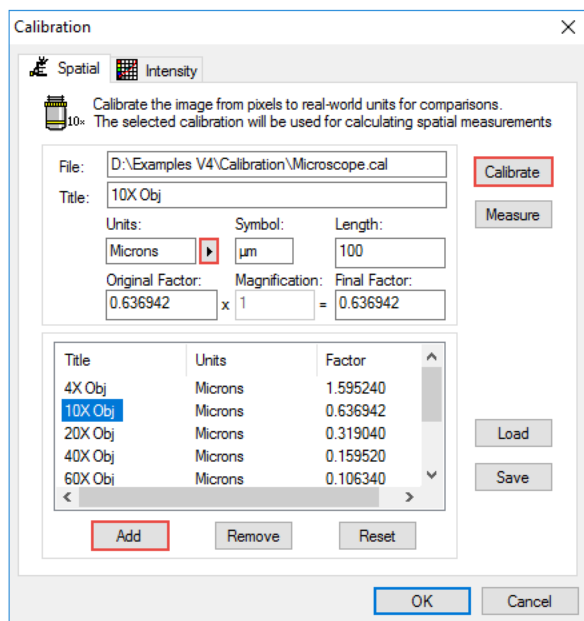


# CALIBRATION

## Calibrate an Image from Pixels to Microns

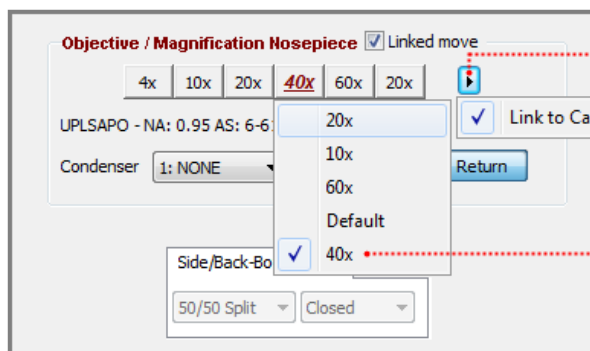
Open or capture an image with some known distance, for example a micrometer. Click on the Calibration Properties icon (  Calibration ) on the Analysis toolbar and follow the instructions below.

- 1 Add Calibration**  
Click Add and enter a title (e.g., 10x)
- 2 Select Units**  
Select Microns from the Units list
- 3 Calibrate the Image**  
Click Calibrate. Draw a line to span the distance to measure
- 4 Enter the Distance**  
Enter the known distance and click OK



## Link Calibration to Objective

To link the calibration to an objective, go to the Microscope Setup panel in the Devices pane and follow the steps below.



- 1 Enable Link to Calibration**  
Click and select Link to Calibration

- 2 Select Calibration**  
Right-click on the 40x objective and select the 40x calibration

## Calibrate a Stage

Before calibrating the stage, make sure to load the correct scale factor for the selected objective and then follow the instructions below.

**1 Define Step Size**  
Go to the Stage Setup tab, located in the XYZ Setup panel and click Step Size

**2 Center Top Left**  
Center an object to the green crosshair and click OK

**3 Center Bottom Right**  
Center the same object to the green crosshair and click OK

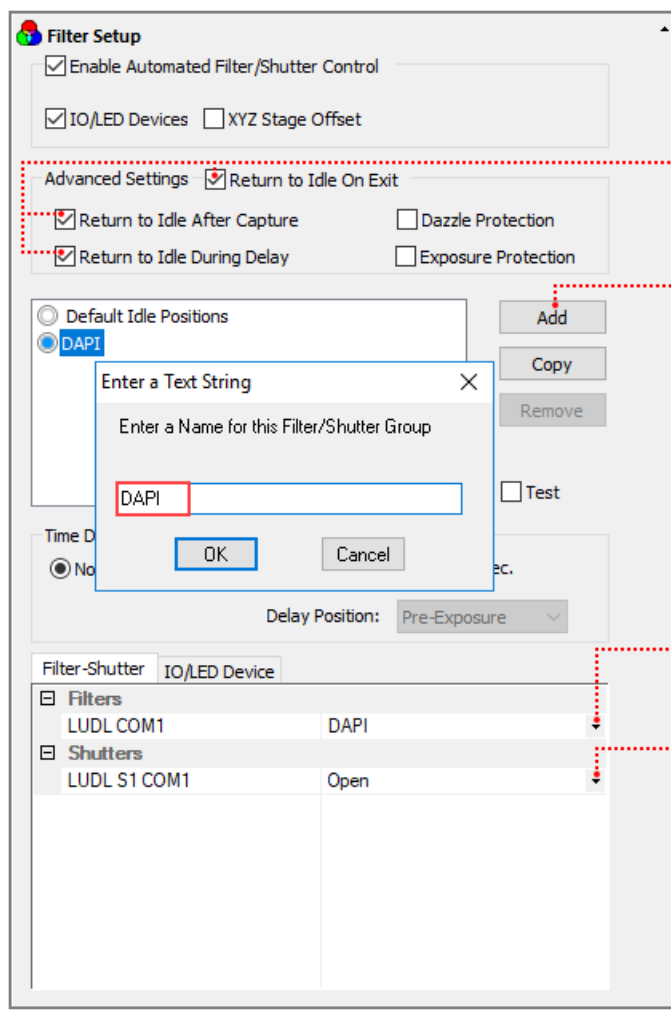
**4 Object Centered**  
If the object moved back to the center of the top left crosshair, the calibration was successful, click OK. If the object did not move to the same location, check the camera orientation to the microscope and repeat Steps 1-3.

## FILTER SETUP

Once the filter device has been added to the profile it will need to be configured in the Filter Setup. The examples below outline the basic steps for configuring two commonly used filter devices, a filter wheel with a shutter and a Lambda DG-4.

### Filter Wheel and Shutter Setup

After the filter wheel and shutter have been added to the profile, go to Filter Setup in the Device pane and follow the instructions below.



The screenshot shows the 'Filter Setup' dialog box. It has several sections: 'Enable Automated Filter/Shutter Control' with checkboxes for 'IO/LED Devices' and 'XYZ Stage Offset'; 'Advanced Settings' with checkboxes for 'Return to Idle On Exit', 'Return to Idle After Capture', 'Return to Idle During Delay', 'Dazzle Protection', and 'Exposure Protection'; 'Default Idle Positions' with a list containing 'DAPI' and buttons for 'Add', 'Copy', and 'Remove'; a 'Time Delay' section with a 'No' radio button; a 'Delay Position' dropdown set to 'Pre-Exposure'; and a table at the bottom for 'Filter-Shutter' and 'IO/LED Device' settings. A small 'Enter a Text String' dialog is open over the 'DAPI' entry in the 'Default Idle Positions' list.

- 1 Enable Return to Idle Conditions**  
Select Return to Idle on Exit, After Capture and During Delay
- 2 Add Filter Group**  
Click Add, enter name and click OK
- 3 Enable Filter Settings**  
Right-click on the filter group that was just created and select a filter tint
- 4 Select Filter Position**  
Select the filter from the list
- 5 Define Shutter Setting**  
Select Open
- 6 Add Remaining Filters**  
Repeat the steps to add the remaining filters
- 7 Define Default Idle Settings**  
Select Default Idle Positions, under Filters select Don't Care and for Shutters select Closed

## Lambda DG-4 Filter Setup as an I/O Device

TTL can be used to control many types of devices. This example explains how to configure a Lambda DG-4 as an I/O Device controlled through the parallel port. In the Device pane go to Filter Setup and follow the instruction below.

**1 Enable IO/LED Devices**  
Select IO/LED Devices

**2 Enable Return to Idle Conditions**  
Select Return to Idle on Exit, After Capture and During Delay

**3 Add Filter Group**  
Click Add, enter name and click OK

**4 Enable Filter Settings**  
Right-click the filter group that was just created and select filter tint

**5 Enable IO Pin Settings**  
Click ellipsis button

**6 Define Pin Settings**  
Select Don't Care (un-check), enable Pin 2 and click OK

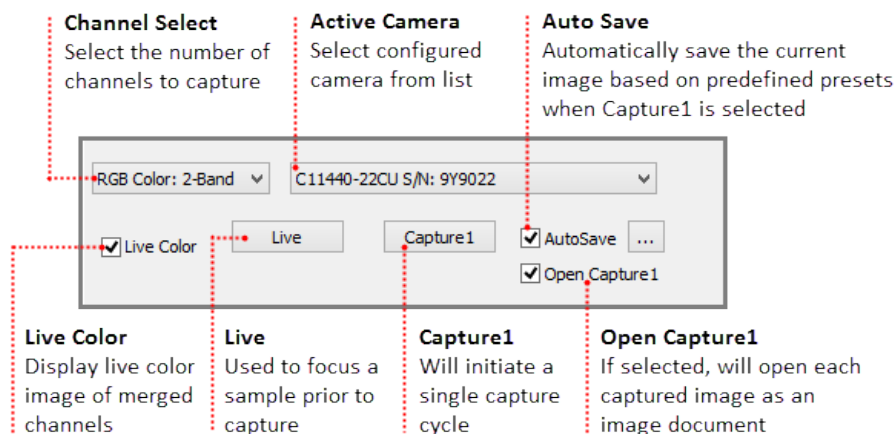
**7 Add Remaining Filters**  
Repeat the steps to add the remaining filters and attenuations using the pin settings in the table below

**8 Define Default Idle Pin Settings**  
Select Default Idle Positions, then for the pin settings select Don't Care (un-check) and click OK

Filter Position	Attenuation		
	100%	50%	33%
1	Pin 2	Pins 2 & 4	Pins 2 & 5
2	Pin 3	Pins 3 & 4	Pins 3 & 5
3	Pins 2 & 3	Pins 2, 3 & 4	Pins 2, 3 & 5
4	Pin 4	Pin 5	Pins 5 & 4

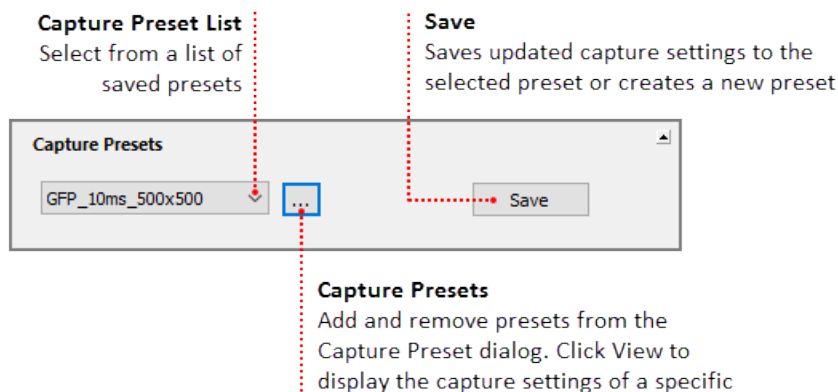
## CAPTURE

The Capture Pane provides a flexible and comprehensive method to access camera features and functionality. The Capture Pane is organized by functionality into panels that can be expanded when in use or collapsed when space is needed. The capture controls at the top of the pane (shown below) are always visible and used for controlling how images are acquired and displayed.



## Capture Presets

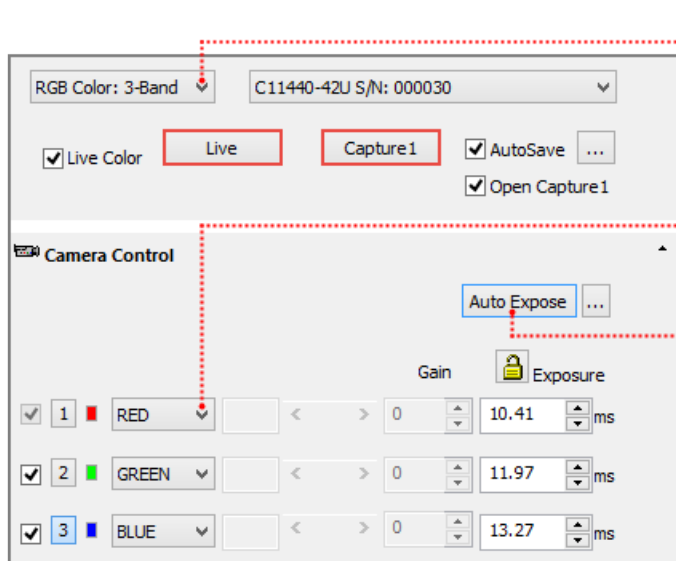
Capture presets save basic settings such as the capture mode, channels, filters, exposure times, as well as output trigger settings and advanced camera properties. For a list of the camera settings that are saved, select a capture preset from the Capture Presets dialog and click View. HCImage will load the capture settings from the previous session when launched.



**Note:** Capture presets are not automatically saved before changing presets or exiting the software. To make changes to a saved capture preset, select the capture preset from the list, adjust the capture settings and click Save.


## Capture a Color Image

Capturing a color image requires filter setup, for instructions on configuring filters, please see "**Filter Setup**" on page 10.



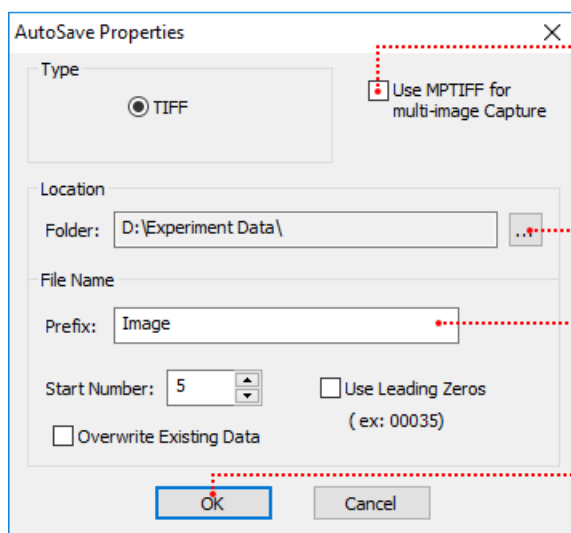
The screenshot shows the 'Camera Control' window. At the top, 'RGB Color: 3-Band' is selected in a dropdown. Below it, 'Live Color' is checked, and 'Live' and 'Capture1' buttons are highlighted with red boxes. To the right, 'AutoSave' and 'Open Capture1' are checked. In the center, 'Auto Expose' is highlighted with a blue box. At the bottom, three filter channels are listed: 1. RED (exposure 10.41 ms), 2. GREEN (exposure 11.97 ms), and 3. BLUE (exposure 13.27 ms). Each channel has a lock icon and a red arrow pointing to it from the 'Exposure' label.

- 1 Select Capture Mode**  
Select RGB Color: 3-Band
- 2 Select Filters**  
Select Red for channel 1, Green for channel 2 and Blue for channel 3
- 3 Adjust Exposure**  
Click Live and adjust the exposure manually or use Auto Expose
- 4 Capture a Color Image**  
Click Capture1

**Hint:** In order to achieve the best possible speed when acquiring color images, set the same exposure for each channel. Once each of the exposures have been entered, click the Exposure Lock icon (  ) to lock the exposure settings. Now any exposure adjustments will be made to all of the channels.

## How to use AutoSave

Enabling AutoSave will automatically save the current image every time Capture1 is selected. The captured image is saved as a TIFF based on the file name and destination directory defined in the AutoSave Properties dialog. Enable AutoSave and then click on the ellipses to open the AutoSave Properties dialog.



The screenshot shows the 'AutoSave Properties' dialog box. At the top, 'Type' is set to 'TIFF'. Below it, 'Use MPTIFF for multi-image Capture' is checked. In the 'Location' section, 'Folder' is set to 'D:\Experiment Data\'. In the 'File Name' section, 'Prefix' is set to 'Image'. At the bottom, 'Start Number' is set to 5, and 'Use Leading Zeros' is checked. The 'OK' button is highlighted with a blue box.

- 1 TIFF or MPTIFF**  
Enable to save as MPTIFF for multiple image capture versus individual TIFF images
- 2 Set Location**  
Click the ellipsis icon and navigate to the destination directory
- 3 Set Default File Name**  
Enter file name
- 4 Save Settings**  
Click OK

## Define a Custom SubArray for Maximum Speed

Click Live, focus on the sample and move the area of interest into the center of the image. Follow the steps below to define a custom subarray.

1

**Define SubArray**  
Click Define and draw the area on the image

2

**Center on Sensor**  
Right-click and select Center on Image

3

**Apply SubArray**  
Click Apply

**Binning and SubArray**

Binning: 1

☒ Adjust Exposure Time

Sub-Array

Preset Sizes 2048 x 2048

Reset

Define

Apply

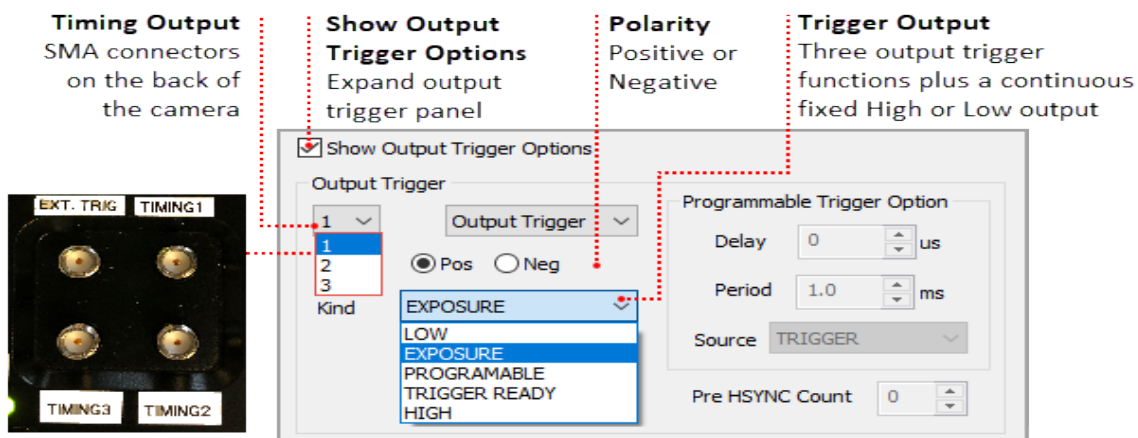
X0 0 Width 2048

Y0 0 Height 2048

**Note:** Centering the subarray for maximum speed is only required for the ORCA-Flash 4.0 series cameras.

## Control an LED using Output Trigger from the Camera

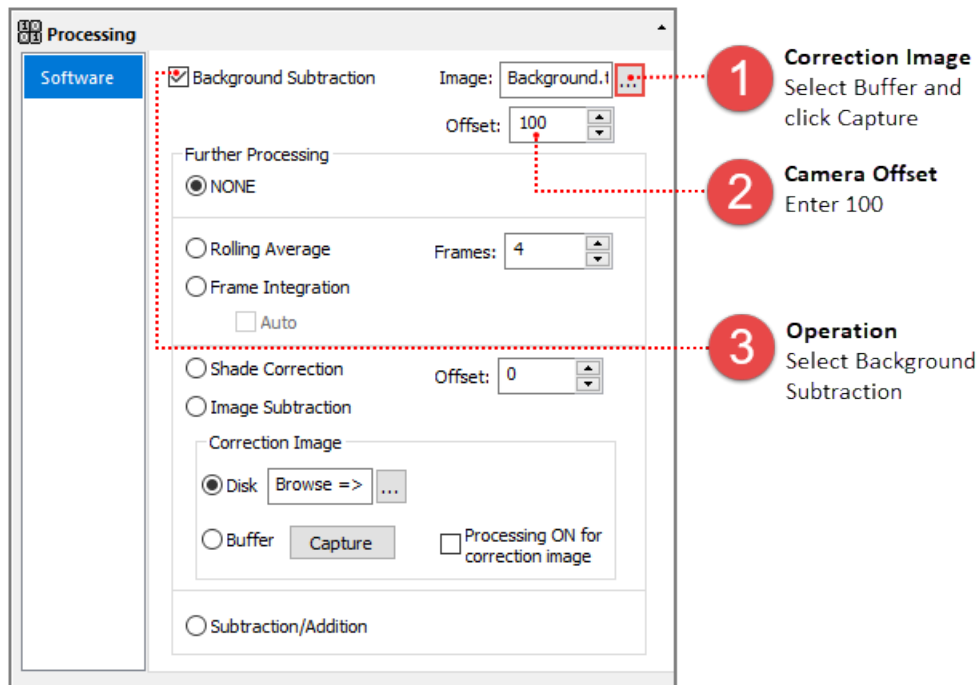
Some cameras provide a range of output trigger signals to synchronize with an external instrument where the camera becomes the master and the external instrument becomes the slave.



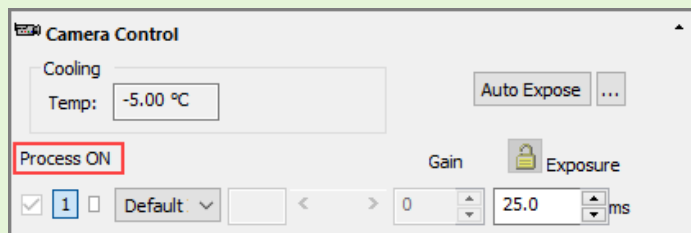


## How to Setup a Background Subtraction

Typically used in fluorescence microscopy, a background subtraction can be used when the image presents a dark non-uniform background. To perform a background subtraction click Live, bring the sample into focus and then move the stage off of the sample so that only the background is visible. Next, follow the steps below, when finished move the stage to bring the sample into view and the background subtraction is applied.

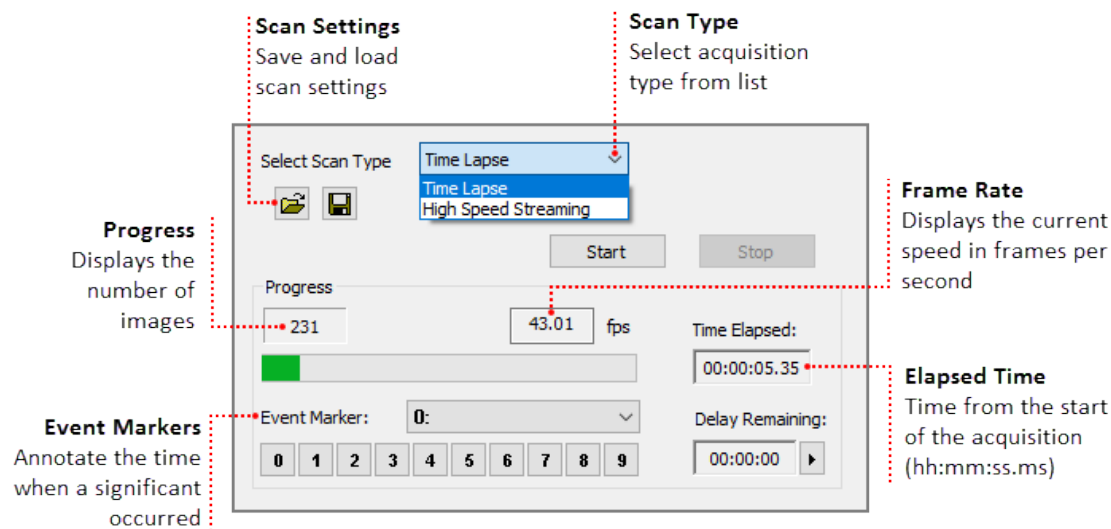


**Hint:** HcImage remembers the capture settings from the previous session, if background subtraction was left enabled, Process ON will be displayed in the Camera Control panel. The display image may appear distorted or black.



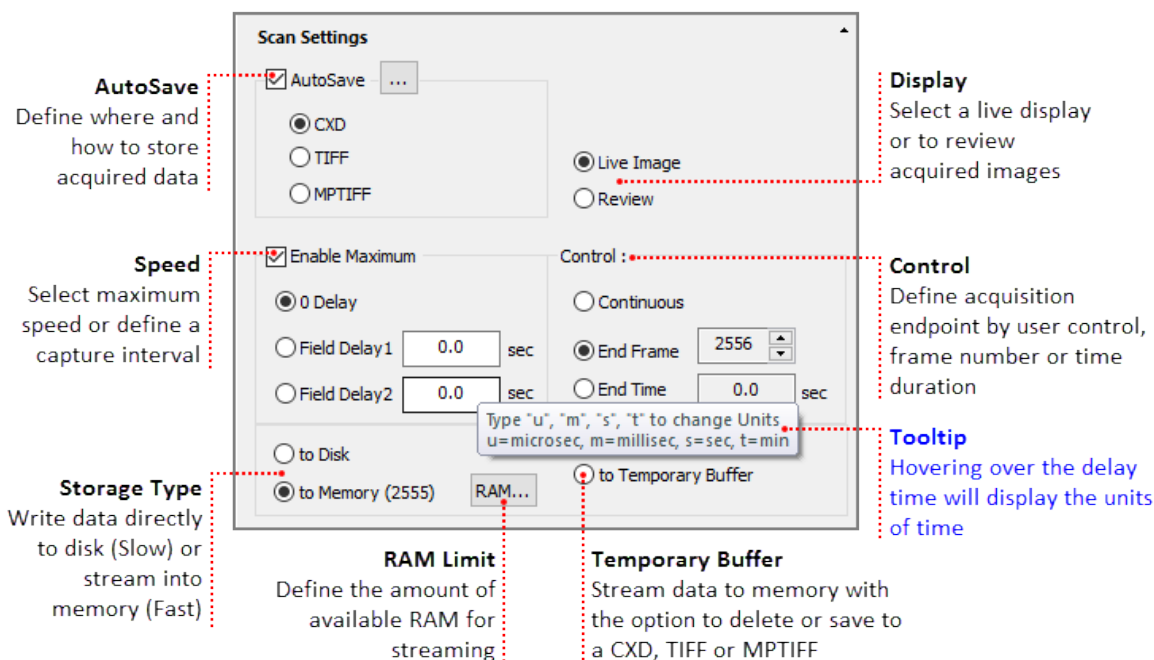
## SEQUENCE

The Sequence pane provides a variety of options for defining a time lapse or high speed streaming. The sequence controls at the top of the pane (shown below) are always visible and used for selecting the scan type and reporting in real time, information about an ongoing sequence. This sections covers the basic steps for setting up a typical time lapse and high speed streaming.



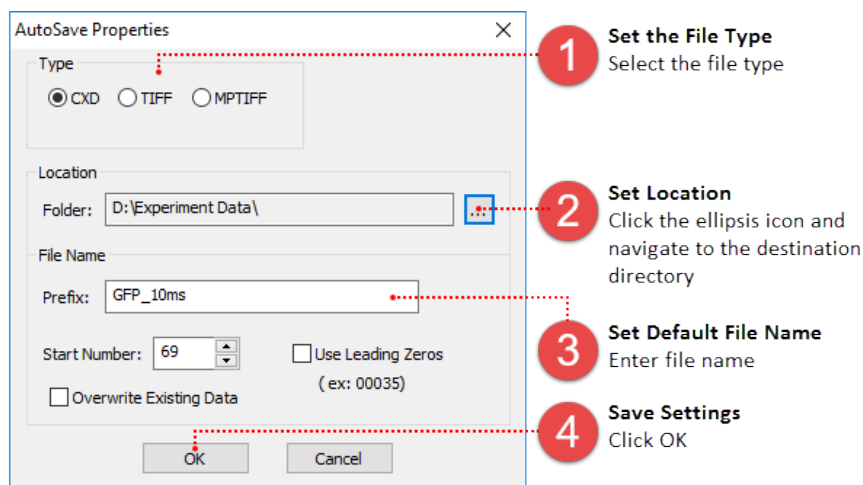
## Setting up a Time Lapse

The Scan Settings panel provides a variety of options for defining a time lapse to fit the needs of your application. This section provides three examples of typical time lapse settings, using each of the storage options.



## How to Use AutoSave

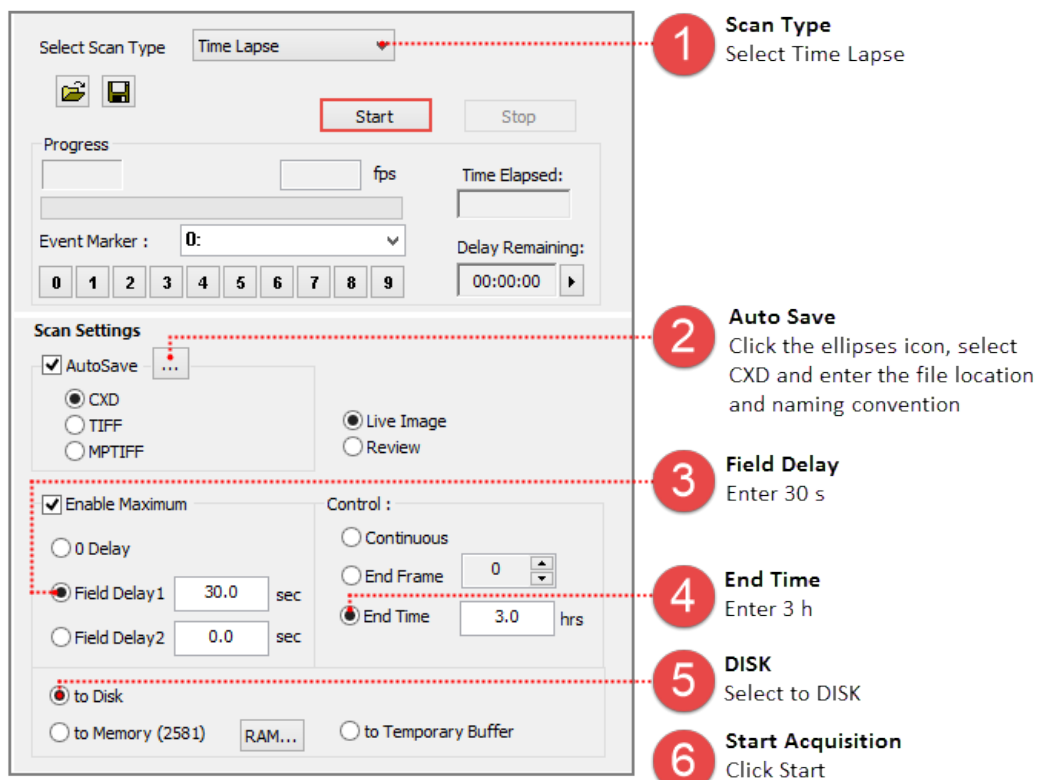
In the AutoSave Properties dialog, the user can determine how and where to store the acquired data. Image data can be saved as a CXD, TIFF or MPTIFF. The example below provides a description of the Auto Save Properties dialog.



**Note:** MPTIFF files have a 65,000 image limit or 4 GB size limit. For image sequences exceeding these limits, multiple MPTIFF files will be saved and numbered sequentially.

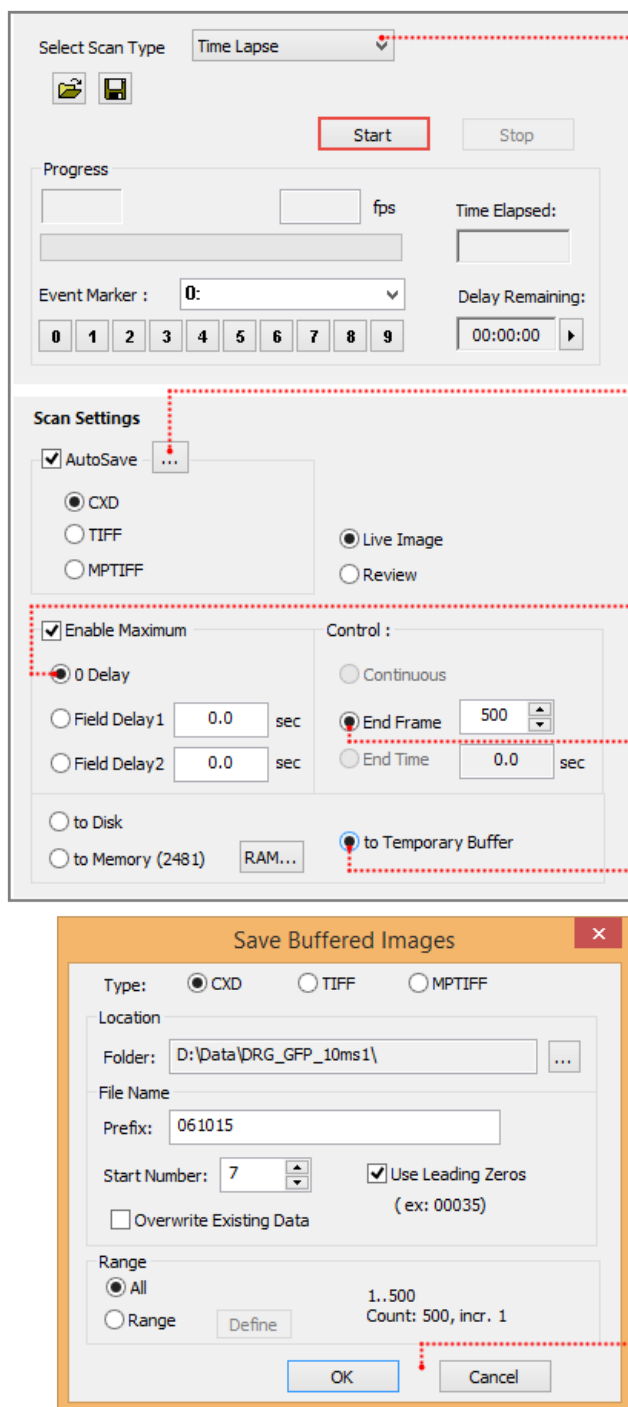
## Setup a Time Lapse - Save to Disk

The time lapse in this example will acquire an image every 30 seconds for 3 hours and the data will be saved as a cxd. Once you are satisfied with capture settings and the sample is in focus, go to the Sequence pane and follow the steps below.



## Setup a Time Lapse - Save to the Temporary Buffer

Acquired data is stored in memory with the option to review the image sequence before saving or deleting it. When Temporary Buffer is selected, End Frame is automatically enabled and display the maximum number of frames that can be streamed to memory. Once you are satisfied with capture setting and the sample is in focus, go to the Sequence pane and follow the steps below.



**1 Scan Type**  
Select Time Lapse

**2 Auto Save**  
Click the ellipses icon, select CXD and enter the file location and naming convention

**3 Field Delay**  
Select 0 Delay

**4 End Frame**  
Enter 500

**5 Temporary Buffer**  
Select to Temporary Buffer

**6 Start Acquisition**  
Click Start

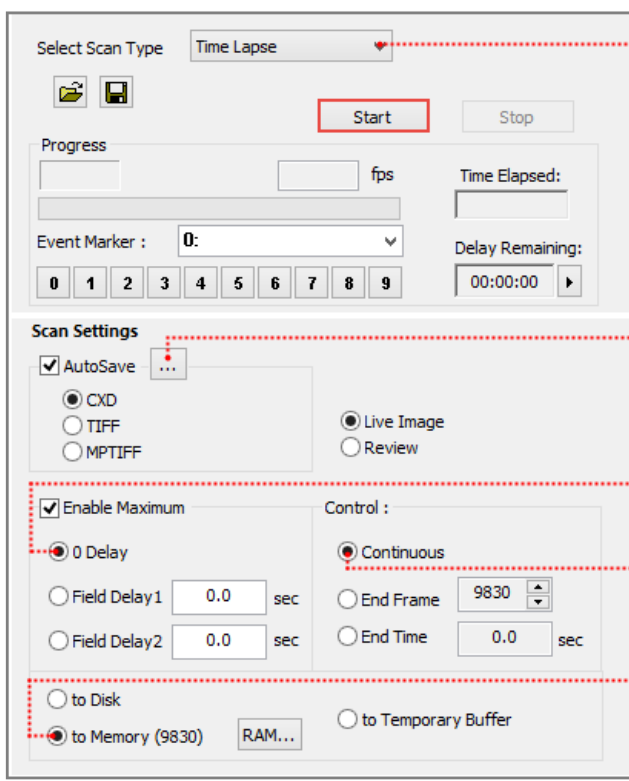
**7 Acquisition Complete**  
Review acquired data using the playback controls in the Image Display

**8 Save or Delete**  
Save - click OK  
Delete - click Cancel

**Note:** Streaming to the Temporary Buffer is very useful because it provides the option to review the image sequence when trying to capture specific event and for demonstrating camera speeds.

## Setup a Time Lapse - Save to Memory

The time lapse in this example will store images in memory until the acquisition is stopped or runs out of memory at which point the acquired images are saved to disk for the remainder of the time lapse. Once you are satisfied with capture settings and the sample is in focus, go to the Sequence pane and follow the steps below.



**1 Scan Type**  
Select Time Lapse

**2 Auto Save**  
Click the ellipses icon, select CXD and enter the file location and naming convention

**3 Field Delay**  
Select 0 Delay

**4 Continuous**  
Select Continuous

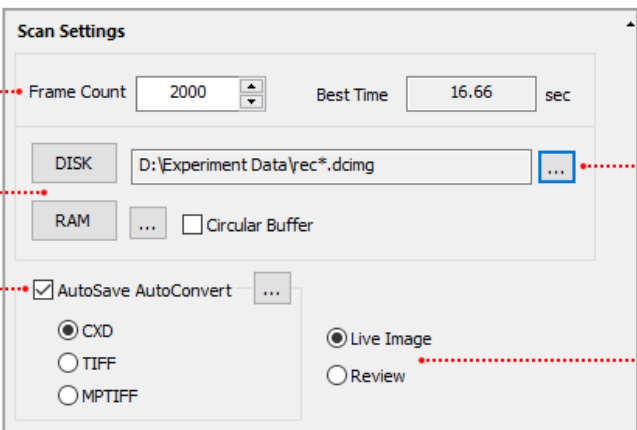
**5 Memory**  
Select to Memory

**6 Start Acquisition**  
Click Start

## High Speed Streaming

High Speed Streaming is used to obtain the fastest acquisition speed from the camera. This scan is optimized for single channel streaming to RAM or directly to the computer's solid state drives (SSD) configured in a RAID 0.

**Note:** Acquisition rates will vary based on the PC configuration, for information about the computer requirements, please see the [PC Recommendations for ORCA-Flash4.0 V3 / LT+](#).



**Control**  
Enter the number of frames to acquire and the approximate end time is displayed to the right

**Stream Type**  
Stream directly to HDD or into memory with option to use Circular Buffer

**AutoSave/AutoConvert**  
Define how streamed data is handled

**DCIMG Location**  
Set a file location for streaming data to DISK

**Display**  
Select a live display or to review acquired images

**Note:** High Speed Streaming does not support multi-channel acquisition, camera registration features (i.e., flip, rotation and pixel shift) or software processing operations (e.g., shade correction and rolling average).

### Steps for Streaming to Disk

When streaming to disk, a temporary file (.dcimg) is created to store the data while it is being acquired, the temporary file location needs to be located on the RAID array, SSD drive, or the fastest drive available. Configure the capture settings, go to the Sequence pane and follow the steps below.

The screenshot shows a software interface for configuring streaming to disk. It includes a 'Select Scan Type' dropdown set to 'High Speed Streaming', a 'Start' button, and a 'Stop' button. Below these are progress indicators for 'Progress' (0 fps), 'Time Elapsed', 'Event Marker', and 'Delay Remaining'. The 'Scan Settings' section contains a 'Frame Count' of 1000, 'Best Time' of 9.9003 sec, and options for 'DISK' (selected) and 'RAM'. The 'DISK' option shows a file path 'D:\Experiment Data\DCIMG\rec\*.dcimg'. There are also checkboxes for 'AutoConvert' (checked) and 'Circular Buffer'. At the bottom, there are radio buttons for 'CXD', 'TIFF', 'MPTIFF', 'Live Image', and 'Review'. Five numbered steps are overlaid on the interface: 1. Select Scan Type (High Speed Streaming), 2. Enter Frame Count (1000), 3. Select Stream Type (DISK), 4. Auto Convert File Type (AutoConvert checked), and 5. Start Streaming (Click Start).

- 1 Select Scan Type**  
Select High Speed Streaming
- 2 Enter Frame Count**  
Enter the number of images to acquire
- 3 Select Stream Type**  
Select DISK
- 4 Auto Convert File Type**  
Enable AutoConvert and select file type
- 5 Start Streaming**  
Click Start

**Note:** To leave the streamed data as a DCIMG file disable AutoConvert.

## Steps for Streaming to RAM

Acquired data is stored in memory with the option to review the image sequence before saving or deleting it. In the AutoSave Properties dialog, the user can determine how and where to store the acquired data. Once you are satisfied with capture settings and the sample is in focus, go to the Sequence pane and follow the steps below.

**Note:** The Circular Buffer stores streamed data in memory, once the frame count has been reached, the previous acquired data is replaced sequentially. The cyclic process repeats until the acquisition is stopped, leaving the most recent images stored in RAM.

The screenshot shows a software window with the following sections and steps:

- 1 Select Scan Type**: A dropdown menu is set to "High Speed Streaming".
- 2 Enter Frame Count**: The "Frame Count" field in the "Scan Settings" section is set to "1000".
- 3 Select Stream Type**: The "RAM" option is selected under the "Stream Type" section.
- 4 Auto Save File Type**: The "AutoSave" checkbox is checked, and the "CXD" file format is selected.
- 5 Start Streaming**: The "Start" button is highlighted with a red box.

Other visible elements include a "Progress" section with a "0" value and "fps" label, a "Time Elapsed:" field, an "Event Marker:" dropdown, a "Delay Remaining:" field with a "00:00:00" timer, and a "Best Time" field with a "9.9003 sec" value.

## IPA OVERVIEW

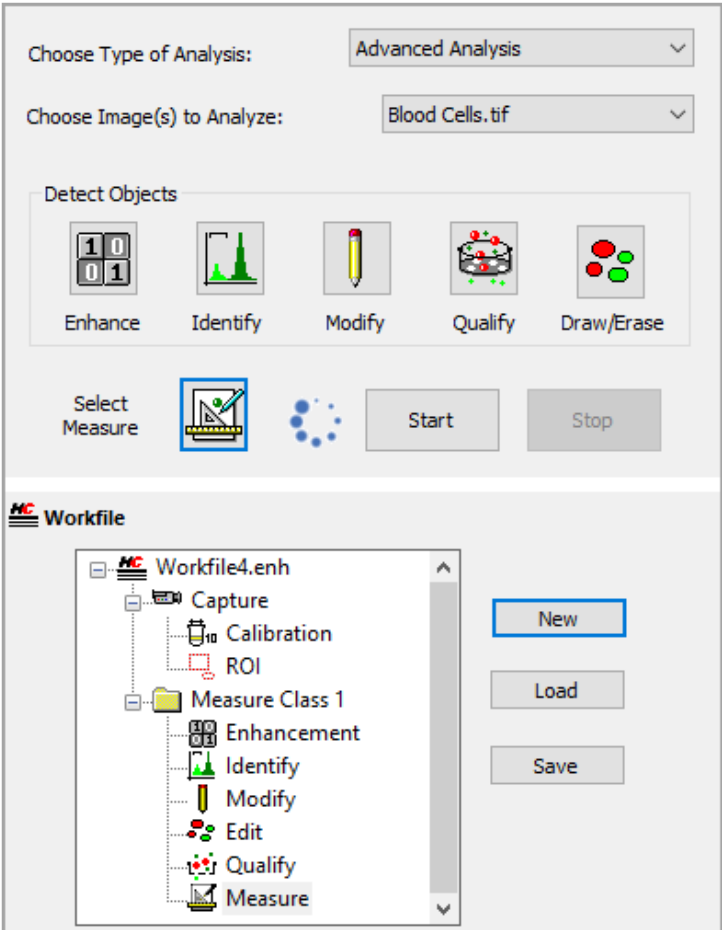
Image Processing and Analysis provide an extensive selection of image processing and image analysis tools to enable quantitative analysis on a wide range of complex image sequences. Imaging tools are selected using customized icons to derive workfiles (macros), which are saved and can be used multiple times. Images are saved with measured data allowing dynamic interaction between images, objects, graphs and tables to provide instant user feedback.

### Understanding the Workspace

The Image Processing and Analysis functionality is accessed through the Analysis pane by selecting Advanced Analysis from the Choose Type of Analysis list. In addition to the Advanced Analysis, Single Image Measure and Sequence Intensity Analysis are also available. For Single Image Measure and Sequence Intensity Analysis the user can select from Simple Analysis and Advanced Analysis modes by going to View in the menu bar, then highlight Analysis Mode and select Simple or Advanced. The Simple modes provides a variety of drawing tools that can be used to identify objects of interest. The Advanced mode described in the example below.

#### Advanced Analysis

For Advanced Analysis, the measurement algorithm is set up by configuring an icon-driven workfile. This is done by adding steps in an interactive process and observing the effects on the identified image objects as the various steps are added and modified as shown below. The procedure is methodical, where the operator selects each option interactively. The steps used can be saved in a workfile (.enh) for later reuse, review, or modification.



The screenshot displays the 'Advanced Analysis' workspace. At the top, 'Choose Type of Analysis' is set to 'Advanced Analysis' and 'Choose Image(s) to Analyze' is 'Blood Cells.tif'. Below, the 'Detect Objects' section contains icons for Enhance, Identify, Modify, Qualify, and Draw/Erase. The 'Select Measure' icon is highlighted. The 'Workfile' pane shows a sequence of steps: Capture, Calibration, ROI, Measure Class 1, Enhancement, Identify, Modify, Edit, Qualify, and Measure. To the right, a series of six images illustrate the process: 1. Original grayscale image of blood cells. 2. Binary image with cells highlighted in green. 3. Binary image with cells highlighted in green, showing improved object size, shape, and connectivity. 4. Binary image with cells highlighted in green, showing objects drawn or erased. 5. Binary image with cells highlighted in green, showing objects qualified based on size, shape, position, etc. 6. Binary image with cells highlighted in green, showing multiple objects measured in a single image.

**Enhance** the image using image processing filters

**Identify** objects of interest with a binary image layer

**Modify** the binary layer to improve object size, shape and connectivity

**Draw/Erase** objects using drawing tools

**Qualify** objects based on size, shape, position, etc.

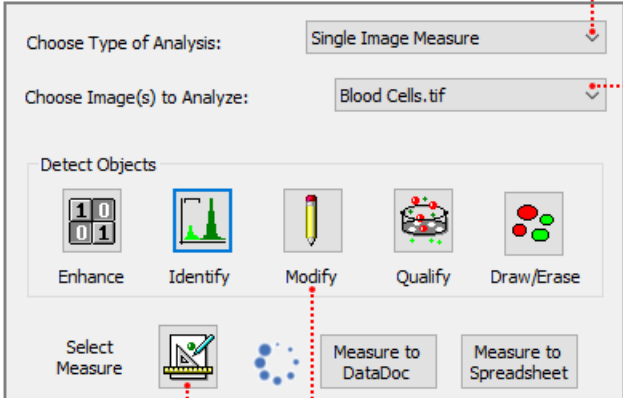
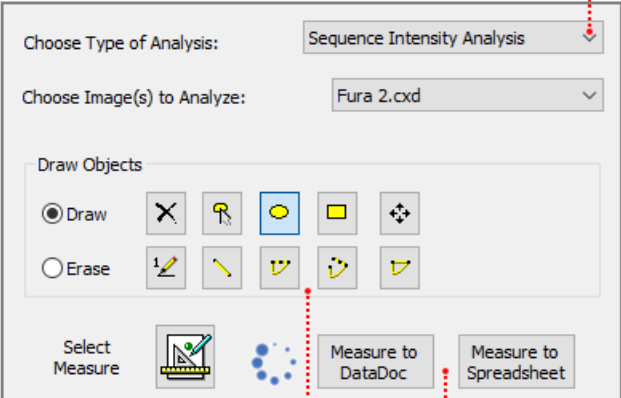
**Measure** multiple objects in a single image

AREA.1	MEAN.1
532.000000	105.492
781.000000	110.521
573.000000	105.324
420.000000	110.206



## Single Image measure

For Single Image Measure, measure the size, shape, intensity, position or create a custom measurement of multiple objects in a single image. The image can be from the Live Scan (image display), part of an image sequence, or a single standalone image. The object of interest can be identified using the Advanced Analysis mode or drawn using the Simple Analysis mode (both shown below). The Simple mode is active by default but can be changed by clicking View on the Menu bar, highlighting Analysis Mode and selecting Advanced. The measured data can be saved to data document (.cxd) or to a spreadsheet.

<b>Single Image Measure</b> Measure the area, length and intensity of multiple objects in a single image	<b>Choose Data to Analyze</b> Select from open files or choose Live Scan to use the image in the Image Display	<b>Sequence Intensity Analysis</b> Measure the intensity of a single object through an image sequence	
			
<b>Measurements</b> Select the measurements to collect	<b>Advanced Analysis Mode</b> Detect large numbers of objects and objects not easily differentiated using an interactive set of tools	<b>Simple Analysis Mode</b> Use the drawing tools to identify objects of interest. The Clone tool is very useful for drawing objects of the same size	<b>Output Format</b> Select whether to save the data to a data document or a spreadsheet

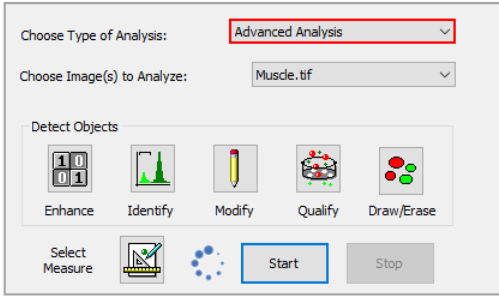
## Sequence Intensity Analysis

For Sequence Intensity Analysis, measure the intensity of a single object over time in an image sequence. The object of interest can be identified using the Advanced Analysis mode or drawn using the Simple Analysis mode (both shown above). The Simple mode is active by default but can be changed by clicking View on the Menu bar, highlighting Analysis Mode and selecting Advanced. If multiple areas are drawn or identified, they are treated as a single object. The measured data can be saved to data document (.cxd) or to a spreadsheet.

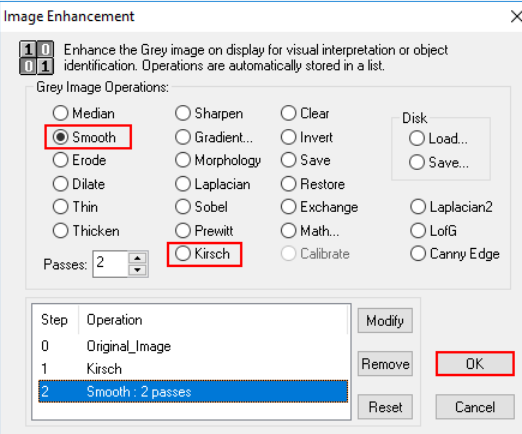
# IPA EXAMPLES


## Advanced Analysis - Muscle Fiber Example

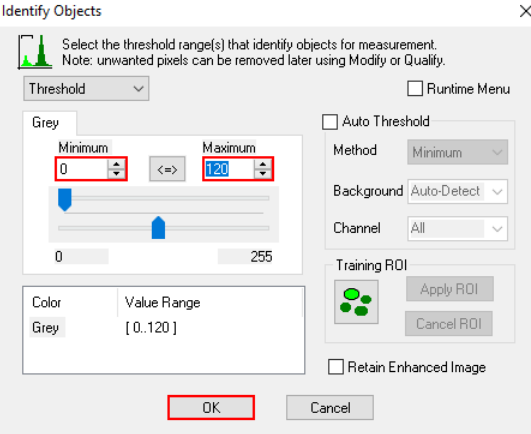
The example below explains how to create a Workfile for measuring the size and shape of muscle fibers.




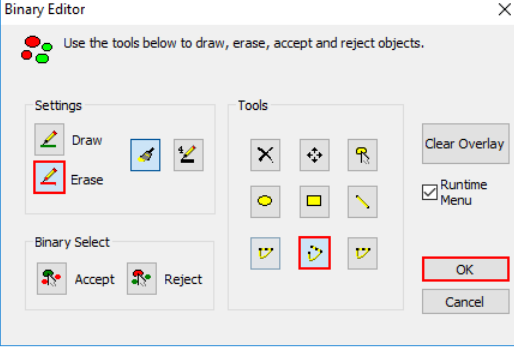
**1 Enable Advanced Analysis**  
Open the Muscle.tif and select Advanced Analysis from the drop-menu.




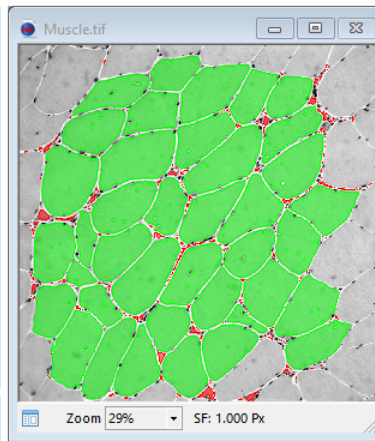
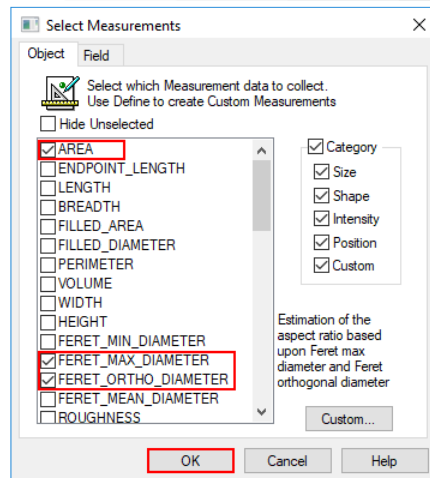
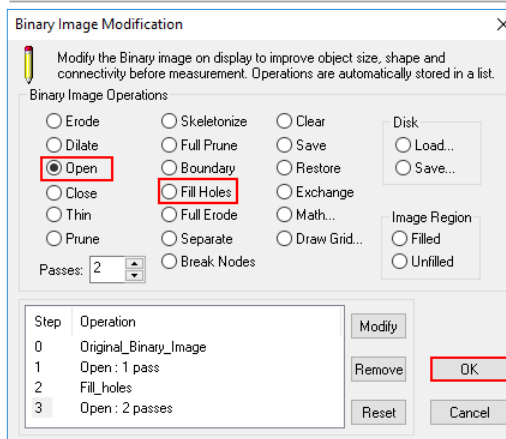
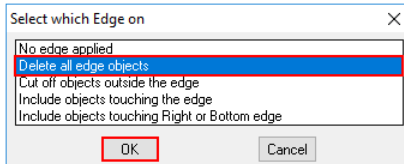
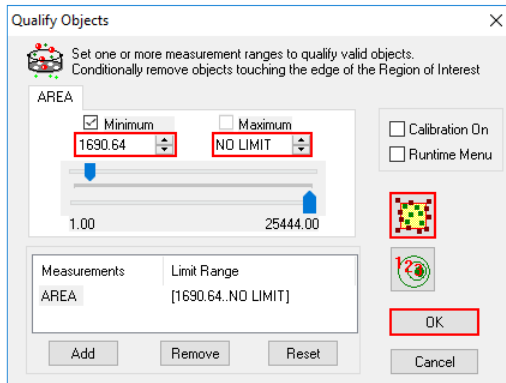
**2 Enhance the Image**  
Click the Enhance icon , apply a Kirsh and two passes of the Smooth filter to the image. Click OK.





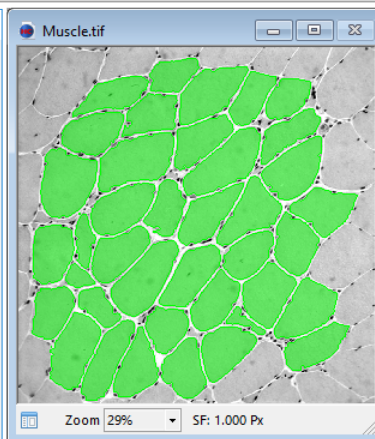
**3 Identify Objects of Interest**  
Click the Identify icon , adjust the min to 0 and the max to 120, covering the muscle fiber with a green overlay and click OK.




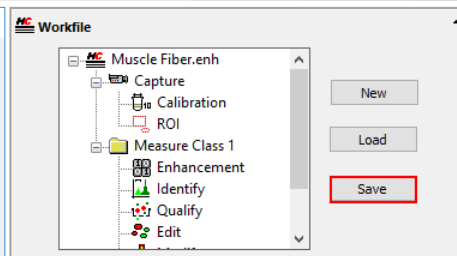
**4 Separate Muscle Fibers**  
Click the Draw/Erase icon , click Erase, select the Open Polygon tool and erase any connections between fibers by drawing a line across the connection and right-click to apply. Click OK.




- 5 Remove Unwanted Objects**  
Click the Qualify icon , click the Edge Objects icon , select Delete all edge objects and click OK. Adjust the min area to 1690 to remove the smaller objects and click OK.



- 6 Modify the Binary Image**  
Click the Modify icon , apply an Open, select Fill Holes, apply Open two passes and click OK.

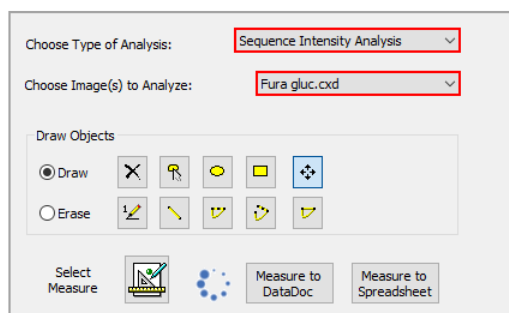


- 7 Select Measurements**  
Click the Measure icon , select Area, Feret Max Diameter, Feret Ortho Diameter, Feret Elongation and Feret Aspect Ratio. Click OK.

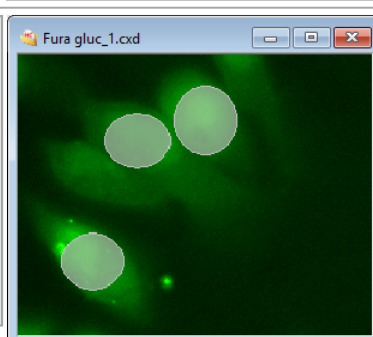
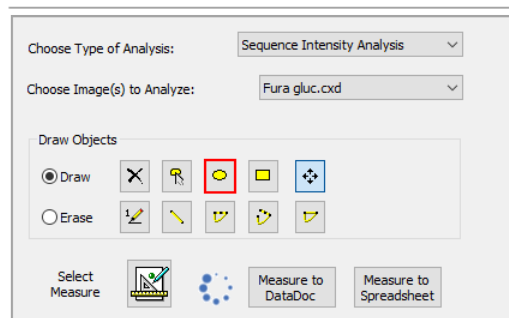
- 8 Save and Run the Workfile**  
Click the Save button and click Start.

## Sequence Intensity Analysis - Simple Mode

Sequence Intensity Analysis will measure the intensity of a single object in the image sequence. If multiple areas are drawn or identified, they are treated as a single object. HImage has two modes, the Simple mode is active by default but can be changed by clicking View on the menu bar, then highlighting Analysis Mode and selecting Advanced.

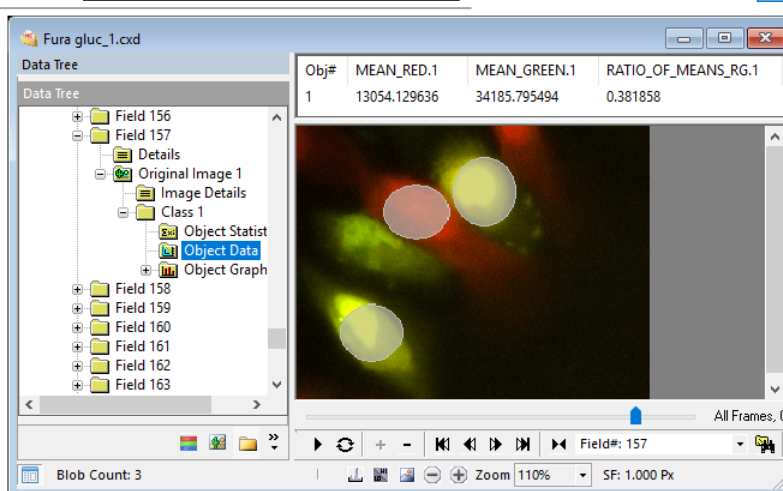
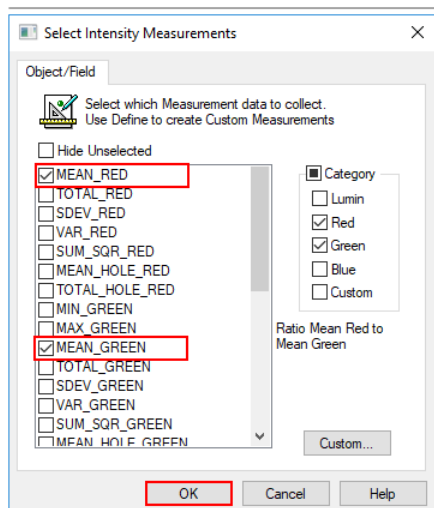


- 1 Enable Sequence Intensity Analysis**  
Open the dataset and select Sequence Intensity Analysis from the drop-menu.

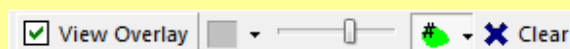


- 2 Draw Object**  
Click the Ellipse icon and manually identify the object of interest.  
**Tooltip**  
Press SHIFT to draw a circle.

- 3 Analyze Objects of Interest**  
Click the Measure icon, select measurements, click OK and select Measure to DataDoc.



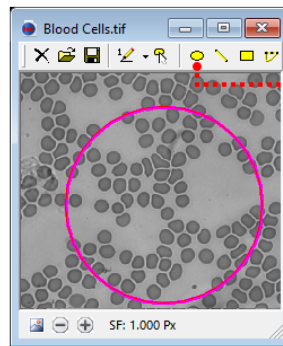
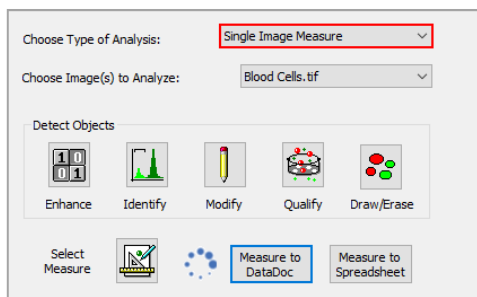
**Note:** Change the overlay color to silver instead of green in the **Change Overlay Color** icon. Use the translucency slider to adjust the overlay transparency or hide it by selecting **View Overlay**. Click **Clear** to delete the overlay.




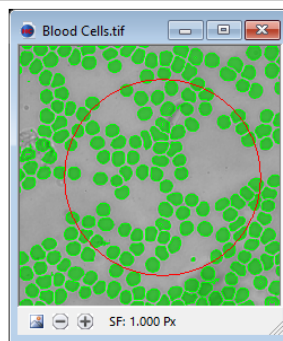
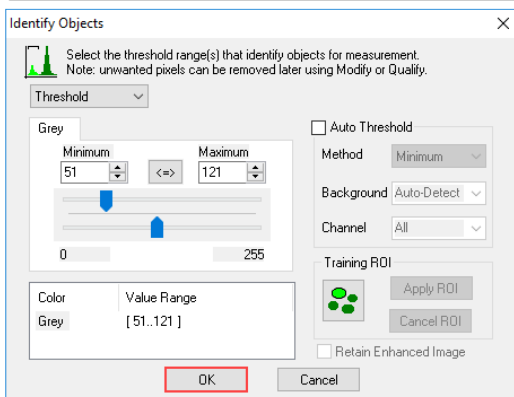



## Single Image - Measure Analyze Objects Inside of a ROI

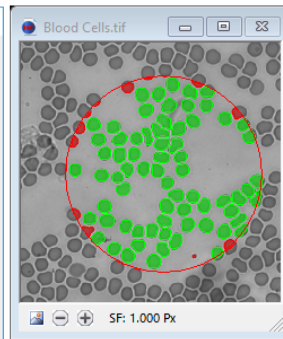
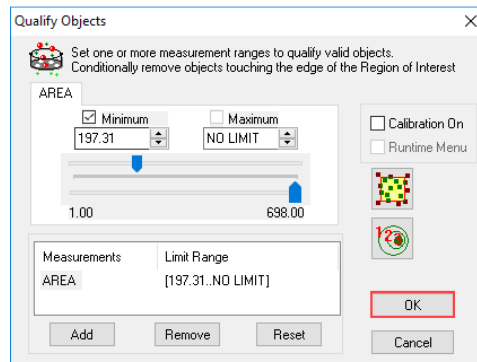
Enable the Advanced Analysis mode by clicking View on the Menu bar, then highlighting Analysis Mode and selecting Advanced. Open an image, go to the Analysis pane and select Single Image Measure from the drop-down list. Click on the image and follow the instructions below.






- 1 Define Region of Interest**  
Activate the Image region layer , click the ellipse icon and draw region of interest

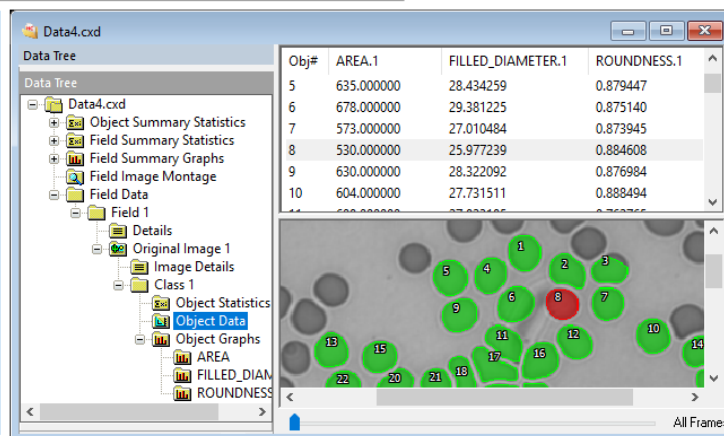
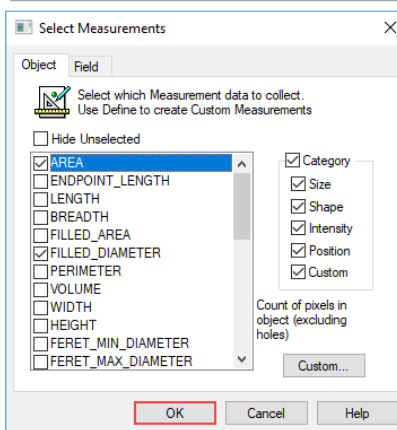


- 2 Identify Objects of Interest**  
Click the Identify icon , adjust the Min and Max sliders until the objects of interest are covered by the green overlay and click OK



- 3 Remove Unwanted Objects**  
Click the Qualify icon , use the Min and Max sliders to reject objects and click OK

- 4 Analyze Objects of Interest**  
Click the Measure icon , select measurements, click OK and select Measure to DataDoc 



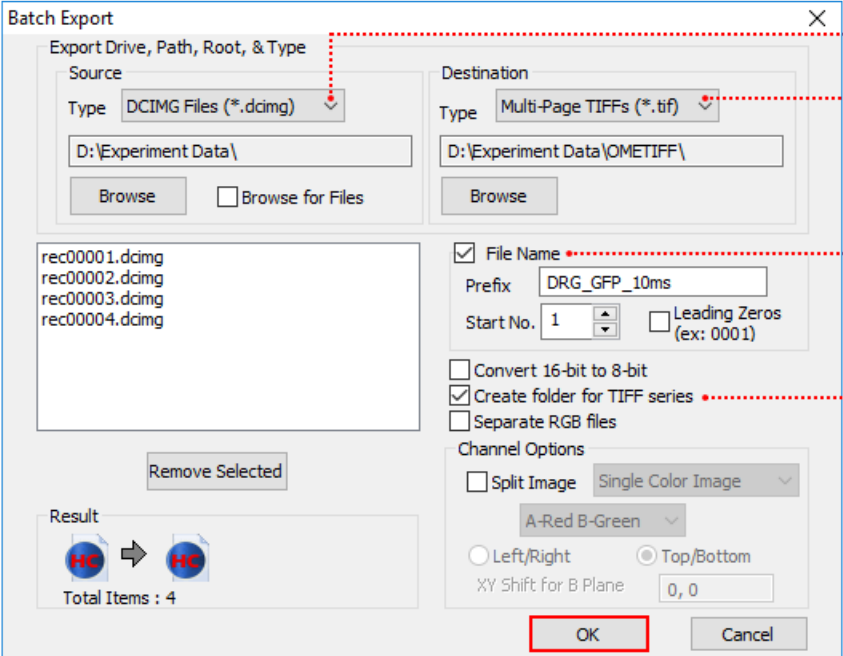
## BATCH EXPORT

When batch exporting all of the source files must be in the same directory and of the same file type. Source file types include: Data documents (.cxd), Movies (.avi), Multi-Page TIFFs (.tif) and DCIMG Files (.dcimg). Destination file types include: Data documents (.cxd), Movies (.avi), TIFF Files (.tif), Multi-Page TIFFs (.tif), OME-TIFF (.tif), Bitmap (.bmp), JPEG (.jpg), and PNG (.png).

**Note:** MPTIFF files have a 65,000 image limit and 4 GB size limit. For image sequences having more than 65,000 images or larger than 4 GB, multiple MPTIFF files will be saved and numbered sequentially.

### Batch Export DCIMG to MPTIFF

In the File menu select Batch Export and follow the instructions below. The exported files are not automatically opened in the software.



The screenshot shows the 'Batch Export' dialog box with the following settings and annotations:

- 1 Enter Source Location:** Type: Select DCIMG Files. Browse: Go to the file directory. (Points to the Source Type dropdown and the Source path field).
- 2 Enter Destination Location:** Type: Select Multi-Page TIFF Files. Browse: Go to output directory. (Points to the Destination Type dropdown and the Destination path field).
- 3 Define Output File Name:** Define the file naming convention. (Points to the File Name section, including Prefix, Start No., and Leading Zeros).
- 4 Enable Create Series Folder:** Select Create folder for TIFF series. (Points to the 'Create folder for TIFF series' checkbox).
- 5 Export to MPTIFF:** Click OK. (Points to the OK button).

Other visible settings in the dialog include:

- Source Type: DCIMG Files (\*.dcimg)
- Source Path: D:\Experiment Data\
- Destination Type: Multi-Page TIFFs (\*.tif)
- Destination Path: D:\Experiment Data\OMETIFF\
- File Name: ☒ File Name, Prefix: DRG\_GFP\_10ms, Start No.: 1, Leading Zeros: ☐ (ex: 0001)
- Convert 16-bit to 8-bit: ☐
- Create folder for TIFF series: ☒
- Separate RGB files: ☐
- Channel Options: ☐ Split Image, Single Color Image (selected), A-Red B-Green (selected), Left/Right (selected), Top/Bottom (selected), XY Shift for B Plane: 0, 0
- Result: HC icon, Total Items: 4
- Buttons: Remove Selected, OK, Cancel


**Note:** Depending on the destination file type, certain options are available.

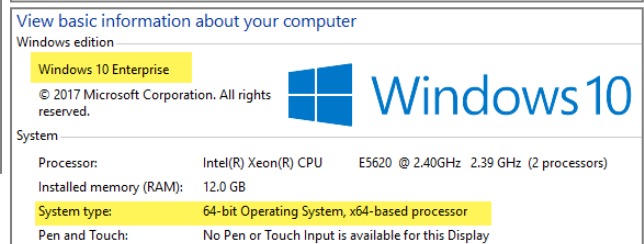
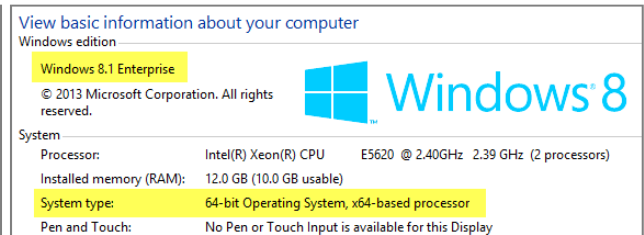
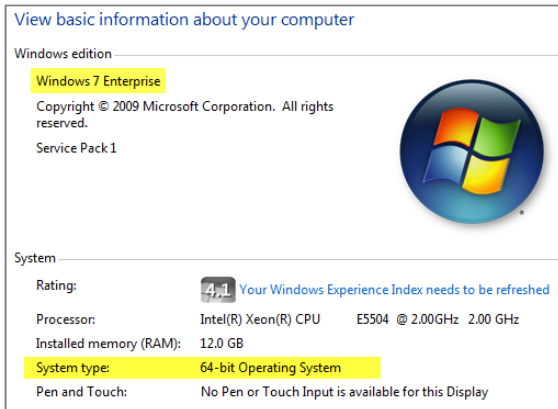
- **Convert 16-bit to 8-bit:** Converts 16-bit monochrome images to 8-bit and will convert 48-bit color images to 24-bit. All movies (.avi) are exported 8- or 24-bit files.
- **Create folder for TIFF series:** Creates a folder for each exported TIFF or Multi-page TIFF series (i.e., one folder is created for each source file). This option is turned on by default but can be disabled.
- **Video Compression:** Use a video compression algorithm when converting to movie file format. The compression algorithms are based on the video codecs installed on the computer. Video compression is only available when exporting to movies (.avi).

# TROUBLESHOOTING

## System Information

### What version of Windows is installed?

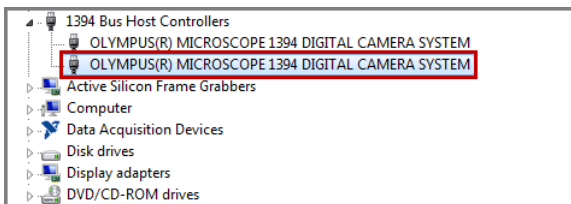
Press the **Windows Logo**  + **Pause/Break** keys to view the System Properties window. The Windows System Properties displays a basic overview of the computer including Windows edition and System type (i.e., 32-bit or 64-bit).



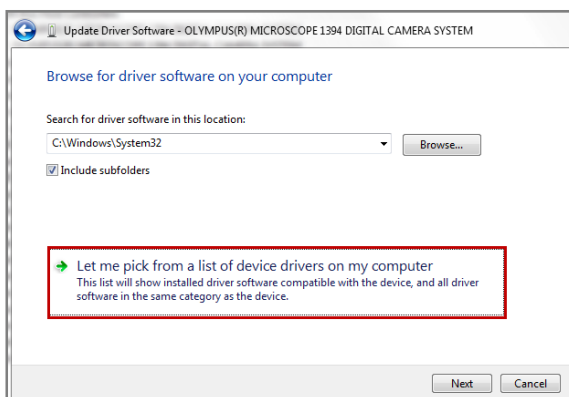
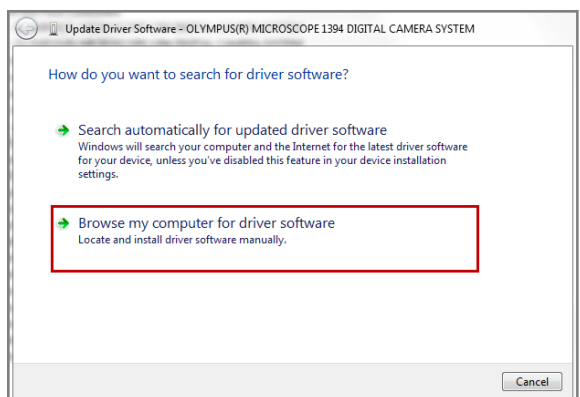
## Unable to communicate with Hamamatsu 1394 camera

### Was the Hamamatsu 1394 driver re-installed after installing the Olympus drivers?

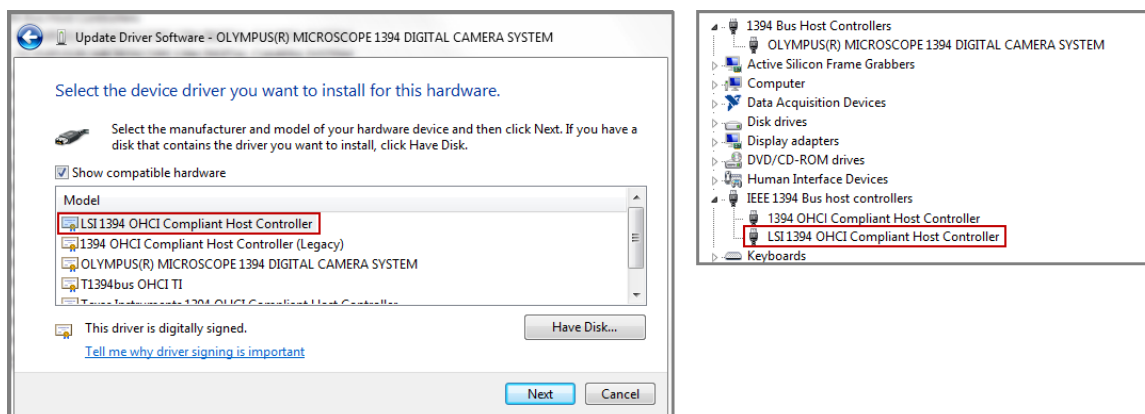
1. Right-click on **My Computer**, select **Manage** and select **Device Manager** in the System Tools list.
2. Right-click on the appropriate 1394 controller and select **Update Driver Software**.



3. Choose **Browse my computer for driver software**
4. Choose **Let me pick from a list of device drivers on my computer**



- Choose the DCAM compatible driver - **LSI 1394 OHCI Compliant Host Controller** and click **Next** to install the driver.



## Light remains on during delay or after capture

### Is the light source shuttered or is it an IO/LED device?

Review the Filter Setup setting below. The example on the left is for a shuttered light source and the example on the right is for an IO/LED device.

