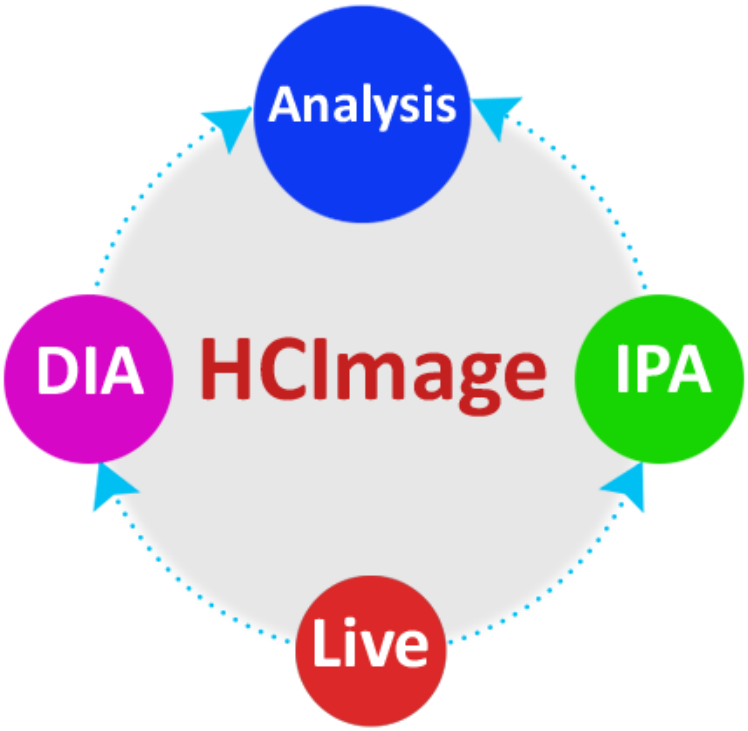
HAMAMATSU
PHOTON IS OUR BUSINESS

HCImage Live

Getting Started Guide



Release 5.0

November 2022

Hamamatsu Corporation
360 Foothill Road, Box 6910
Bridgewater, NJ 08807-0910
USA
+1 908.231.0960
sales@hamamatsu.com
https://camera.hamamatsu.com/us/en/index.html

Software Support
hcsupport@hamamatsu.com
www.hcimage.com

Table of Contents

INSTALLATION

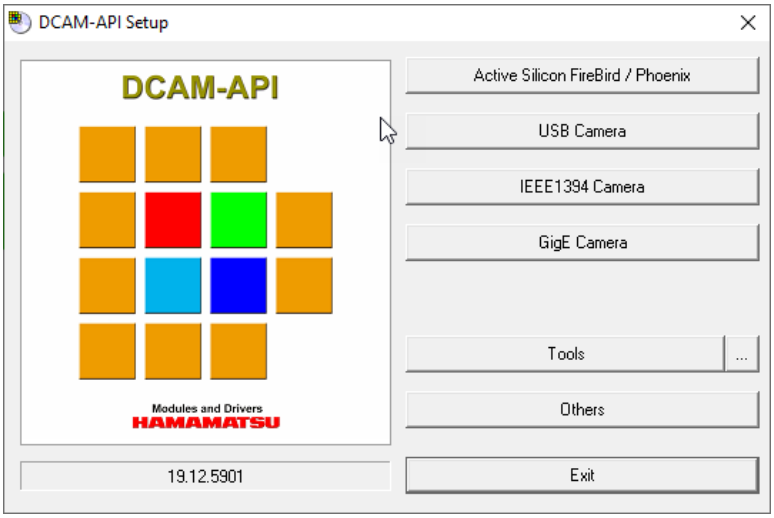
HCImage Live

1. Insert the HCImage Live installation DVD into the DVD-ROM drive. If autoplay is enabled, the HCImage Live setup will run automatically. If autoplay fails to start, locate your DVD-ROM drive and double-click **setup.exe**.
2. Click **Yes**, if prompted by the User Account Controls.
3. Review the Software License information and click **Yes**.
4. Review the README section for up-to-date information on software compatibility and support. When you are ready, click **Yes**.
5. On the Personalize screen, enter your registration information and click **Next**.
6. Choose the Destination Folder and click **Next**. It is recommended to install the software in the default path.
7. If you are ready to proceed with the installation, click **Install**.
8. Follow the instructions on each installation page.
9. Click **Finish**, when the installation is complete.
10. Install the appropriate DCAM-API drivers, see the instructions below, then turn the camera on before launching HCImage Live. If the drivers have not been installed, or the camera is not turned on before launching HCImage Live, the camera will not be available in the software.
11. Click the **HCImage Live** icon on the Desktop to launch HCImage Live.

Install DCAM-API Drivers

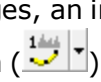
Before installing the camera driver, make sure that the camera is turned off.

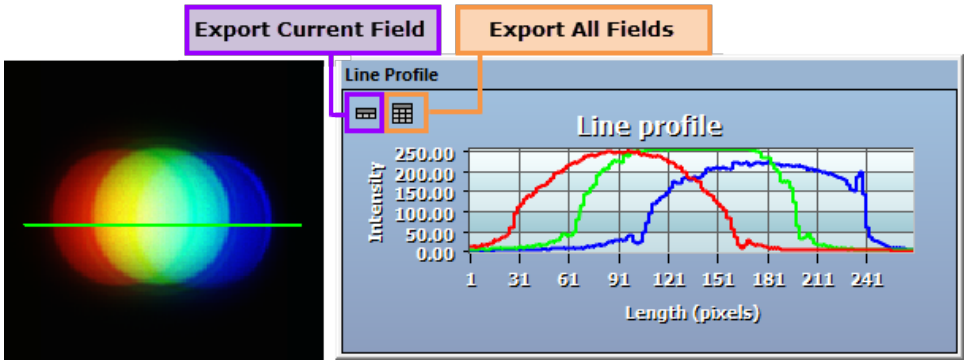
1. After installing HCImage Live from the DVD, you will be prompted to install DCAM-API, click **Yes**. If you downloaded HCImage Live, please go to http://www.dcam-api.com/ and download the DCAM-API drivers for Windows.
2. Click **Yes**, if prompted by the User Account Controls.
3. Select the appropriate 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**.



4. Click **Next** to begin the installation.
5. Follow the instructions on each installation page.
6. Click **Finish** when the installation is complete.

Line Profile

The Line Profile is a useful tool that allows users to draw a line on the image and see the corresponding intensity values plotted on a graph. The line profile may be used on a live or a captured image or image sequence. For two and three channel color images, an intensity profile is plotted for each channel as it's respective color. Click the Line Profile icon () and then draw a line on the image. See the Line Profile Properties below for a list of drawing tools. The Line Profile may be toggled on/off by clicking the Line Profile icon .



Line Profile Properties

The Line Profile icon is located in the Annotations toolbar, click the Line Profile drop-menu to view the line properties. The Line Profile Properties are defined below.

- **Line Thickness**: adjust the line thickness from one to five pixels, the selected thickness is displayed on the toolbar icon
- **Single**: left-click, hold and draw a single straight line
- **Segmented**: create an open polygon by left-clicking to define a starting point, then left-click to create an end point for each segment, and right-click to complete the line
- **Free-hand**: left-click and hold, then using the mouse trace the line on the image
- **Clear**: delete the current line profile

Viewing the Data

The intensity values for the line are plotted in a graph. The graph can be undocked and resized for optimal viewing. It is continually updated, when live or during playback. The intensity data can be exported to a spreadsheet and saved as a .csv (comma separated values) file. If a calibration was used, the scale factor values will be included in the exported data. There are two options for exporting data to a spreadsheet:

1. **Export Data to Spreadsheet**: export the intensity values for the current image
2. **Export Data to Spreadsheet (all fields)**: export the intensity values for all of the fields in the data set

Note: When using the ORCA-Flash4.0 LT, ORCA-Flash4.0 V2 or the ImagEM X2, the pixel values for line profile will be plotted in gray levels and electrons for monochrome images. For color images, the user can toggle between the two by clicking the Electron Count icon (). Both pixel values will be exported.

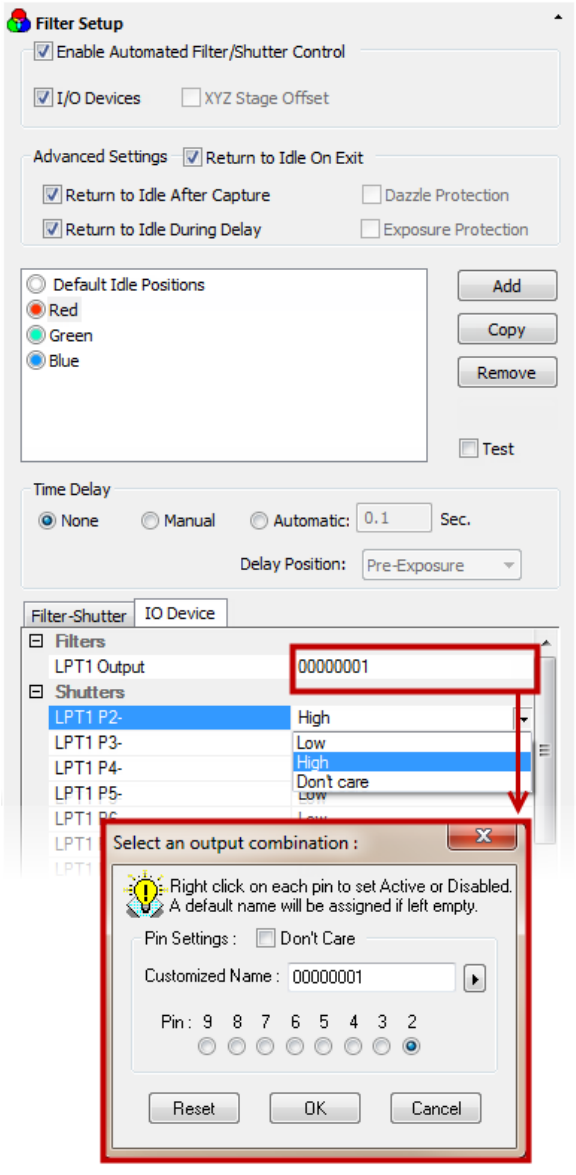
Filter Control using TTL

TTL can be used to control many types of devices, this example explains how to use the parallel port as an I/O device and configure it to control an LED light source. LED light sources, like the Lumencor Spectra and the CoolLED pE-2, provide high-speed switching between wavelengths and don't require a shutter.

Filter Setup

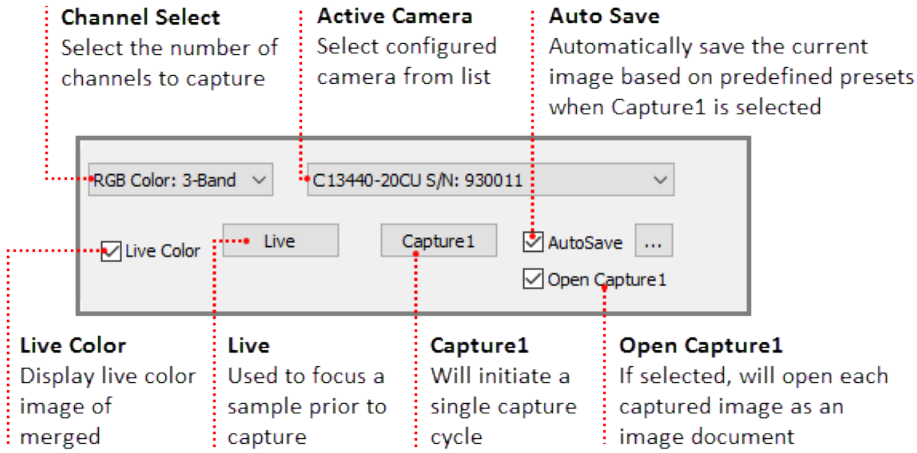
In this example, the LED turns on when the pin state is high and off when the state is low. Pin 2 controls the Red LED, Pin 3 the Green LED and Pin 4 the Blue LED.

1. Go to the **Devices** pane, expand the **Filter Setup** panel enable **I/O Devices**.
2. Next, make sure that **Return to Idle on Exit**, **Return to Idle After Capture** and **Return to Idle During Delay** are enabled.
3. Select **Default Idle Positions**, then select **LPT1 Output** under Filters in the I/O Device tab.
4. Click the **ellipses** to the right of Don't Care in the first line.
5. Enable pin settings by clicking **Don't Care** (should be unchecked).
6. Click **OK**. For the default state, all of the LEDs should be off.
7. Under **Shutters,** select **LPT1 P2**, use the drop menu to the right of Don't Care and set the state to **Low**.
8. Set the pin state to **Low** for LPT1 P3 and LPT1 P4.
9. Click **Add**, enter **Red** as the filter name and click **OK**.
10. Right-click on the filter and select Red from the tint list.
11. Select **LPT1 Output** under Filters in the I/O Device tab and click the **ellipses**.
12. Uncheck **Don't Care**, enable Pin 2 and click **OK**.
13. Go to **Shutters** in the I/O Device tab and set the LPT1 P2 state to **High** and the state to **Low** for LPT1 P3 and LPT1 P4.
14. Now add the **Green** filter, tint it green, enable Pin 3, then set the LPT1 P3 state to **High** and the state to **Low** for LPT1 P2 and LPT1 P4.
15. Add the **Blue** filter, tint it blue, enable Pin 4, then set the LPT1 P4 state to **High** and the state to **Low** for LPT1 P2 and LPT1 P4.



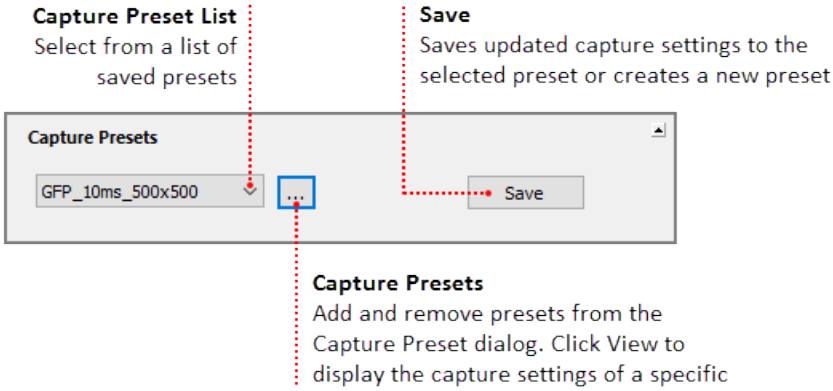
The Capture Pane

The Capture Pane provides a flexible and comprehensive method to access the camera's 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

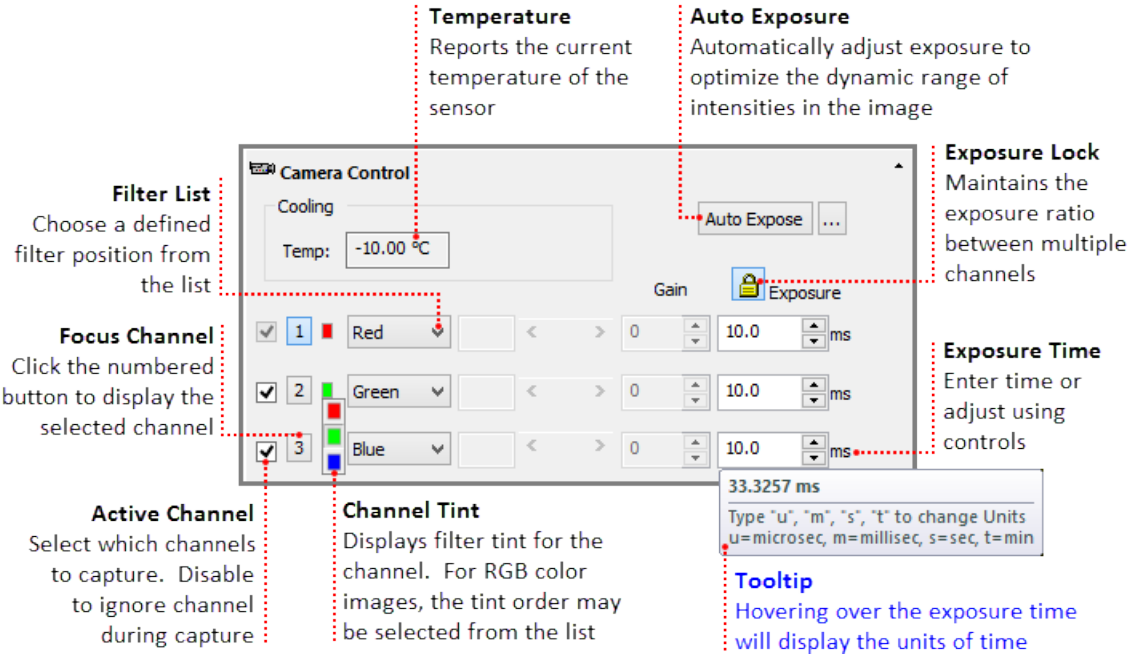
Capture settings can be saved as presets and then loaded when needed. Create multiple capture presets to easy change between frequently used capture settings. Capture presets may be selected from a list of saved presets available in the Capture Presets panel, located at the top of the Capture pane. To add, remove, rename or view the settings of a preset, click the ellipsis to the right of the list, to open the Capture Presets dialog. 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.

Camera Control

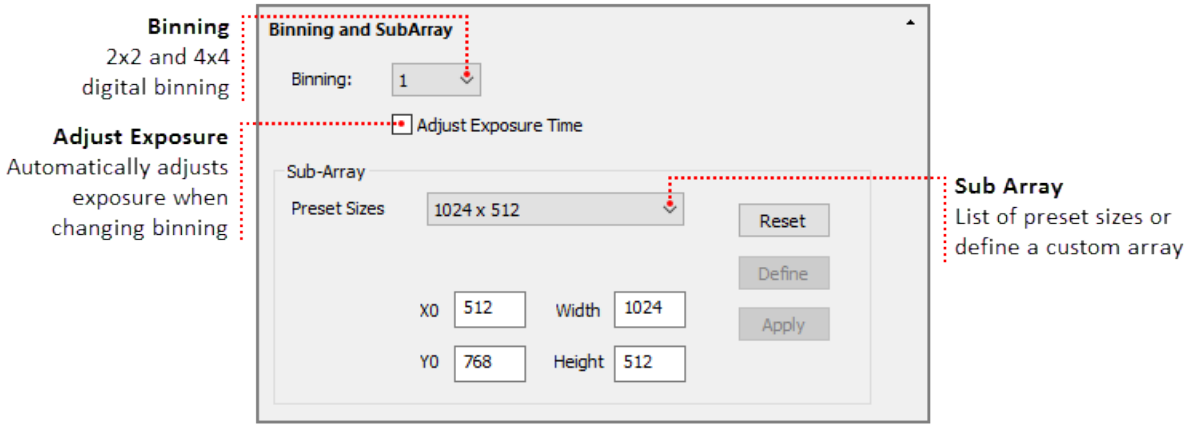
Manage capture settings using the individual channel and exposure controls.



Hint: In order to achieve the best possible acquisition speed when acquiring color images, set the same exposure for each channel. Once 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.

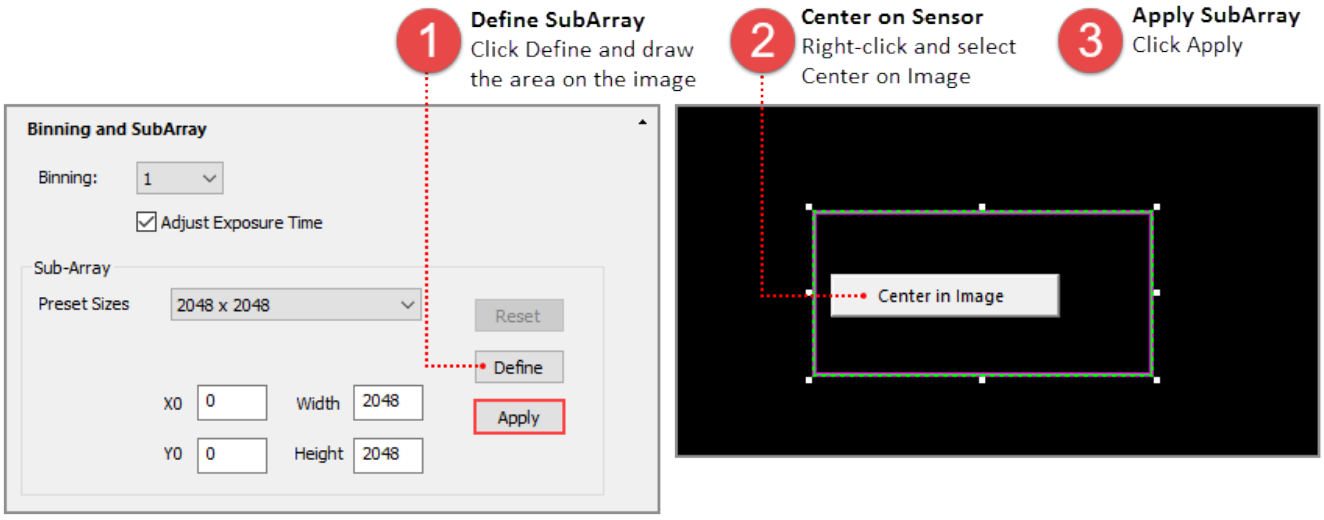
Binning and SubArray

With a CCD camera, 2x2 binning increases the signal to noise ratio by a factor of four and increases the speed of image acquisition by a factor of about two. With an sCMOS camera binning is purely digital, 2x2 binning increases the signal to noise ratio by a factor of two. Digital binning does not increase the speed of image acquisition. Adjust the spatial resolution using a subarray preset for increased speed and less data throughput. For sCMOS cameras a subarray must be centered on the camera sensor in order to achieve maximum speed. The subarray preset sizes for in the list are automatically centered (for sCMOS) but custom arrays are not. To center a custom array, see the example below.



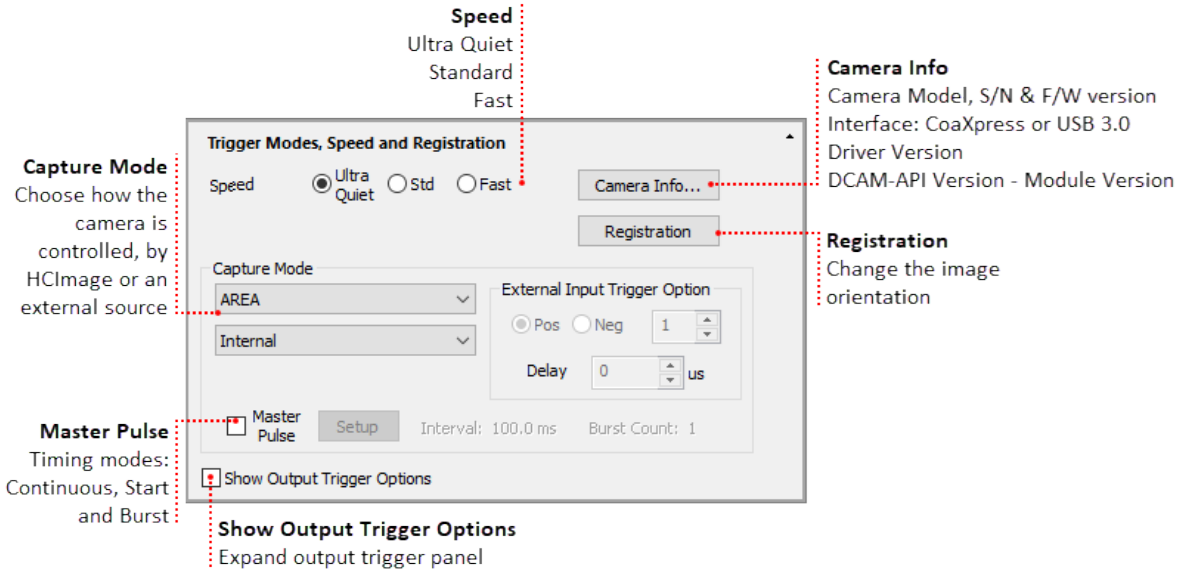
Define a Custom SubArray for Maximum Speed (ORCA-Flash)

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.



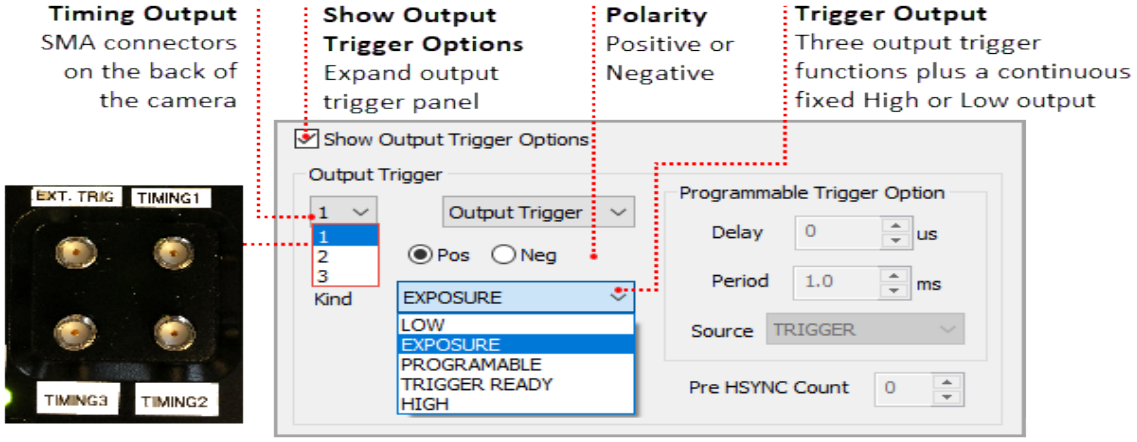
Trigger Modes, Speed and Registration

By default the camera is controlled through software but some cameras offer advanced triggering features allow the camera to control external devices or be controlled by them. The speed, capture mode and output trigger settings can be adjusted based on the needs of the application. The example below describes options for the ORCA-Fusion.



Output Trigger Options

The camera provides a range of trigger output signals to synchronize with an external instrument where the camera becomes the master and the external instrument becomes the slave. There are three different trigger output functions, as well as a continuous High output (High output fixed) or continuous Low output (Low output fixed). For a detailed description of each of the output trigger options, please see "**Camera Trigger Output**" on page 1.



Advanced Camera Properties

DCAM Properties provide a list of camera parameters reported by DCAM. The camera properties and reported values are specific to the connected camera and in some cases provide access to additional functionality based on the capture mode. These properties are referenced in text and screenshots as needed for setting specific camera modes. Most of the camera properties in the list display values that cannot be changed and appear grayed out.

Processing

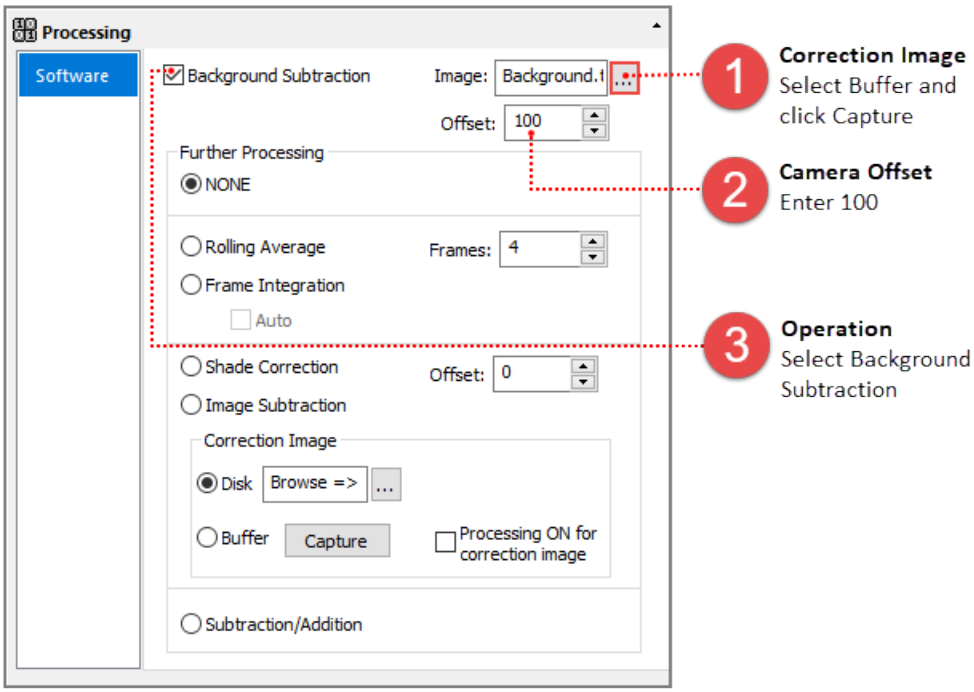
The Processing Panel provides the opportunity to enhance images during focus and acquisition by incorporating image-processing operations during or immediately after image Capture. To select an Image Processing operation, first expand the Process Pane and then select the Operation Type. Rolling Average and Frame Integration are used for noise reduction. Use the image arithmetic functions like Shade Correction, Background Subtraction or Image Subtraction to remove artifacts from the incoming image. Clicking Capture1 will initiate image capture with the selected image processing operations applied.

Note: For Image Correction or Arithmetic, the user must first choose a source or background image. The image may be the current image saved in a buffer or one previously saved to disk. To use the current image, make sure Processing is OFF, select Buffer, click Capture and then select Shade Correction, Background Subtraction or Image Subtraction. Use the same method when using an image from Disk.

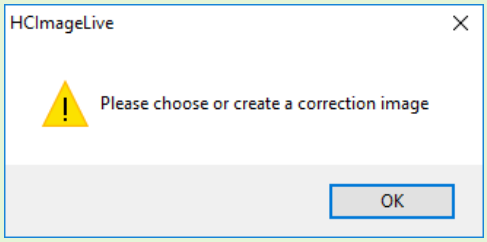
Hint: Enable Processing ON for correction image when you would like to capture a correction image using Rolling Average or Frame Integration. When you are ready to capture the correction image, select Rolling Average and enter the number of frames, enable Processing ON for correction image and then click the Capture button to the right of Buffer. The captured averaged image is stored in the buffer and ready to use a correction image.

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.

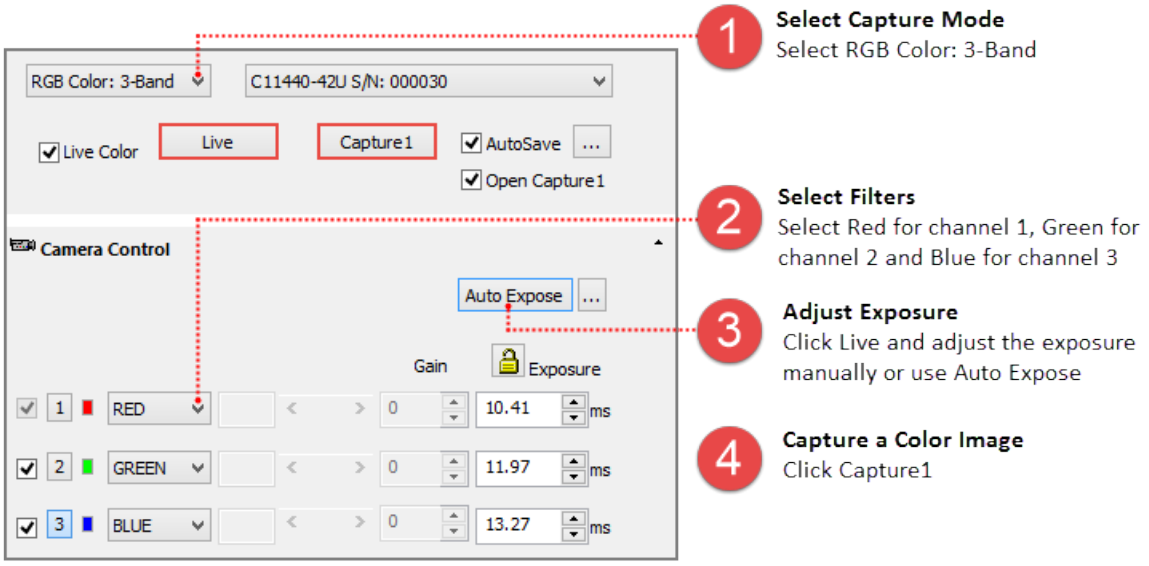


Hint: HCImage remembers the capture settings from the previous session, if background subtraction was left enabled, the following message will appear the next time HCImage is launched.



Capture a Color Image

Capturing a color image requires filter setup, for instructions on setting up an LED light source in HCImage Live, please see "**Filter Control using TTL**" on page 5.

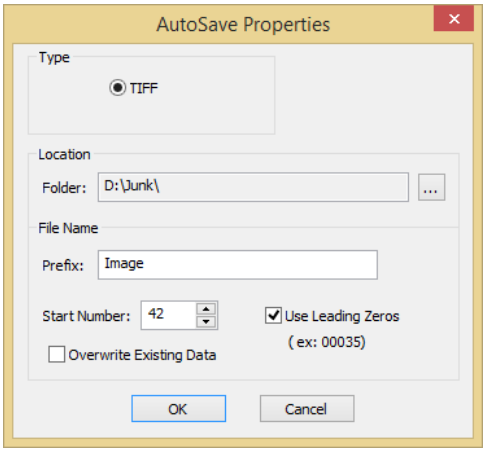


Hint: In order to achieve the best possible speed when acquiring color images, set the same exposure for each channel. Once the exposures have been entered, click the Exposure Lock icon () to lock the exposure settings. Now any exposure adjustments will be made to all of the channels.

Using 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.

1. Enable **AutoSave** and then click on the ellipses to open the AutoSave Properties dialog.
2. Enter or navigate to the destination directory.
3. Enter the file name and the starting image number, subsequent captures will be number sequentially.
4. Click **OK**.



W-VIEW and the ORCA-Flash4.0 LT

HCImage Live supports the W-VIEW GEMINI for simultaneous dual wavelength image acquisition using the ORCA-Flash4.0 LT. The W-VIEW GEMINI uses image splitting optics to project the two wavelengths of interest (side by side) onto the sensor of the camera. This means that the effective size of the field of view is approximately half the sensor size. The W-VIEW GEMINI was designed to take advantage of the large field of view of the ORCA-Flash4.0 providing approximately 2000 x 1000 pixels for each image. HCImage Live incorporates W-VIEW mode, a multi-view functionality, allowing the ORCA-Flash4.0 LT to function in a similar manor as using dual cameras. The W-VIEW mode allows for independent exposure time settings, independent readout directions and separate position offset for subarray.

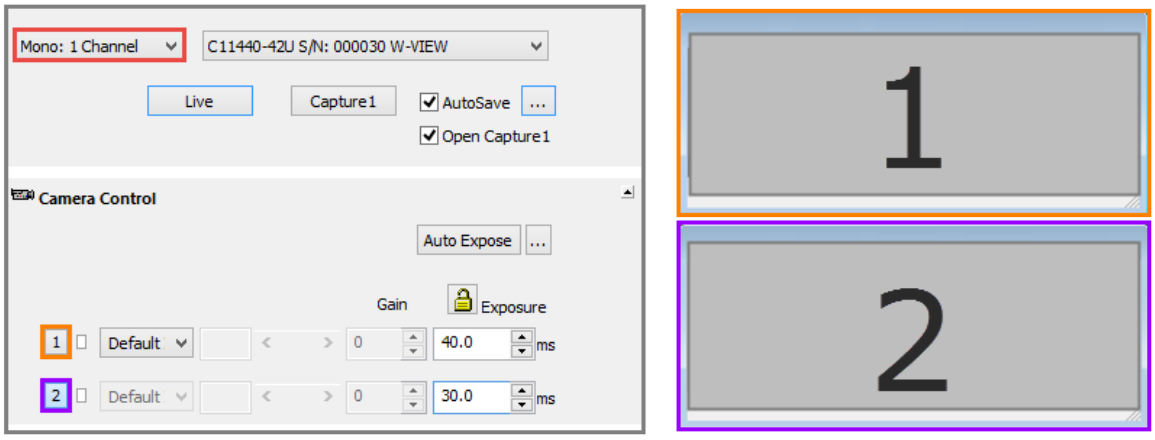
Note: With W-VIEW mode, the readout direction in the upper and lower half of the sensor can be setup separately. The readout direction for View 1 (top half) and for View 2 (bottom half) can be set to Forward or Backward under DCAM Properties in the Advanced Camera Properties panel.

Capture Modes

HCImage Live will automatically detect the ORCA-Flash4.0 LT as two cameras, a normal camera and as a camera in W-VIEW mode. Select C11440-42U S/N: #### for normal mode or C11440-42U S/N:#### W-VIEW for W-VIEW mode from the Capture Device list. W-VIEW capture modes include: Mono 1 Channel, RGB Color 2-Band and Mono 2 Channel. To select a capture mode go to the Capture panel and click on the drop-menu above the Live button. The Camera Control pane is modified based on the capture mode selected. The capture modes are explained in detail below.

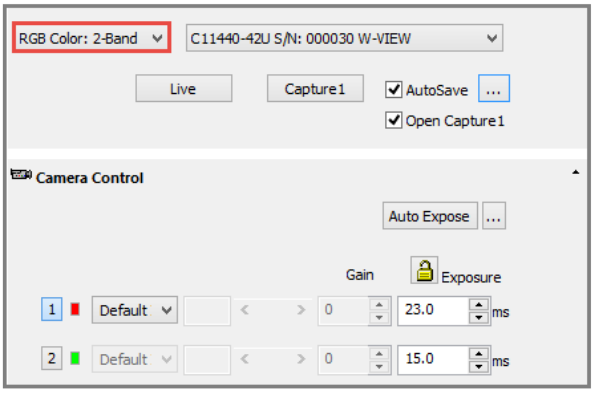
Mono 1 Channel

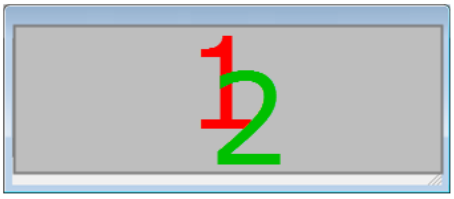
In the single channel monochrome mode, the user can select which image to display, only one image will be displayed at a time. Click on the 1 or 2 button to select which image will be displayed.



RGB Color 2-Band

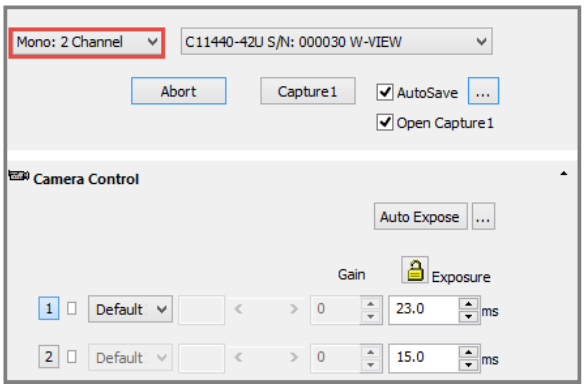
The RGB Color 2-Band mode displays a merged red-green image from image 1 and 2.





Mono 2 Channel

In the two channel monochrome mode, both images 1 and 2 are displayed (i.e., the whole camera sensor is displayed).



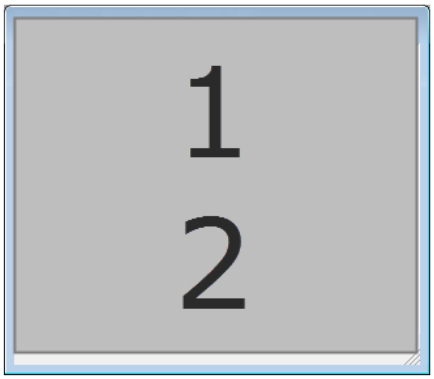
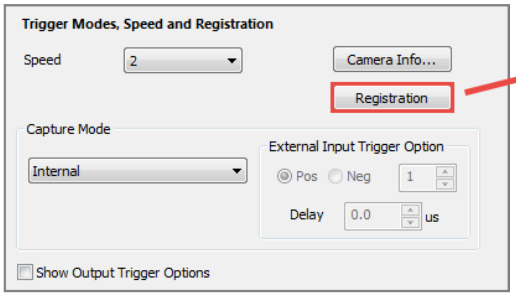
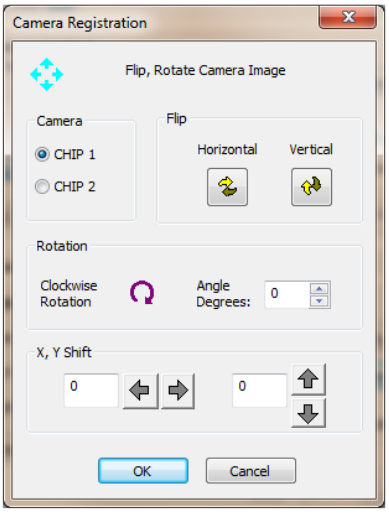


Image Alignment

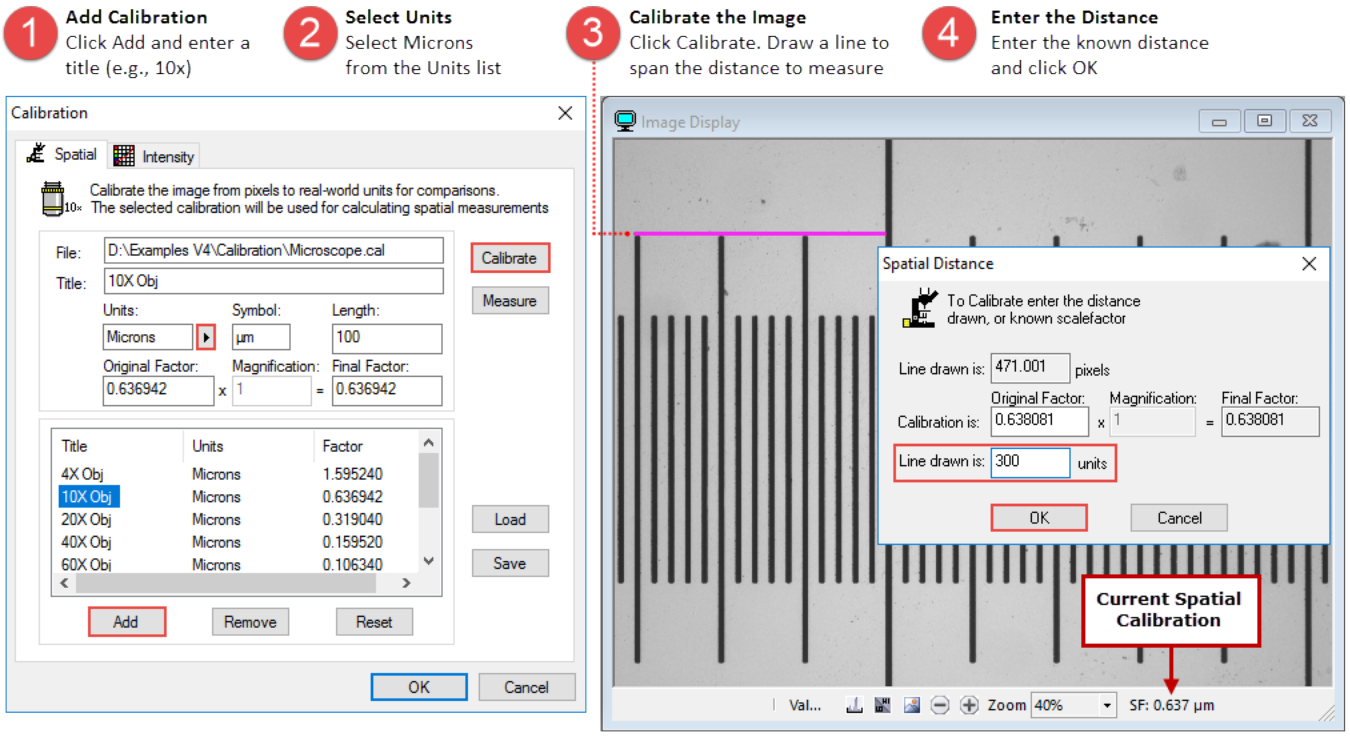
The Camera Registration feature allows the users while Live to flip and rotate the image. Click on the Registration button in the Trigger Modes, Speed and Registration pane to open the Camera Registration dialog.





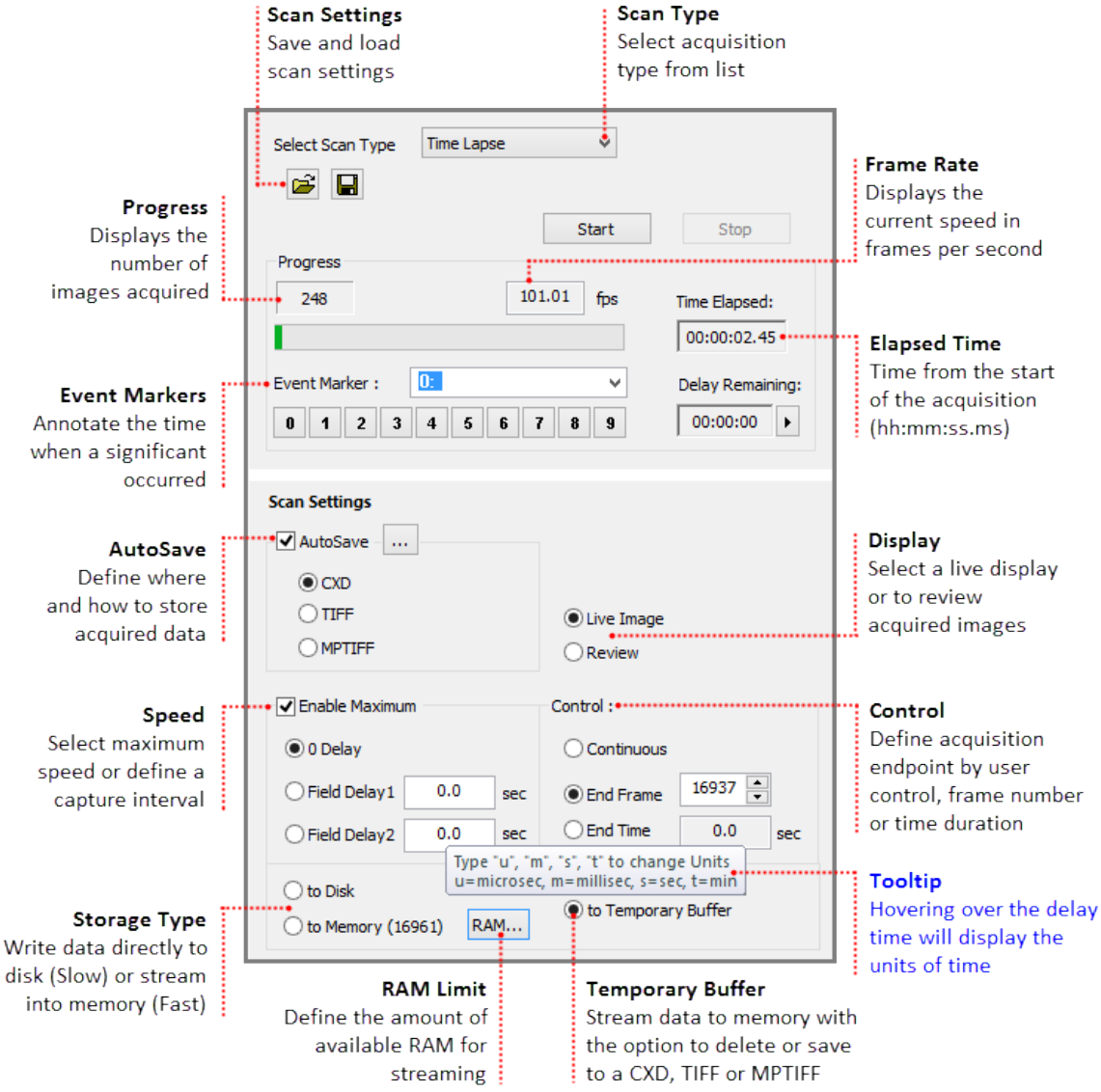
Calibrate an Image from Pixels to Microns

1. Open or capture an image with some known distance, for example a micrometer.
2. Click on the **Calibration Properties** icon (Calibration) on the Analysis toolbar.
3. Enter a Title for the calibration file (e.g., 10x). Select **Microns** from the **Units** drop-menu.
4. Click **Calibrate**. Move the cursor to the start of a known distance; click and drag a line to span the distance to measure.
5. Enter the known distance of the line and click **OK**. The Calibration Factor in the Spatial Calibration Menu will be updated.
6. Click **Add** and repeat the previous steps for adding additional calibrations.
7. **[Save Calibration]** Click **OK** > Select file path > File name > Save. The Spatial Calibration is displayed in the lower right-hand corner of the image file.



Capture a Time Lapse Image Sequence

The Time Lapse scan provides flexibility and a variety of options for defining a time lapse to fit the needs of your application.



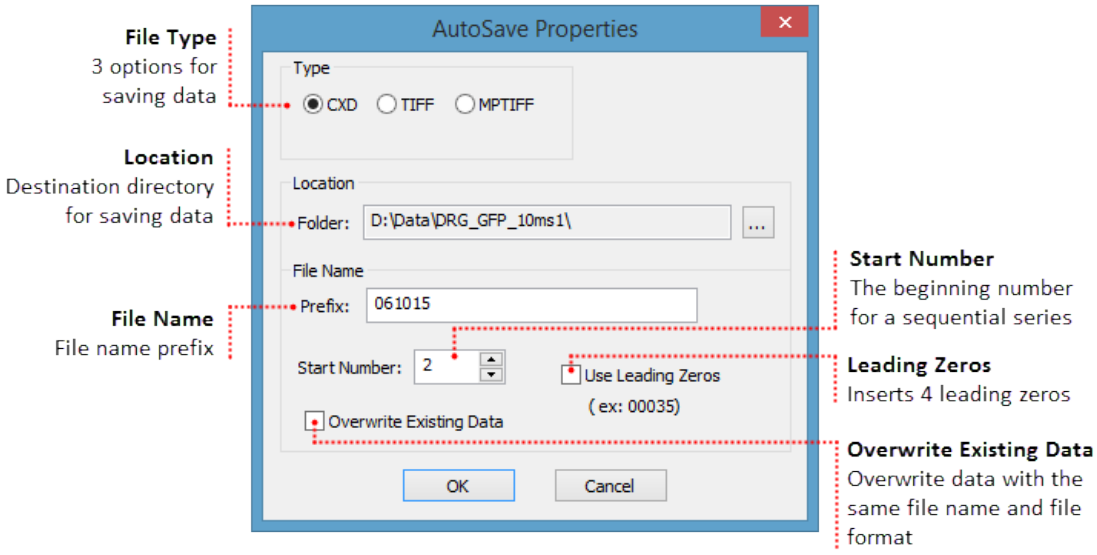
Scan Settings

The Scan Settings panel provides multiple options for defining speed, storage, duration and output settings. Scan settings can be saved for future use.

Note: Select Enable Maximum to acquire at maximum speed. During maximum speed, items which slow down acquisition will be ignored.

Auto Save

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 a 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.

Storage Options

The three options for storing acquired data during a time lapse include saving to Disk, Memory or Temporary Buffer.

Save to Disk

Acquired data is written directly to the hard drive. Frame rates vary based on the PC configuration, including the type and speed of the hard drive(s) being used.

Save to Memory

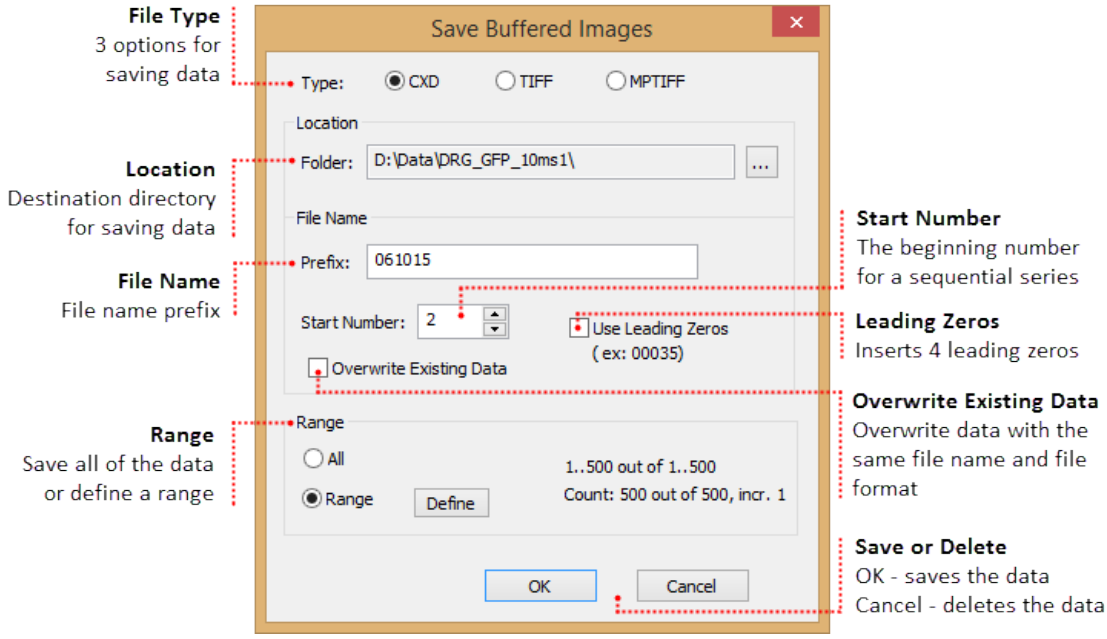
Acquired data is stored in memory and then written to disk when the time lapse is complete or stopped. When the system runs out of memory during a time lapse, acquired data is written to disk for the remainder of the sequence. Saving to memory typically provides a higher frame rate with less timing variation then saving to disk. The maximum number of images that can be acquired depends upon the amount the RAM in the system and the RAM limit set in HCImage. This number is displayed to the right of the memory storage option. When Memory is selected, End Frame automatically displays the maximum number of frames that can be streamed to memory, although any number less than the max can be entered. The Status Bar, located in the bottom left corner of the application window, displays the maximum number of frames that can be streamed to memory.

Save to Temporary Buffer

Acquired data is stored in memory with the option to review the image sequence before saving or deleting it. Storage is limited to the amount of system memory without the option to write to disk when the memory is full. The maximum number of images that can be acquired depends upon the

amount the RAM in the system and the RAM limit set in HCImage. When Temporary Buffer is selected, End Frame is automatically enabled and display the maximum number of frames that can be streamed to memory, although any number less than the max can be entered.

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.

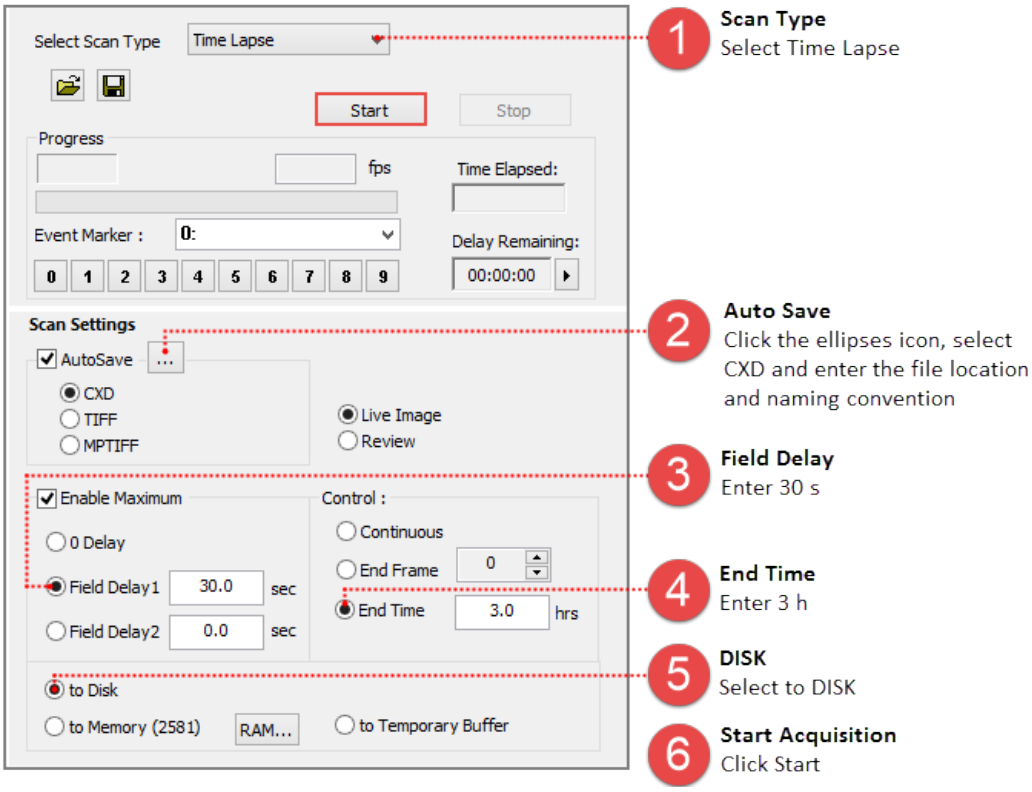


Setting up a Time Lapse

This section provides three examples of typical time lapse settings, using each of the storage options.

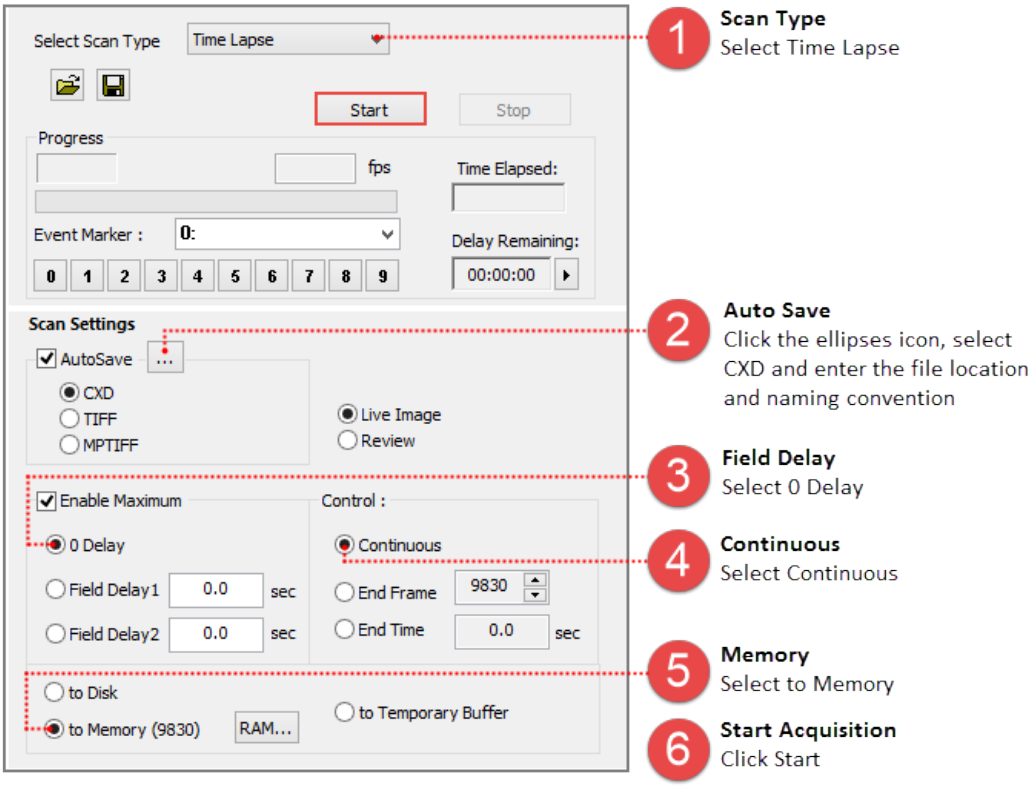
Setup a Time Lapse - Save to Disk

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 your are satisfied with capture settings and the sample is in focus, go to the Sequence pane and follow the steps below.



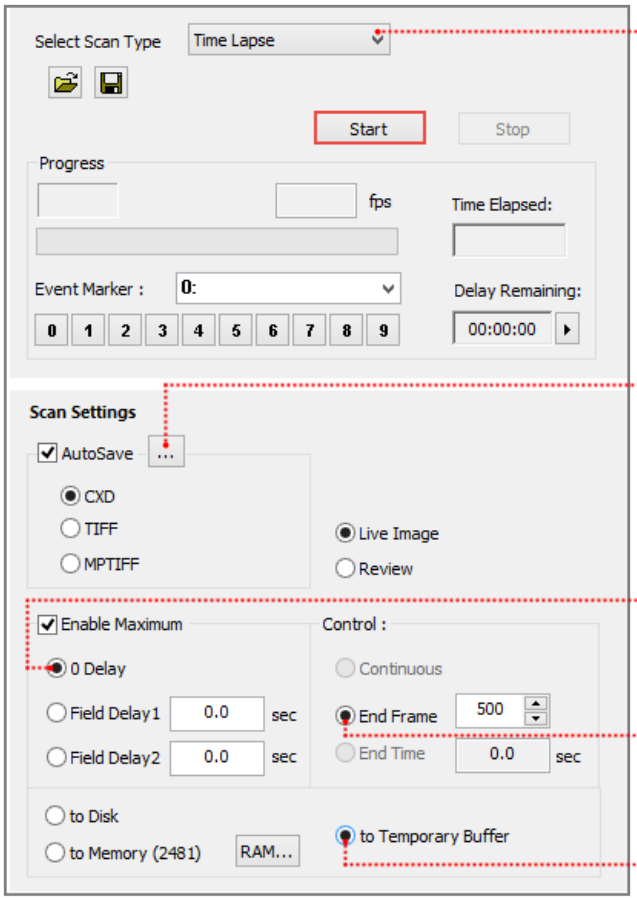
Setup a Time Lapse - Save to Memory

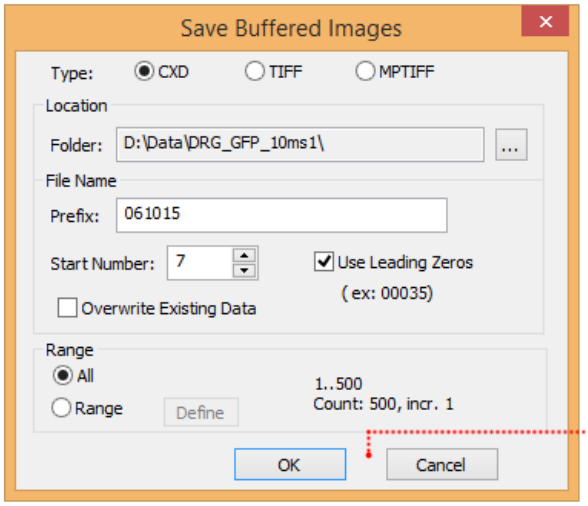
The time lapse in this example will store images in memory until the acquisition is stopped or runs out of memory at which point the acquired images are saved to disk for the remainder of the time lapse. Once your are satisfied with capture settings and the sample is in focus, go to the Sequence pane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



Setup a Time Lapse - Save to the Temporary Buffer

Once your 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

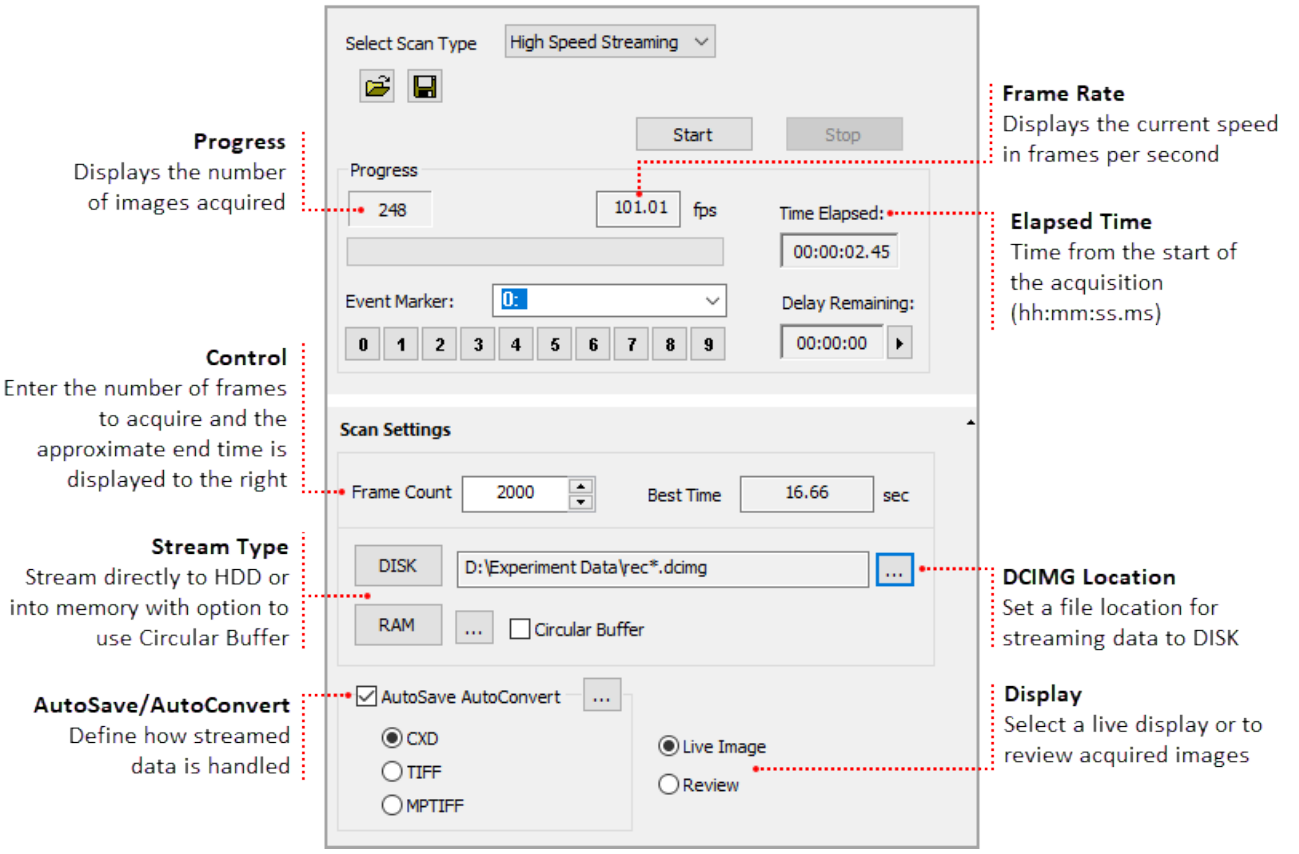
7 Acquisition Complete
Review acquired data using the playback controls in the Image Display

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

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 V2.



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).

Stream to RAM

When streaming to RAM, the image data is stored in memory and then the user has the option to save as either CXD, TIFF, MPTIFF or to delete the data. Up to 80% of the systems available memory will be used for storing streamed data. The Status Bar, located in the bottom left corner of the application window, displays the maximum number of frames that can be streamed to memory. In the AutoSave Properties dialog, the user can determine how and where to store the acquired data. Once the acquisition is complete, the data stored in memory can automatically be saved as a CXD, TIFF or MPTIFF.

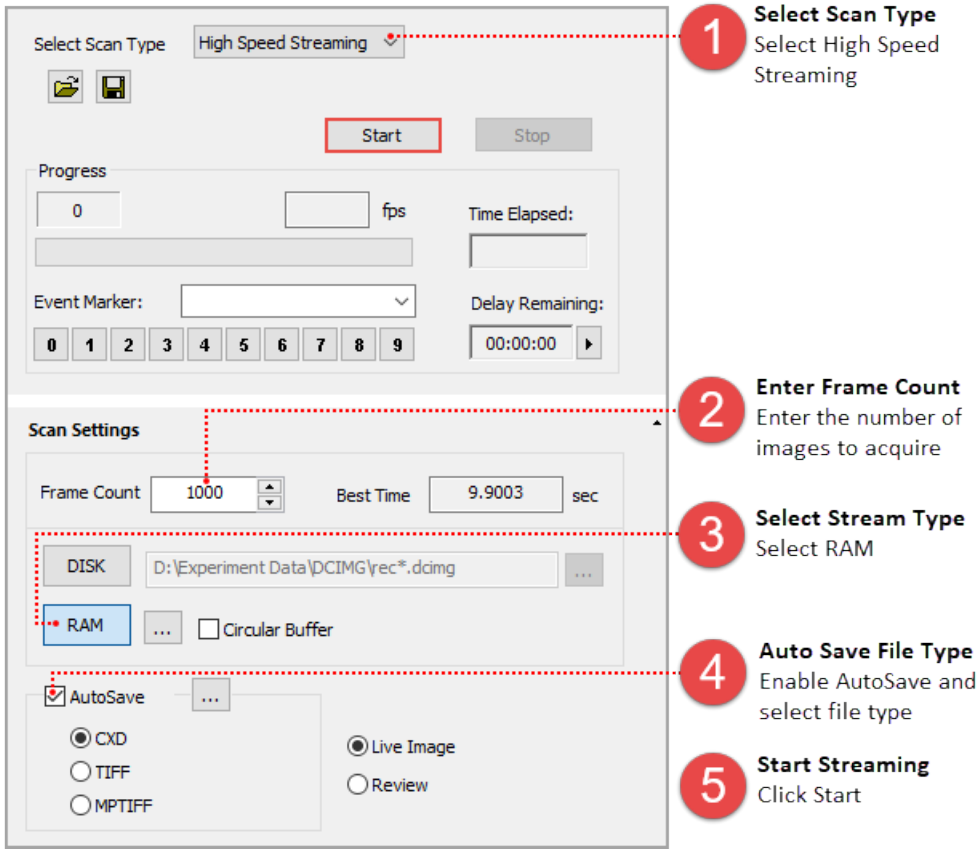
Note: MPTIFF files have a 65,000 image limit or 4 GB size limit. For image sequences having more than 65,000 images or larger than 4 GB, multiple MPTIFF files will be saved and numbered sequentially.

Circular Buffer

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.

Steps for Streaming to RAM

Once your are satisfied with capture settings and the sample is in focus, go to the Sequence pane and follow the steps below.

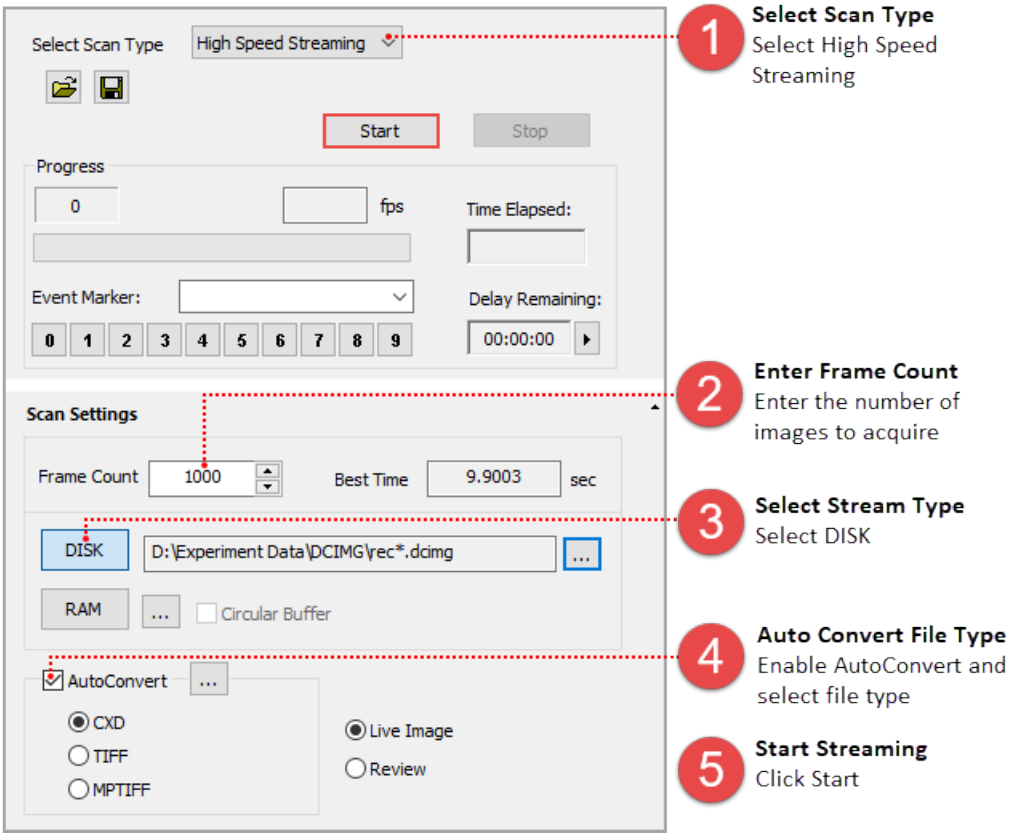


Stream to Disk

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.

Steps for Streaming to Disk

Configure the capture settings, go to the Sequence pane and follow the steps below.



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

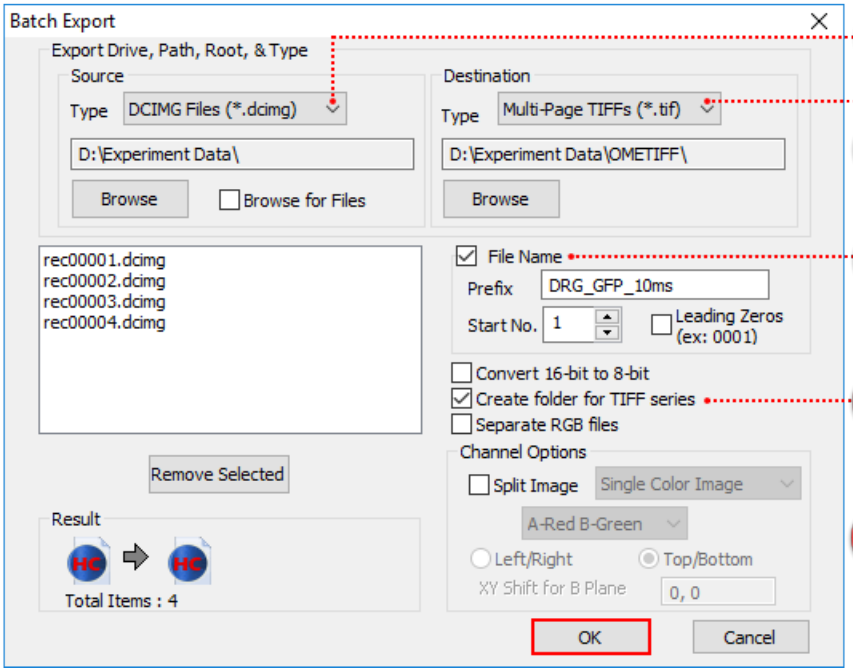
4 Auto Convert File Type
Enable AutoConvert and select file type

5 Start Streaming
Click Start

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

Steps for Batch Export DCIMG to MPTIFF

Go to the File menu, select Batch Export and follow the instructions below.



1 Enter Source Location
Type: Select DCIMG Files
Browse: Go to the file directory

2 Enter Destination Location
Type: Select Multi-Page TIFF Files
Browse: Go to output directory

3 Define Output File Name
Define the file naming convention

4 Enable Create Series Folder
Select Create folder for TIFF series

5 Export to MPTIFF
Click OK

TTL Input & Output

The example below will provide a quick overview on how to configure the parallel port to start and stop a sequence, trigger capture events by receiving TTL pulses and how to output TTL pulse based on capture events. Go to the Device panel and click I/O Setup. The Capture Events tab is divided into Capture Inputs and Capture Outputs.

Note: The description and instructions below only cover some of the types of capture events, for more information please consult the Help in the software or on the HCImage website (http://hcimage.com/help/index.htm).

Capture Events

For Capture Inputs, Sequence Start and Stop, choose input pins and pin state. To start a sequence, click under Frequency to choose when to trigger. Each click will cycle through the list of choices:

- **Each Field** - Waits for an input trigger at the beginning of every capture cycle
- **First Field** - Waits for an input trigger at the start of the sequence
- **Each Pass** - Waits for an input trigger before the first field of each sequence pass

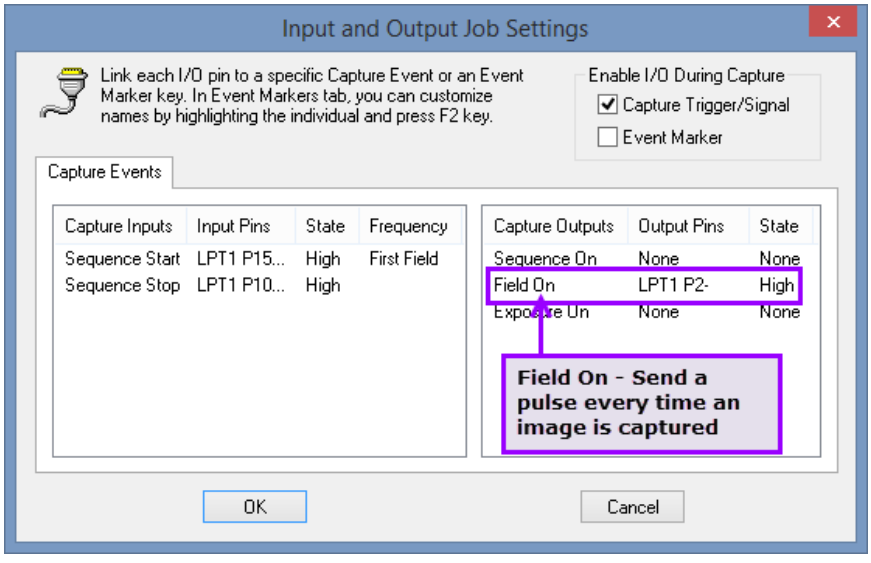
For Capture Outputs:

- **Sequence On** - Will set the chosen state of the pin at the beginning of the capture. This pin will remain at the specified state until the sequence has completed.
- **Field On** - Will set the chosen state of the pin at the start of a field's exposure. This pin will signal on and off as each field is captured.
- **Exposure On** - The TTL output for the duration of each exposure.

Configure Capture Events

The table below provides the basic steps for setting up capture events.

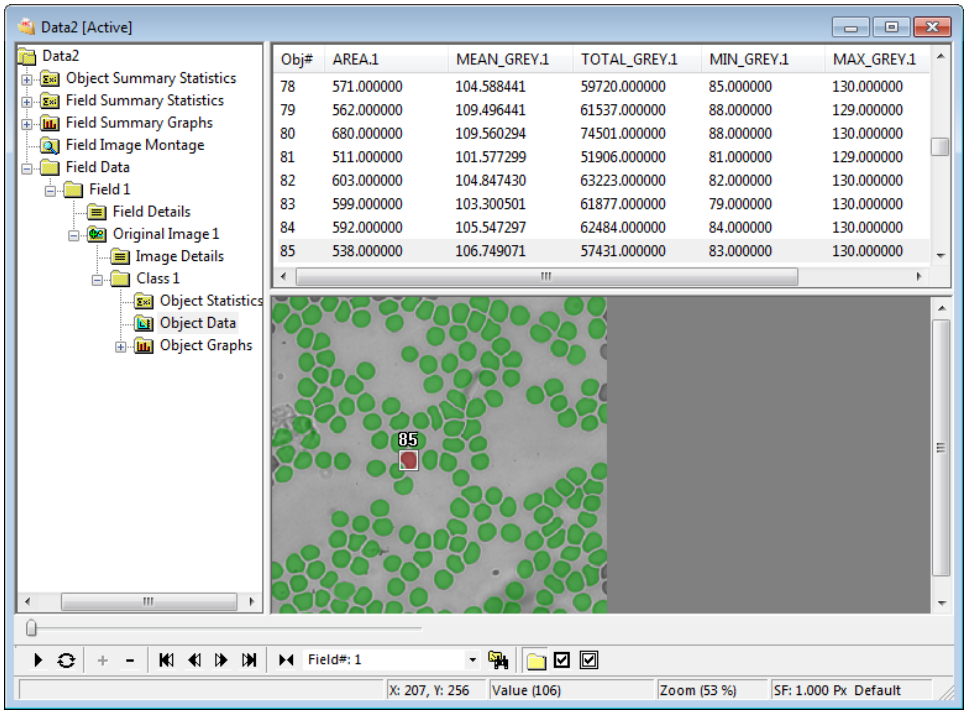
Setup Capture Inputs:	Setup Capture Outputs:
1. Select input pin	1. Select the capture output type
2. Set the input pin state	2. Select the output pin
3. Select the capture frequency	3. Set the output pin state



Analyze a Single Image - Advanced Mode

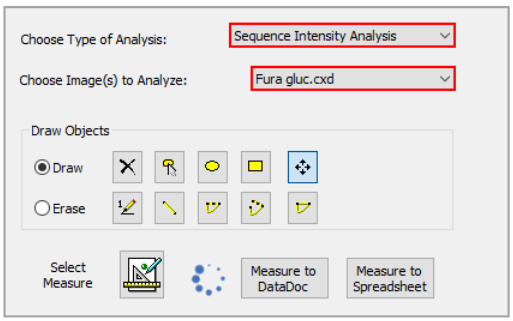
Using the advanced mode, detect and analyze the size and the intensity of multiple objects of interest in a single image.

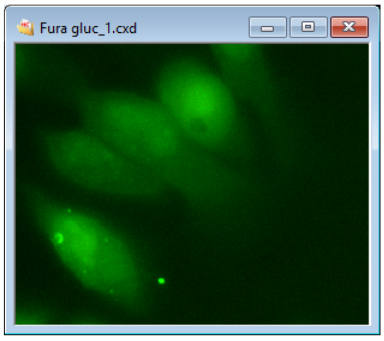
1. Enable the Advanced Analysis mode by clicking **View** on the menu bar, then highlighting **Analysis Mode** and selecting **Advanced**.
2. Open or capture an image, click on the **Analysis** pane and select **Single Image Measure** from the **Choose Type of Analysis** drop-menu.
3. **[Threshold the Image]** Press the **Identify** icon under **Detect Objects**. Set the intensity threshold by adjusting the Min. and Max. sliders until the objects of interest are highlighted by a green binary overlay. Click **OK**.
4. **[Modify the Binary Image]** Click **Modify** to edit the binary overlay. Select any binary operation you need to modify the binary overlay with a binary filter, such as Erode, Dilate, Close, Open, etc; click **OK**.
5. **[Remove Unwanted Objects]** Click the **Qualify** icon. Reject unwanted objects by area by adjusting the Min. and Max. sliders. Objects to be removed will appear red. Additional qualifying parameters can be added such as Mean_Grey and Length, by clicking the **Add** button.
6. **[Measure the Image]** Click **Measure** > Select the type of Measurements > **OK** > Click **Measure to DataDoc** > Save data file.
7. **[Review the Measurements]** Click on a data value to identify the object that corresponds to the data value and vice-versa. The data document contains Object Summary Statistics, Object Summary Graphs, Field Summary Statistics, Field Summary Graphs, Field Image Montages and Field Data. Data can be printed or exported to a spreadsheet by selecting **Copy to Spreadsheet** under the Edit menu.



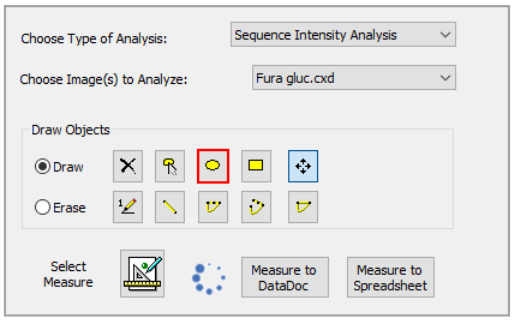
Sequence Intensity Analysis - Simple Mode

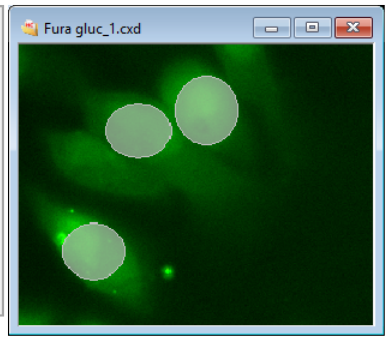
Sequence Intensity Analysis will measure the intensity of a single object in the image sequence. If multiple areas are drawn or identified, they are treated as a single object. HCImage has two modes, the Simple mode is active by default but can be changed by clicking View on the menu bar, then highlighting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





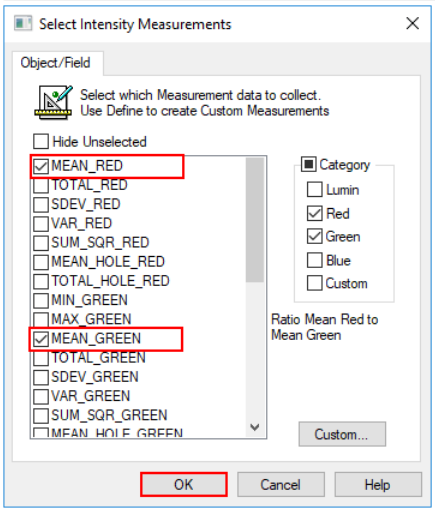
1 Enable Sequence Intensity Analysis
Open the dataset and select Sequence Intensity Analysis from the drop-menu.

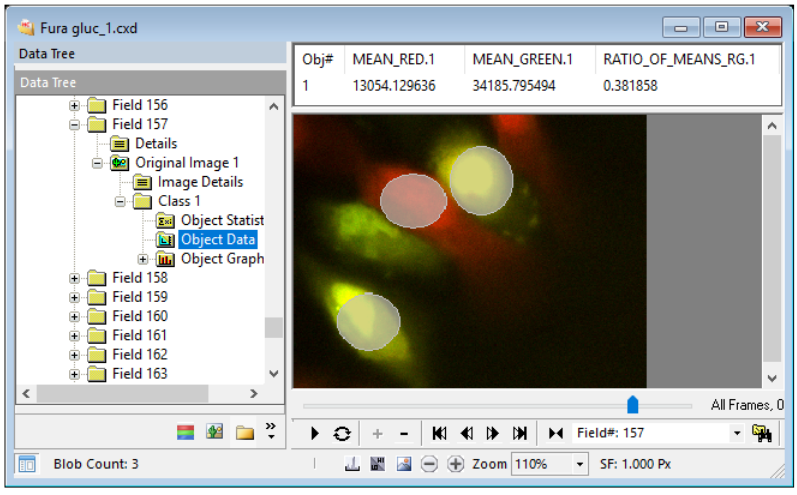




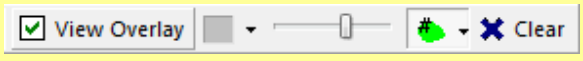
2 Draw Object
Click the Ellipse icon and manually identify the object of interest.
Tooltip
Press SHIFT to draw a circle.

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





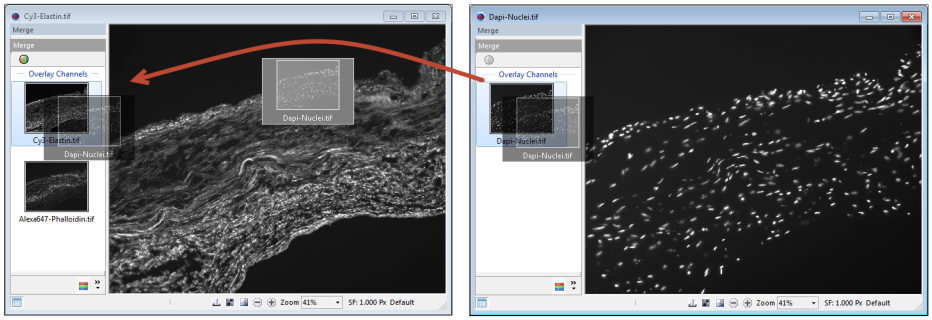
Note: Change the overlay color to silver instead of green in the **Change Overlay Color** icon. Use the translucency slider to adjust the overlay transparency or hide it by selecting **View Overlay**. Click **Clear** to delete the overlay.



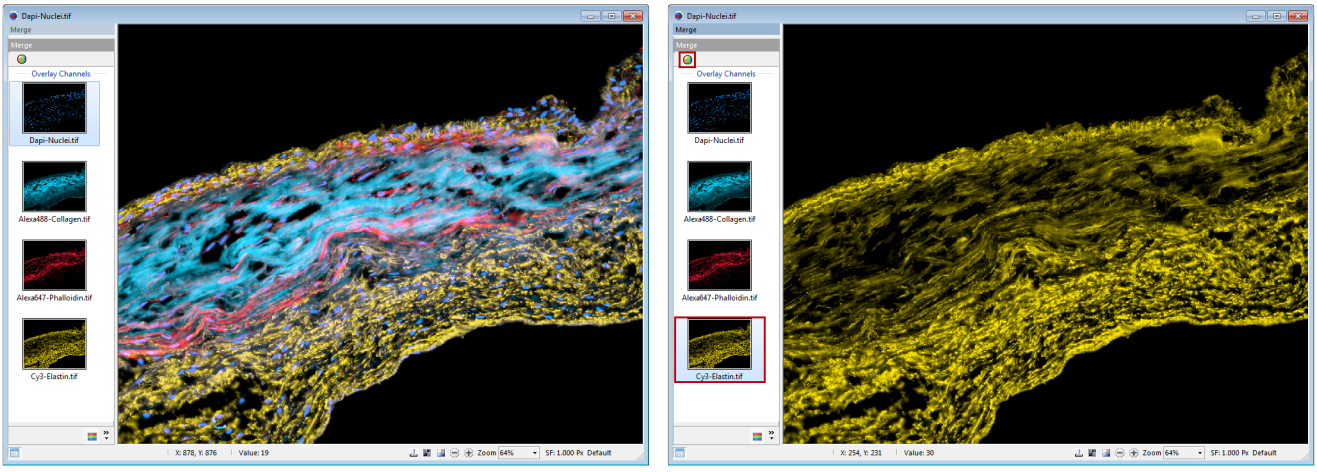
How to Merge Several Monochrome Images

In HCImage, there are a couple of ways to create a merged image. The procedure outlined below describes the drag and drop method of combining multiple images into a single merged image. Keep in mind that the original image is not altered, the merged image is layer that can be turned on or off. In order to keep the merged image, the display image must saved, see below.

1. Open all of the images that will be merged.
2. Click the **Toggle SideBar** icon ( ) if the SideBar is not visible. The Toggle SideBar icon is located in the Status Bar at the bottom left corner of the image.
3. Select a source image and then drag and drop the thumbnails of the other images on to the thumbnail of the source image. The merged image is displayed along with thumbnails of each of the overlay channels.



4. Apply color to an image, right-click on a thumbnail and select a tint from the **Tint** drop-down menu. Repeat the process for each image.
5. Adjust the contrast for the individual overlay channels, right-click on the thumbnail and select **Contrast**. Make adjustments using the sliders in the **Display Contrast** dialog.
6. Click the **Split Channel** icon ( ), located in the top left corner of the SideBar, to enable viewing of the individual overlay channels. When Split Channel mode is enabled, click on a thumbnail to display the corresponding overlay channel. Click the **Split Channel** icon to view the merged image.
7. **[Merged Image On/Off]** Right-click on a thumbnail and select **Merge On** or click the Apply Merged Display icon ( ) in the Display toolbar.
8. **[Save the Display Image]** Right-click on the image > Save Image to File > Display Image > Enter file name > Save.



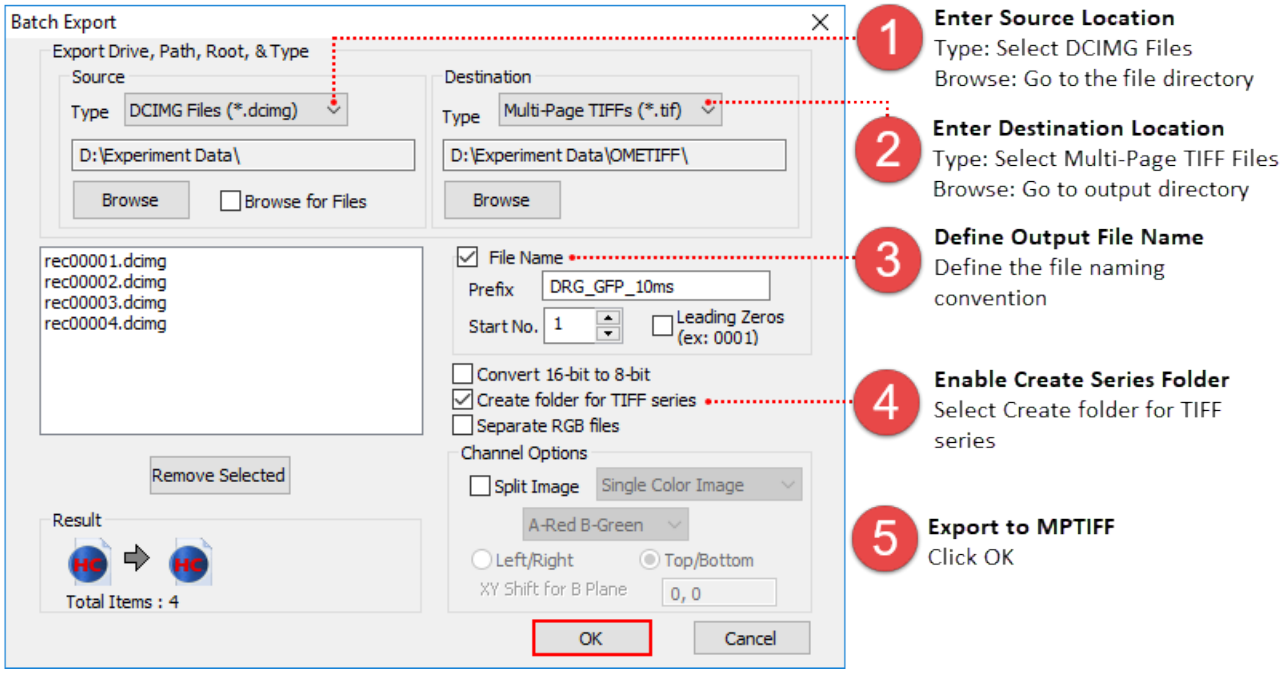
BATCH EXPORT

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),), 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.



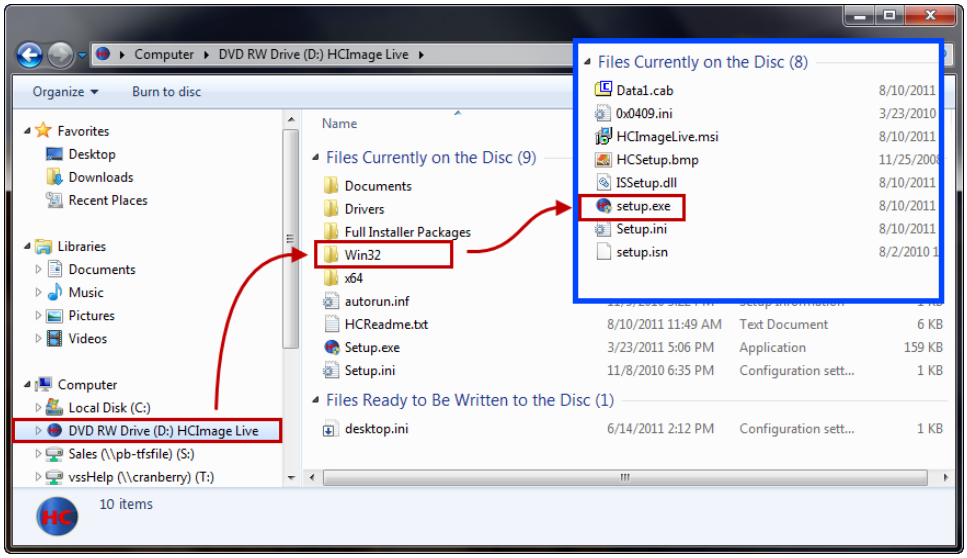
Note: Depending on the destination file type, certain options are available.

- **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).

Install HCImage Live 32-bit on a 64-bit Operating System

There are some instances were the 32-bit version of HCImage Live will need to be installed on a 64-bit machine. This is the case for the **C10633 (inGaAS)** cameras, which are only supported with Windows 32-bit support on any Windows 64-bit (x64) operating system. The instructions below provide the steps for installing HCImage Live 32-bit on a 64-bit operating system.

1. Cancel the Installation Wizard by clicking **Cancel**, click **Yes** to exit setup and then click **Finish** to close the Wizard.
2. Open **My Computer**, select the media drive with the HC icon, open the **Win32** folder and double click **Setup.exe**.
3. Follow the instructions on the Installation Wizard to install HCImage Live.



Hint: If you are unsure if the operating system is 32-bit or 64-bit, press and hold the **Windows Logo + Break** keys to view the System Properties window. Look for the **System Type** to find out which version of the operating system is installed.