



**PHOTOMETRICS**

CMOS, EMCCD & CCD CAMERAS FOR LIFE SCIENCES



**OCULAR<sup>®</sup>**

Scientific Image Acquisition Software

2.0 Quick Start Guide



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# Ocular Interface

The screenshot shows the Ocular software interface with several components labeled:

- Quick Access Toolbar**: Located at the top left of the main window, containing icons for Live View, Acquire Image, Abort, and Memory.
- Control Tabs**: Located below the Quick Access Toolbar, including FILE, ACQUIRE, MOVIE, EDIT, and MEASURE.
- Desktop/Workspace**: The main area of the software, currently displaying a grayscale image of a biological specimen.
- Live View Window**: A separate window titled "Live View (Prime sCMOS)" that provides a detailed view of the camera feed and associated controls.
- Live View Tabs**: Located at the top of the Live View window, including LIVE, TOOLS, and LAYOUT.
- Camera Controls**: A panel on the right side of the Live View window containing various settings and monitoring tools.

The Camera Controls panel includes the following sections:

- Exposure Settings**:
 

Exposure Time	200 ms
Binning	No Binning
Readout	100 MHz, 16 Bits, System Gain 1
Clearing Mode	Pre-Sequence
- Histogram (Full Frame)**: A graph showing the intensity distribution of the image.
- Statistics (Full Frame)**:
 

Minimum	175
Maximum	26186
Mean	3688.42
Standard Deviation	2447.33

# Live View Window

The screenshot shows the 'Live View (Prime sCMOS)' window. At the top, there are tabs for 'LIVE', 'TOOLS', and 'LAYOUT'. Below these are icons for 'Snap to Desktop', 'Publish to Desktop', 'Acquire Movie', and 'Auto Expose'. The main area is divided into 'Field of View', 'Intensity', and 'Overlays'. The 'Camera Feed' shows a grayscale image of a biological sample. On the right, there are panels for 'Exposure Settings', 'Histogram (Full Frame)', and 'Statistics (Full Frame)'. The 'Exposure Settings' panel includes fields for 'Exposure Time' (200 ms), 'Binning' (No Binning), 'Readout' (100 MHz, 16 bits, System Gain 1), and 'Clearing Mode' (Pre-Sequence). The 'Histogram' panel shows a graph with a red vertical line at approximately 10,000. The 'Statistics' panel shows: Minimum: 175, Maximum: 24775, Mean: 3563.09, Standard Deviation: 2219.80. At the bottom left, it displays '5.0 fps' and '2048 x 2048'.

Callouts on the right side of the image point to the following features:

- Live View Tabs
- Intensity/Image Scale Tools
- Snap/Publish/Movie Tools
- Exposure Control
- Histogram Display
- Frame Rate and Frame Size



## Exposure Settings

- **Exposure Time, Binning, Readout and Clearing Mode** can be set on either the **Acquire** or **Movie** tabs or directly from the **Live View** window.
- Parameters dynamically link between tabs and the **Live View**.
- **Ocular** sets **Binning** and **Readout** to the selected cameras default values.
- **Clearing Mode** is set to **Clear Pre-Sequence** by default, allowing for maximum frame rates.
- **Auto Exposure** exists as an option in both the **Live View** window and **Acquire/Movie** tabs.
- **Auto Exposure** is ideal for brightfield images but is not recommended for low light fluorescence studies.

The screenshot displays the software's control interface. At the top, a menu bar includes FILE, ACQUIRE, MOVIE, EDIT, MEASURE, and VIEW. Below the menu, a toolbar contains icons for Live View, Acquire Image, Abort, Memory, Auto Name, Auto Exposure, and Trigger. The Exposure Settings panel is open, showing the following parameters:

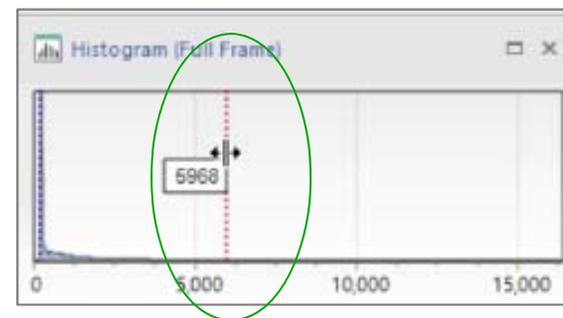
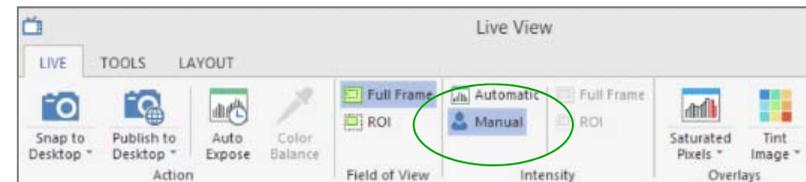
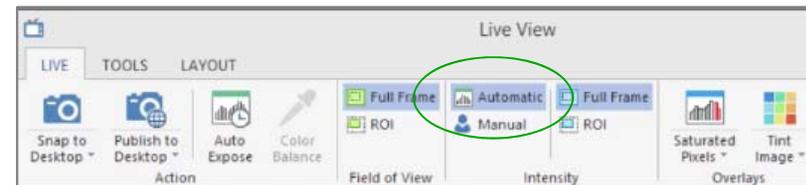
Exposure Time	200 ms	Readout	50 MHz, 14 bits, System Gain 2
Binning	No Binning	Clearing	Pre-Sequence
Readout	50 MHz, 14 bits, System Gain 2		
Clearing Mode	Pre-Sequence		

Below the settings panel, a Histogram (Full Frame) window shows a distribution of pixel intensities with a red vertical line indicating the mean value. At the bottom, a Statistics (Full Frame) window provides the following data:

Minimum	184
Maximum	16383
Mean	1644

## Intensity/Histogram Scale Settings

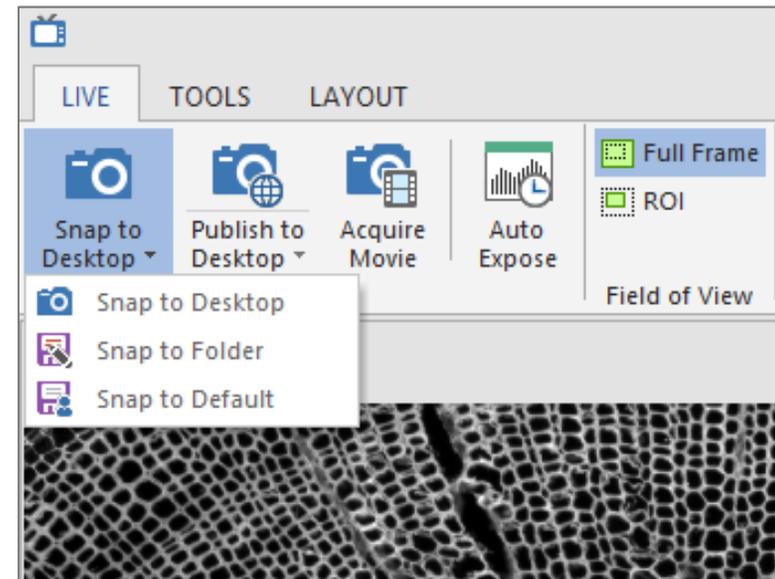
- Ocular sets the display scale to "Best Fit" to ensure the signal being recorded by the camera is scaled for the user to observe it.
- Best Fit Image display is on by default as indicated by the highlighted option **Automatic**.
- By left clicking on the histogram, the slider can be manually adjusted. This intervention automatically sets the Intensity measurement to **Manual**.
- To return to automatic intensity scaling simply reselect **Automatic**.





## Snap or Publish

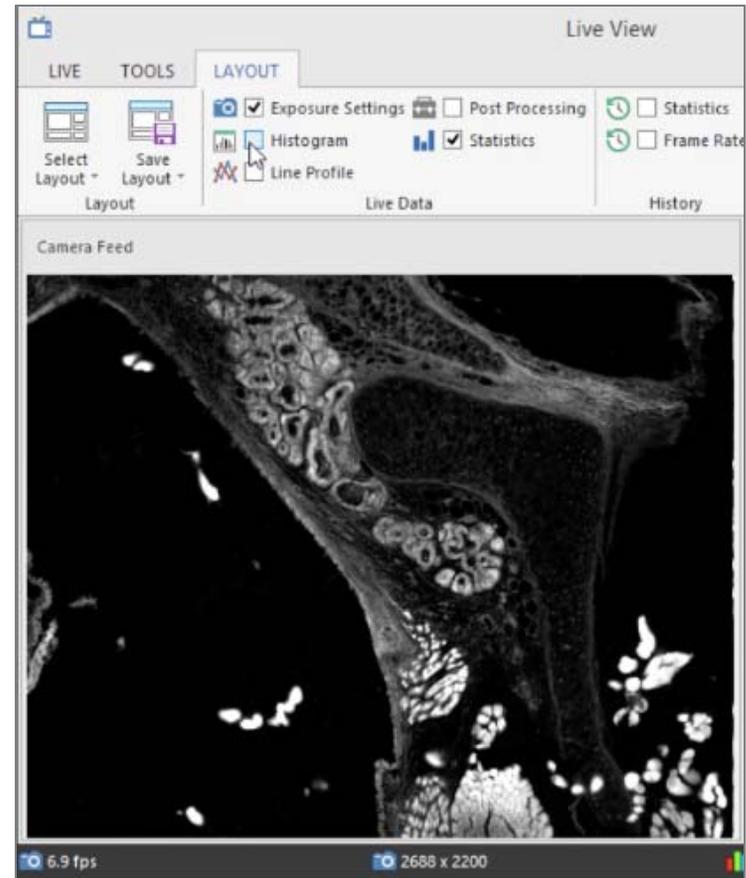
- **Snap** acquires a full bit depth image with the intensity scale set for display only.
- **Publish** acquires the image as a 24-bit image with the intensity scale set to reproduce the current display settings.
- Published images can be saved as compressed formats such as .JPEG.
- Users can **Snap** or **Publish** images to the Desktop or be prompted to save to a specific or default folder.





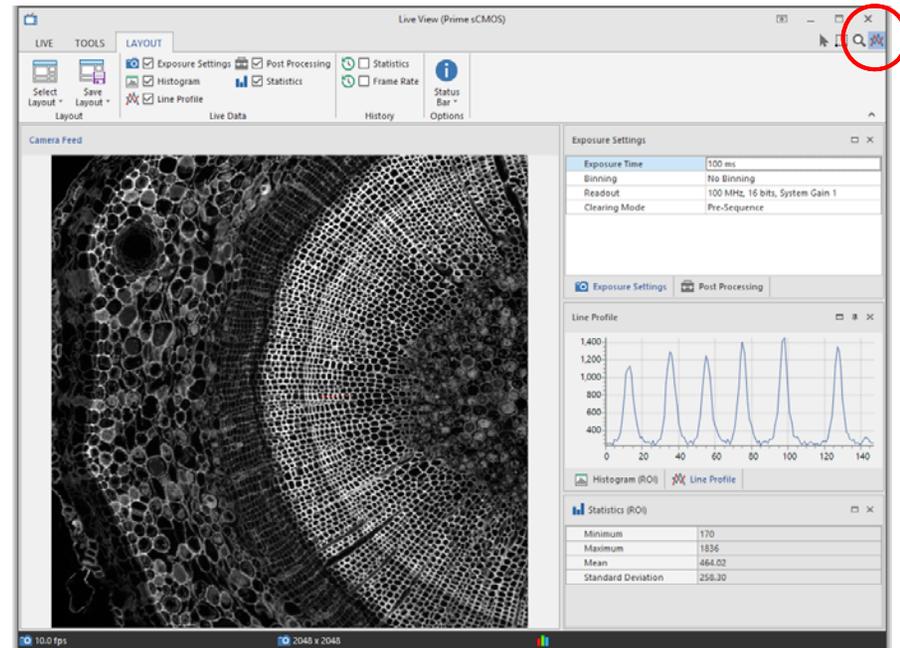
## Customize Live View

- The location and view of all elements of the **Live View** window can be arranged or hidden as needed.
- Under the Layout tab users have the option to select and deselect components, such as histogram and statistics. This allows an increased area for the camera feed.
- Users can select and drag to alter the arrangements of the components.
- **Save Layout** and **Select Layout** saves and loads user preferences.
- At any stage the **Live View** window can be scaled to a user-defined size.



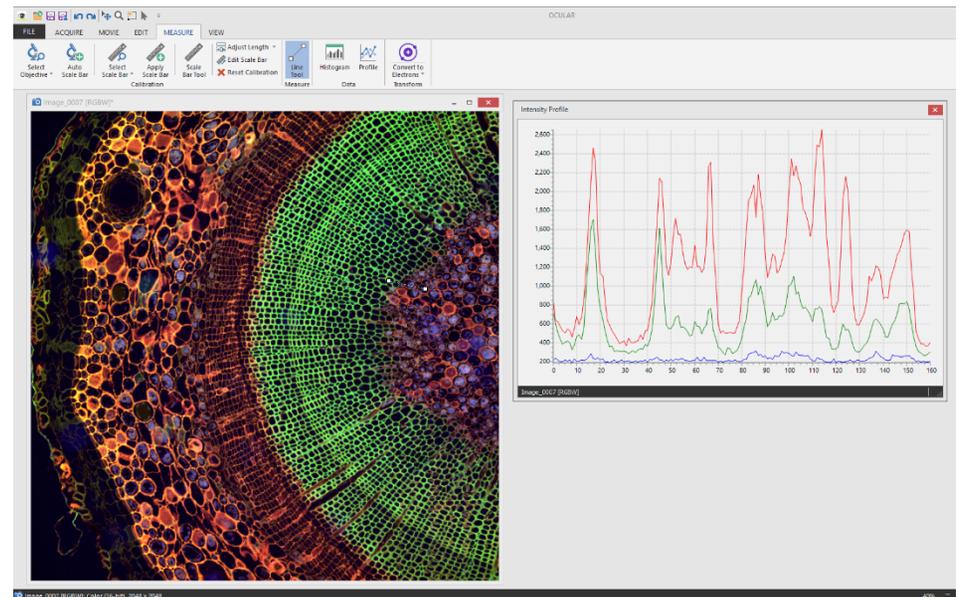
## Live Line Profile

- Users can view a live line profile by making sure **Line Profile** is activated in the **Live Data** section of the **Layout** tab.
- Then a line can be drawn with the line tool in the top right corner of the Live View window.
- The line can be removed by clicking once anywhere on the image with the line tool.



## Post-Acquisition Line Profile

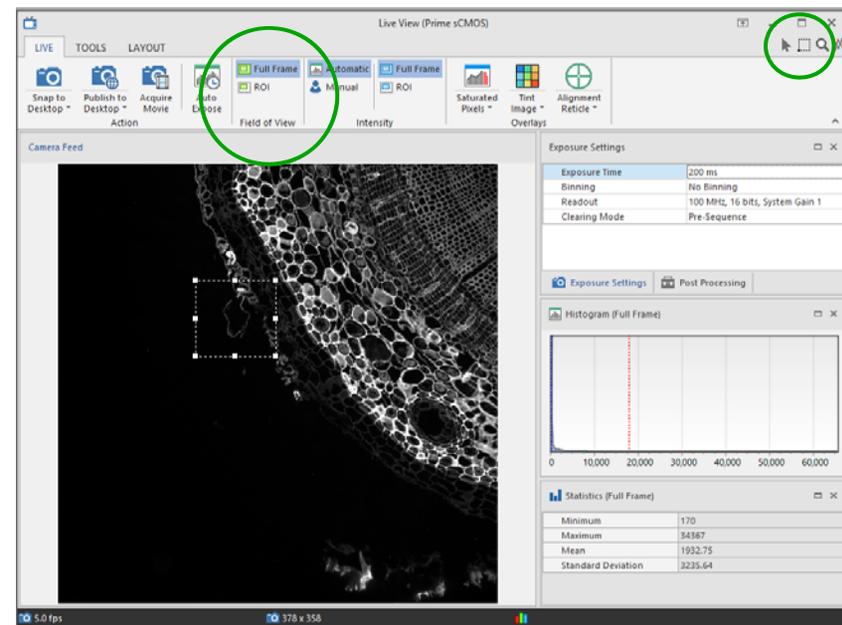
- On the Ocular desktop, a line profile can be drawn by selecting the **Line Tool** from the **Measure** tab.
- A line can now be drawn on the image. (The distance measurement label can be removed by holding Ctrl and clicking the center of the label.)
- Next, select **Profile**.
- Multiple lines can be drawn by selecting the desired lines to display with the mouse pointer tool.





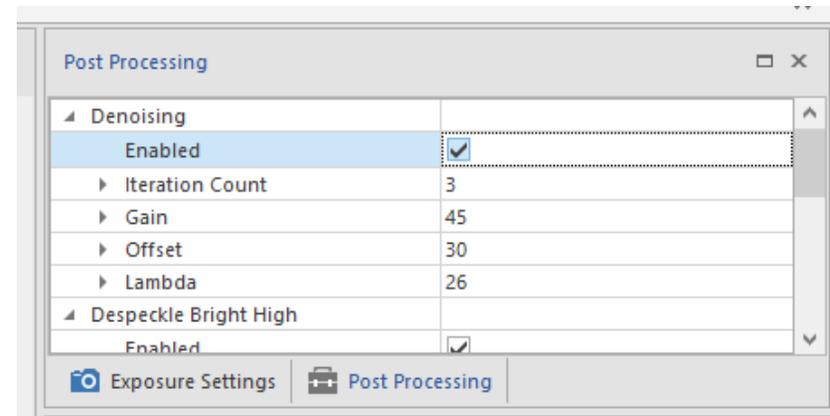
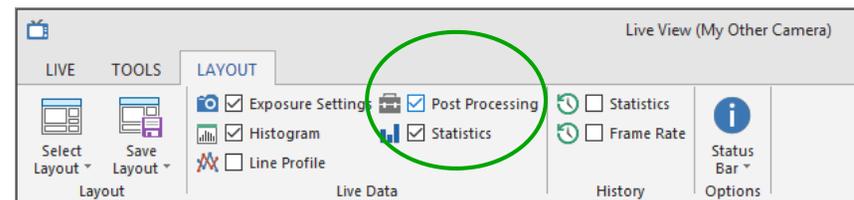
## Region of Interest (ROI) Control

- ROI's can be selected from top right of the **Live View** window.
- Users can left click and drag a selected region onto the image.
- The image with the ROI can be viewed in place or by zooming in by selecting **Full Frame** or **ROI** from the **Field of View** options of the **Live View** window.
- The **ROI** can be deleted by highlighting it and selecting the **Delete** option on the keyboard.



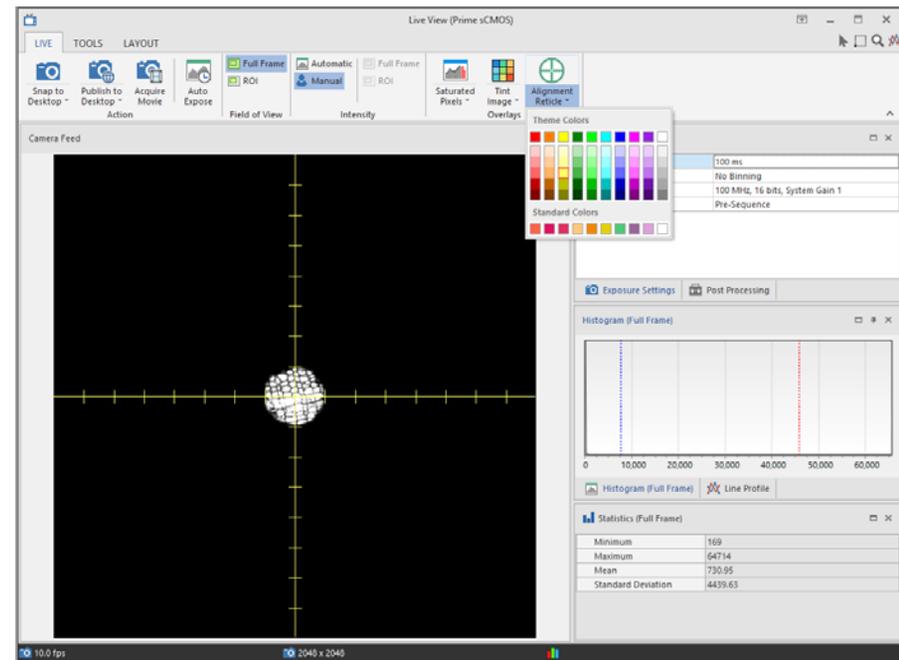
## PrimeEnhance Denoising

- The **PrimeEnhance** denoising algorithm can be activated for compatible cameras by first activating the **Post Processing** options tab, via the **Layout** tab within the **Live View**.
- Within the Post Processing Tab, Find Denoising → Enabled, and check the box.



## Alignment Reticle

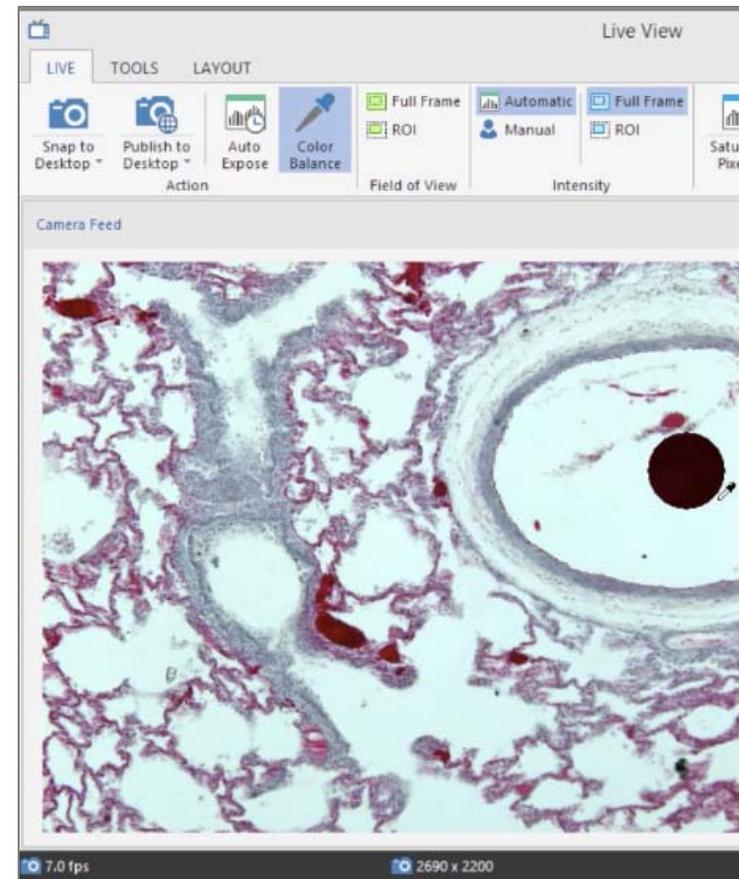
- For some applications an alignment reticle can be very useful. For example this can aid alignment of apertures for Kohler illumination.
- This can be activated by choosing **Alignment Reticle** from the Live View window.
- Then, a color can be selected by clicking the drop-down arrow.





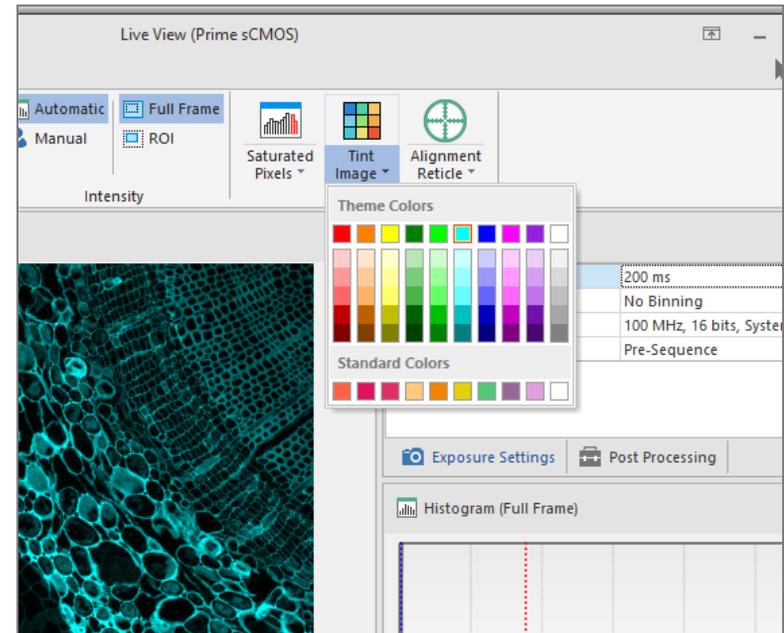
## Color and White Balance

- The best color images can be captured by setting up the microscopes correctly for the user's eye.
- Selecting **Auto Exposure** ensures the camera is using its bit-depth.
- Then **Color Balance** can be selected by left clicking and dragging over to the white area.
- Releasing the left click automatically white balances the image.



## Live Tint

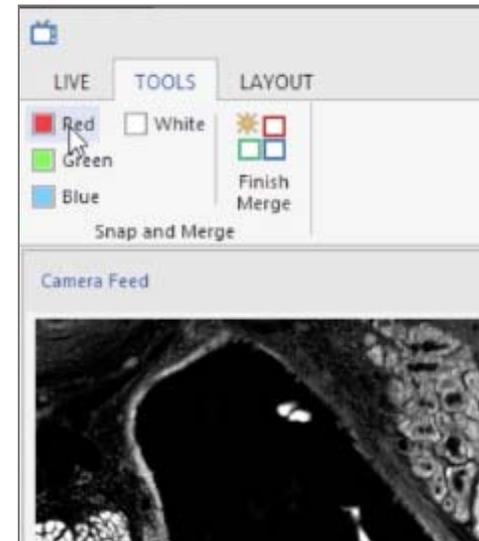
- Images can be matched with colors that are seen down the eye piece by selecting **Tint Image** from the **Live View** window.
- Select the color of choice from the palette.
- The image will now display live in the selected color and **Snap** or **Publish** can be used with the same color scheme.
- **NOTE:** The tint will only be visible outside Ocular if **Publish** is selected.





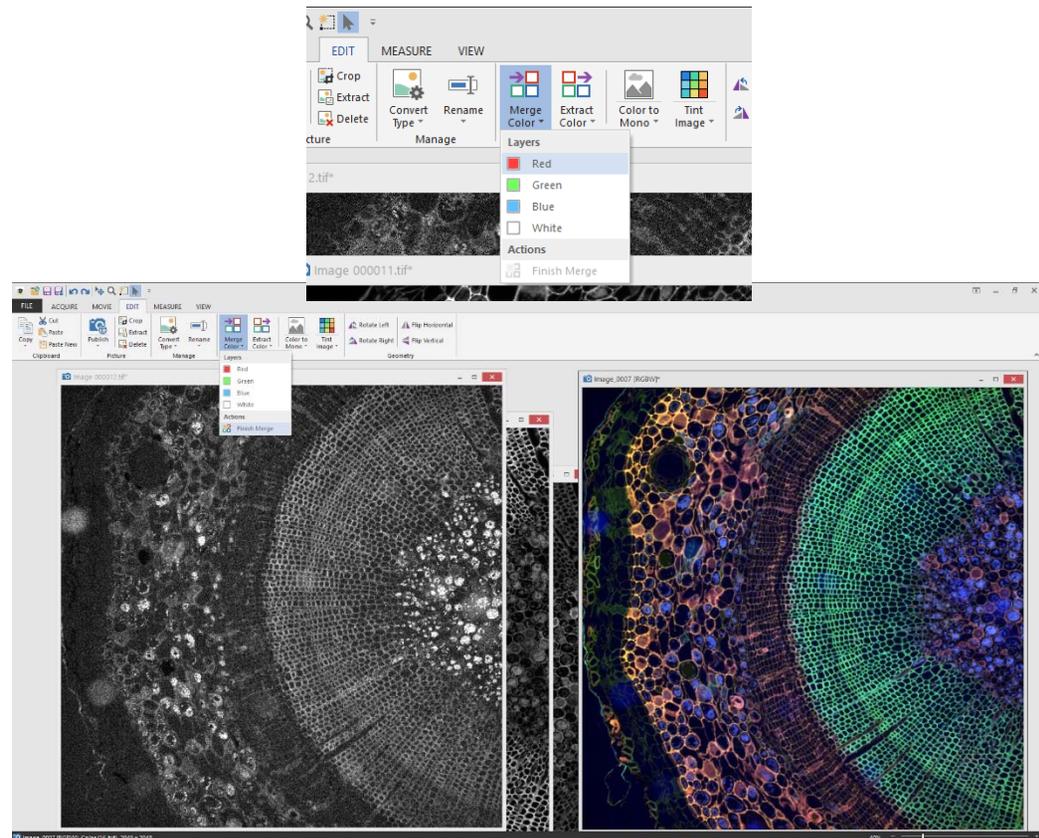
## Tint and Merge

- The **Snap and Merge** tools are accessible from both the **Acquire** Tab and the **Live View** window in the **Tools** tab.
- Users can acquire an image into the workspace with the corresponding tint by selecting a color.
- The image is captured with the current histogram settings.
- Clicking a different color merges the new color into the initial image.
- Clicking on the same color updates the channel in the initial image.
- Selecting **Finish Merge** moves to the next acquisition.



## Post-Acquisition Tint and Merge

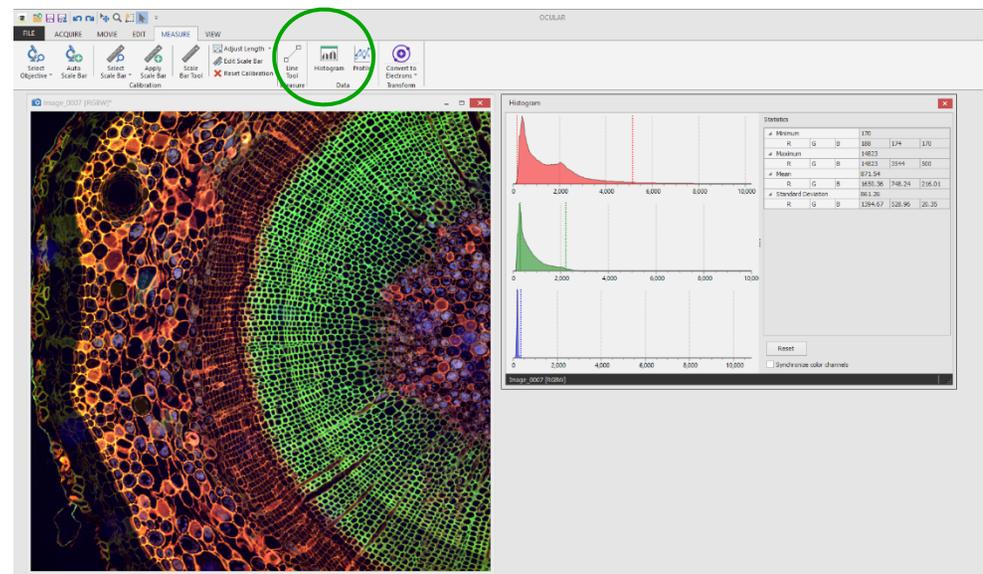
- The **Merge** tool for acquired images is available in the **Edit** tab of the Ocular desktop.
- For each channel of the multi-channel image, first select the monochrome image,
- Then select Merge Color → Red / Green / Blue / White.
- Once all desired channels have been added to the image, choose **Finish Merge**.





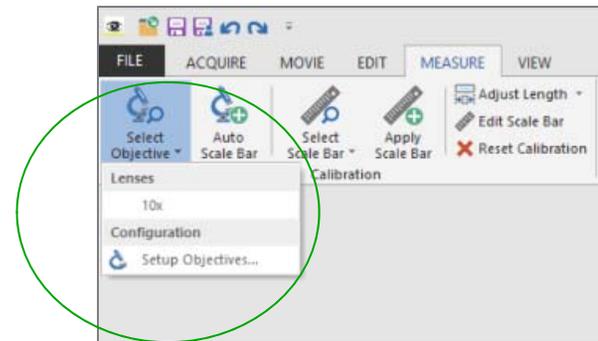
## Histograms – Acquired Images

- View the histogram of an acquired image by selecting Measure, Histogram from the Ocular desktop.
- Adjust the display scaling for the image and view image statistics.
- If the image is color, histograms will be displayed for each color channel separately.
- These can be adjusted independently, by choosing 'Synchronize color channels' to adjust all three simultaneously.



## Calibrations – Set Up

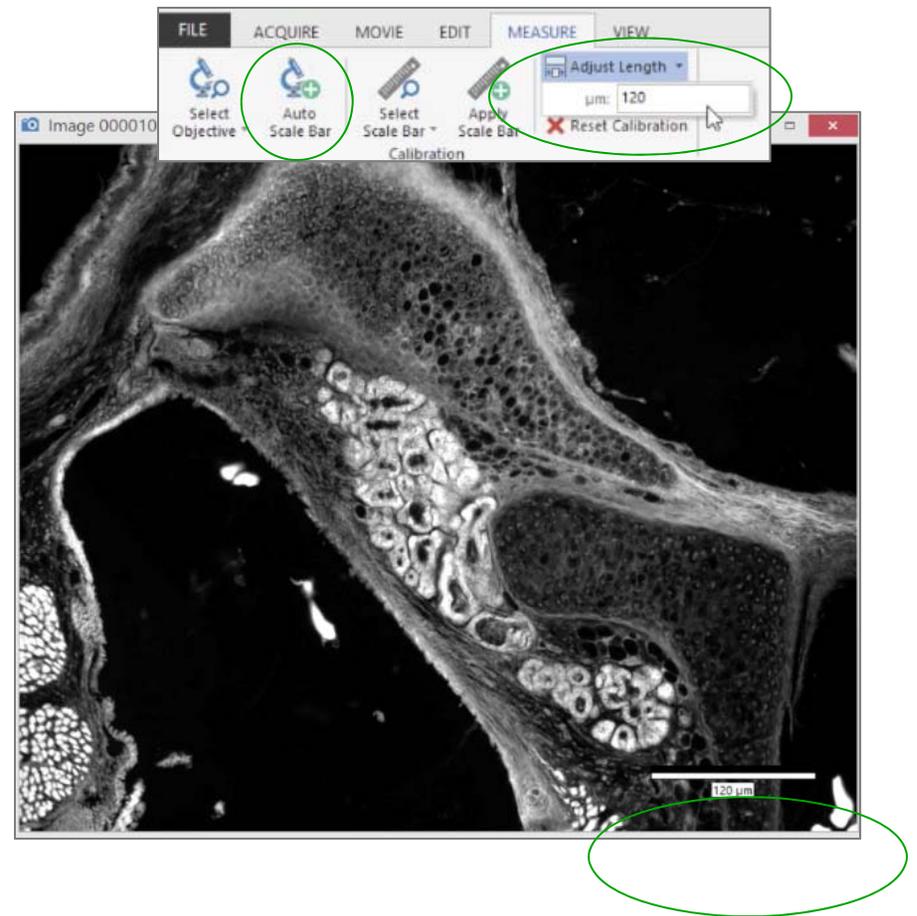
- Before using marker bars, the **Setup Objectives** under the **Select Objectives** option on the **Measure** tab must be completed.
  1. Name the Objective
  2. Select the Magnification of the Objective
  3. Extra magnification refers to any other optics such as a c-mount coupler
  4. Select **Add New** to add it to the List
- Once completed, the Objective will appear in the list of Objectives on the left of this dialog and in the **Lens List** under the **Select Objectives**.





## Calibrations – Auto Scale Bar

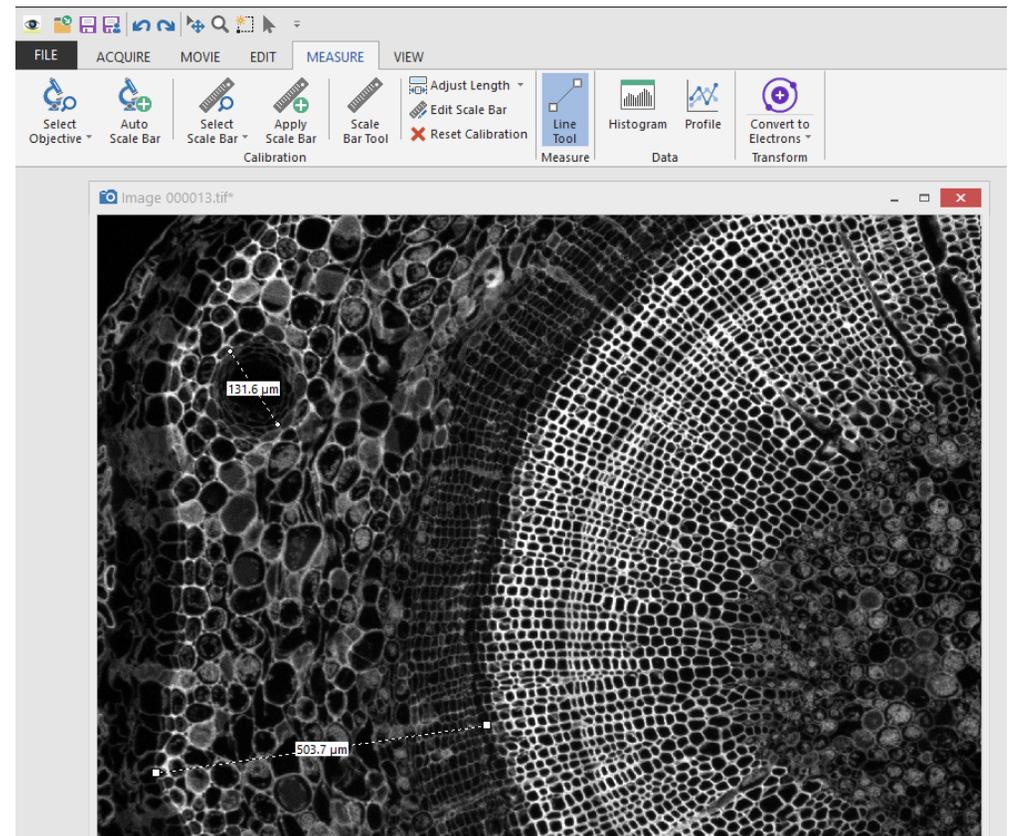
- Add an **Auto Scale Bar** by simply selecting the objective from **Select Objective** and the selecting Add **Auto Scale Bar**.
- This loads a scale bar to the bottom portion of the image.
- The scale bar will be 15% of the field of view in x.
- Length can be adjusted by selecting **Adjust Length** and entering a number.
- The image can be saved with the calibration by selecting the **Publish** option on the **Edit** Tab.





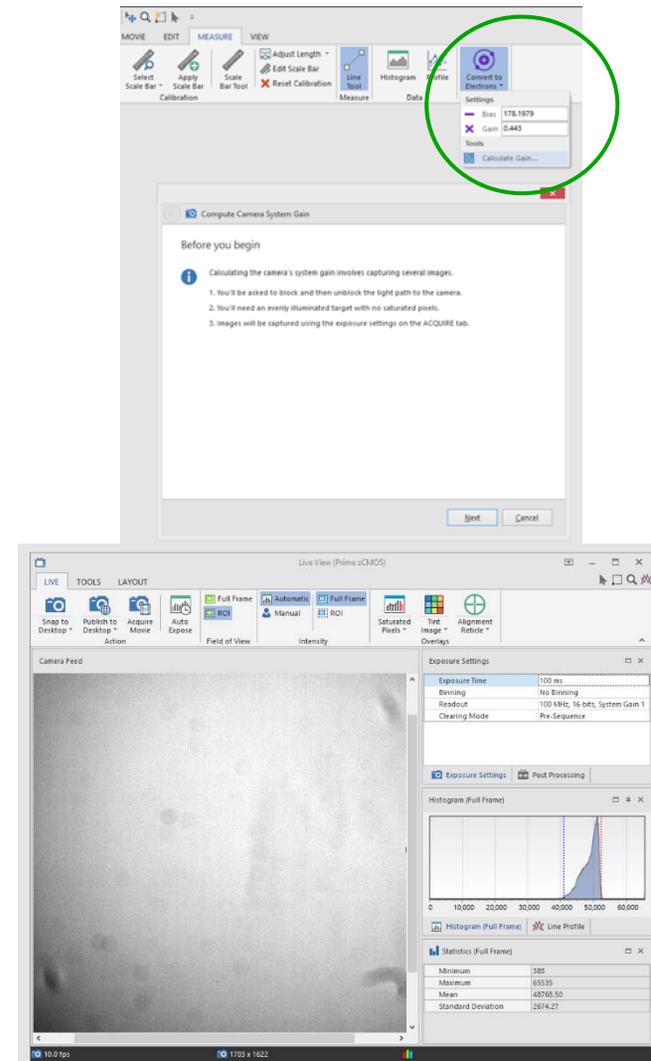
## Measuring Distances

- A line can be drawn by selecting the **Line Tool** from the **Measure** tab.
- For a calibrated image, these distances will be displayed in the chosen units.
- If the image is not yet calibrated, distances will be measured in pixels.
- Drawn lines and measurements can be included in a published image by choosing Edit → Publish, or right clicking the image and choose 'Publish Image'.



## Conversion to Electron Units

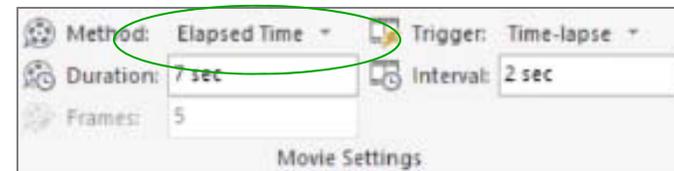
- In many circumstances (such as calculating signal to noise) it can make sense to convert the display units from greyscales to photoelectrons.
- From the Ocular desktop, choose **Convert to Electrons** from the Measure tab.
- If you the camera Bias and Gain settings (such as from a supplied Certificate of Performance), these should be inserted.
- Running the **Calculate Gain Wizard** enables the ability to calculate bias and gain and provides further instructions.
- Note: At one stage, the users will be asked to take flatly illuminated image. A flatter image will yield a better result. An out-of-focus Brightfield illumination of a blank slide is recommended.





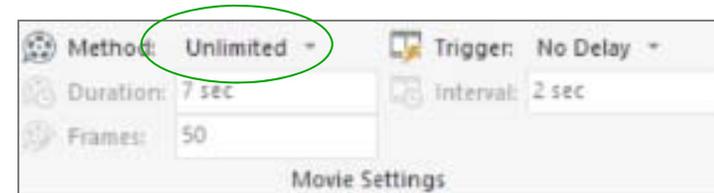
## Movie Capture – Time-Lapse or No Delay

- Ocular allow users to quickly capture Time-lapse or No Delay (Streamed data) Movies.
- From the **Movie Menu** select the **”Trigger”** (time-lapse or no delay).
- From **Method** Select either **Elapsed Time** or **Frame Count**.
- For **Elapsed Time** enter a desired **Duration**.
- For **No Delay** enter a value for **Frames**.
- Movies can then be reviewed using simple re-play tools.



## Start Stop Movies

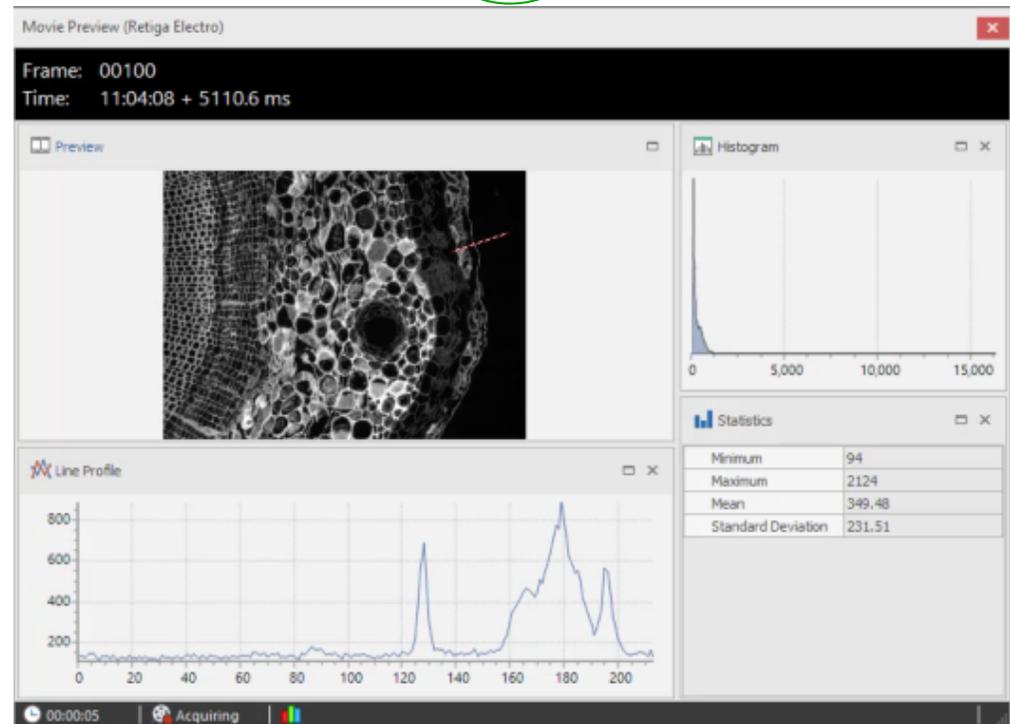
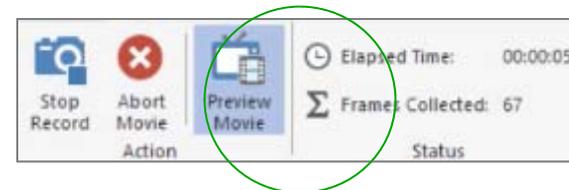
- If **Duration** or **Frames** of experiment is unknown, select **Unlimited** from **Method**.
- This allows the user to **Start Recording** and **Stop Recording** as needed.
- Both the **Elapsed Time** and **Frames Collected** are displayed in the Movies Menu during acquisition.





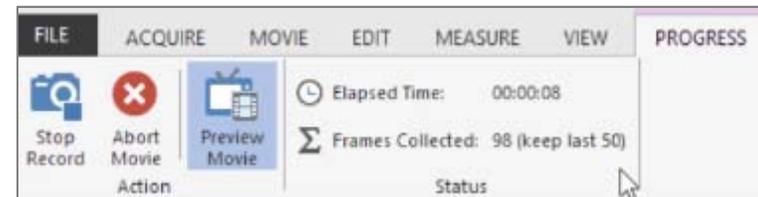
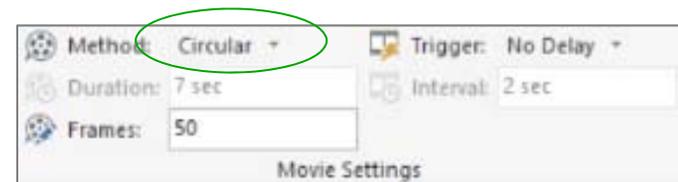
## Movie Preview Window

- To monitor experiments during a movie acquisition, the **Preview Mode** can be activated.
- **Preview Mode** can be selected once acquisition is started and will remain on until deactivated.
- **Preview Mode** initiates a second image display monitor which can be positioned as needed.
- New for 2.0: **Line profiles, statistics** and image **histograms** are also displayed.
- Note: Ocular's **Preview Mode** does not affect recording performance.



## Circular Movies

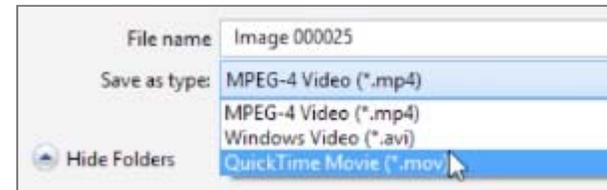
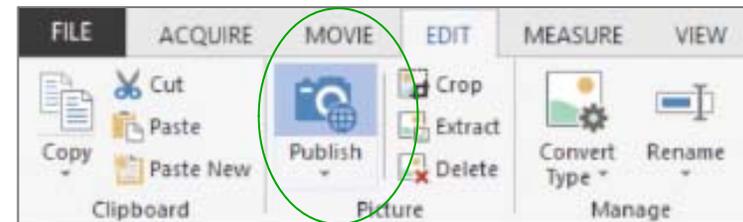
- **Circular Movies** allows users to capture a set number of frames before selecting **Stop Record**, enabling the ability to see the previous  $X$  number of frames.
- **Circular Movies** can be used by selecting it from the **Method** drop down menu.
- Select the number of frames to record –  $X$ .
- Start Recording and conduct the experiment by monitoring in the **Preview Movie Window**.
- When ready, select Stop Record and the resulting movie will be the last  $X$  frames.





## Saving Movies

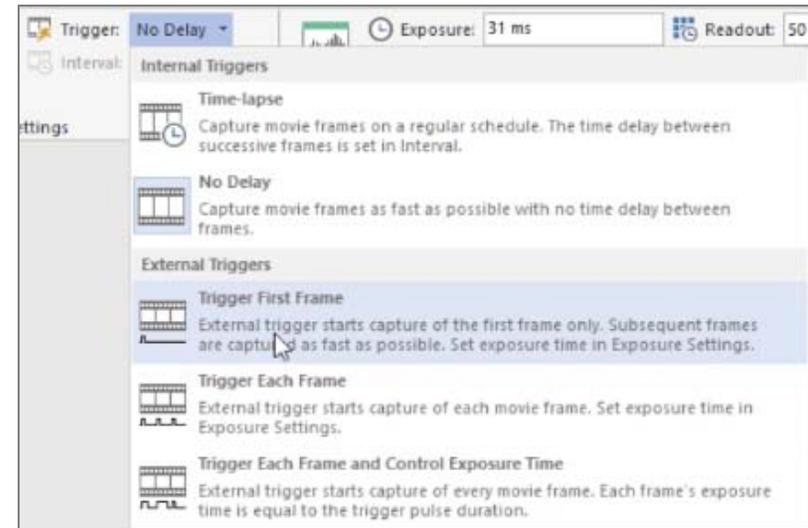
- Movies can be saved for analysis by selecting the **File Menu** or folder icon.
- Acquired movies can be saved in convenient formats such as MP4, AVI or MOV by selecting **Publish** from the **Edit File Menu**.
- Once selected the file is converted to a 24-bit color image, the user will be prompted to save the file in a user-defined format.





## Triggered Movies

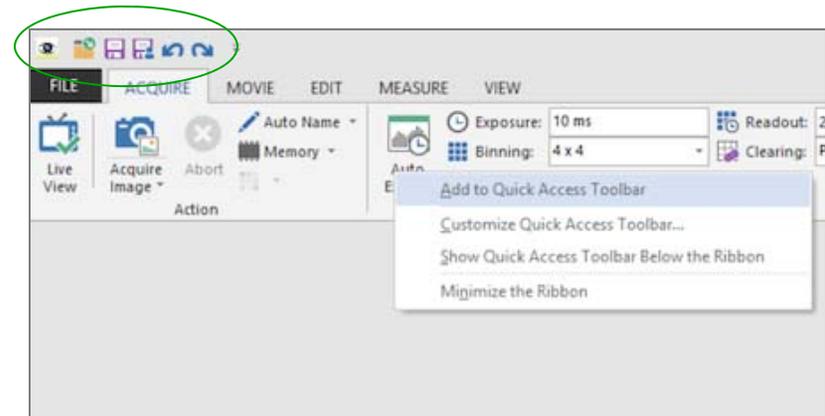
- **Triggers** refers to the initiator of an acquisition.
- **Internal Triggers** refers to software triggers that are sent to the camera to acquire a single image from a **Time-lapse** or by starting a **No Delay** movie.
- **External Triggers** refers to the triggers that are sent to the camera via the camera's trigger in-put.
- The software can be set to expect the triggers to start an acquisition of the **First Frame**, for **Each Frame** or to control the exposure through the input Trigger width.





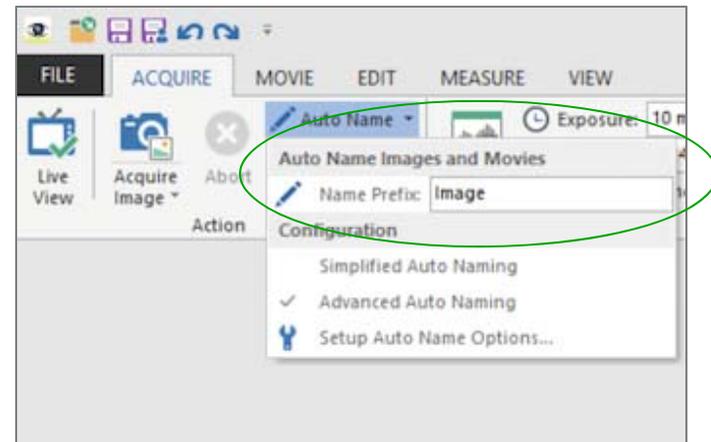
## Quick Access Toolbar

- To add workflow, tools can be added to the **Quick Access Toolbar** (highlighted).
- Tools can be added by right clicking on the tool and selecting **Add to Quick Access Toolbar**.
- Items can be removed from the **Quick Access Toolbar** by right clicking and selecting **Remove from Quick Access Toolbar**.



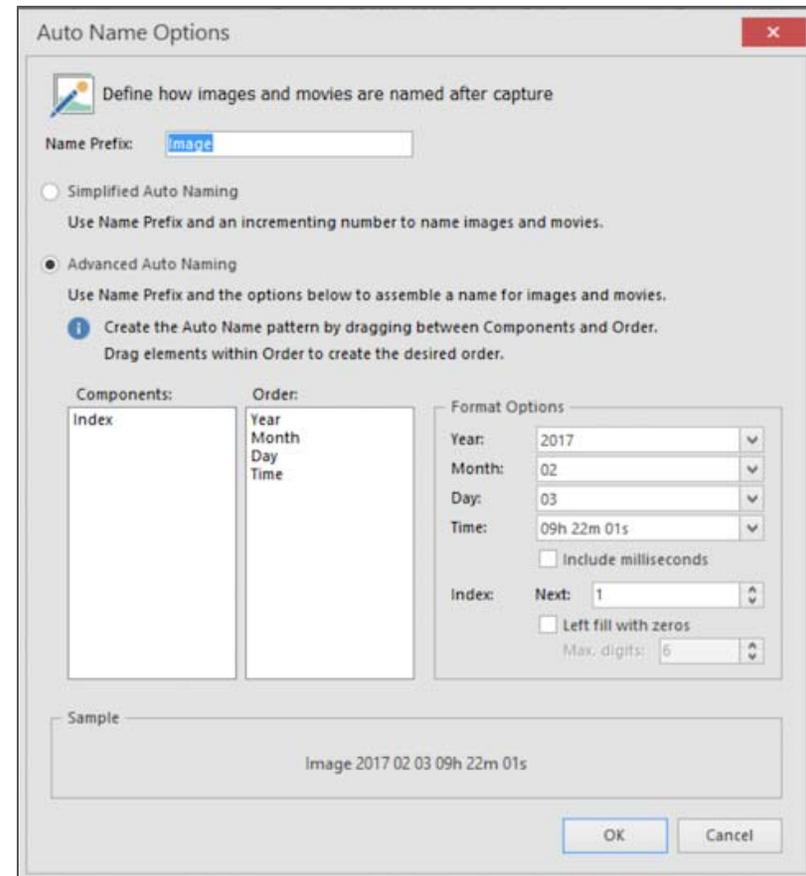
## Auto Naming - Basic

- **Ocular** uses a naming convention of “Imaging0000”. As images are acquired, they are given the name Image0000 which incrementally increases as more images are taken.
- By selecting **Auto Name** from the **Acquire** or **Movie** tab, users can enter a user-defined **Name Prefix**.



## Auto Naming - Advanced

- More advanced auto naming options are available by selecting **Setup Auto Name Options**.
- To aid users who need a more advanced nomenclature, **Ocular** allows users to select parameters such as **Year, Month, Day** and **Time** that are added to the **Name Prefix**.
- Once selected all images will be acquired with the Prefix name, followed by the selected parameters.





## Selecting Between Available Cameras

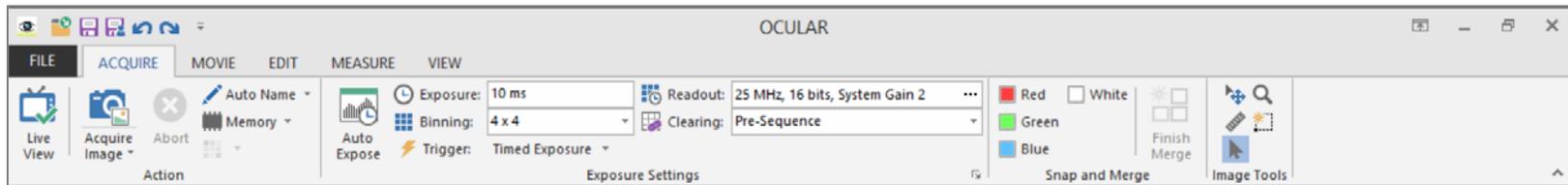
- If multiple compatible cameras are running, users can choose between them via **File** → **Cameras**.
- All available cameras will be listed alongside some useful properties and information.

The screenshot shows the 'Cameras' menu in the software. The menu is open, displaying a list of cameras and a 'Properties' section. The 'Cameras' list includes two entries: 'My Camera' (Qimaging SciCamPro, 1376 x 1024) and 'My Other Camera' (2048 x 2048). The 'Properties' section displays a comparison of various camera specifications for both cameras.

	My Camera	My Other Camera
Camera Firmware Version	14.22	17.3
Camera Head Serial Number	Q42217	A17D202002
Chip Name	ICX825	C152020F
Color Capable	No	No
Device Driver Version	2.0.0	2.0.0
Full Well Capacity	240000 e	30000 e
PCI Firmware Version	1	1
Product Name	SciCamPro	
Sensor Size	1376 x 1024	2048 x 2048
Vendor Name	Qimaging	



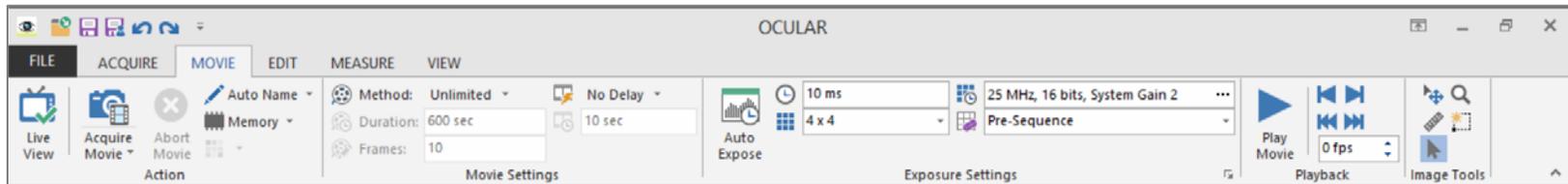
## Acquire Tab



- **Live View** - Re-opens the **Live Window** if closed
- **Acquire Image** – Acquires an image into the workspace
- **Auto Name** – Allows a personalized image naming convention
- **Exposure Settings** – Replicates **Live View** tools
- **Snap and Merge** – Replicates **Live View** tools
- **Image Tools** – Zoom, Region, Scale and Select



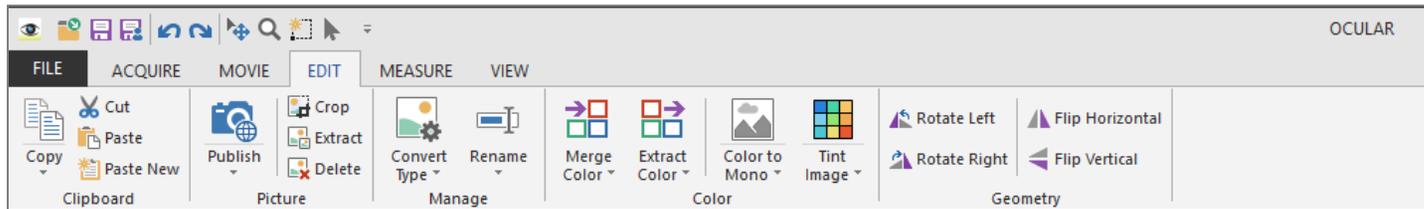
## Movie Tab



- **Live View** - Re-opens the **Live Window** if closed
- **Acquire Movie** – Acquires a movie into the workspace
- **Abort Movie**– Aborts the movie mid acquisition
- **Auto Name** – Allows a personalized image naming convention
- **Movie Settings** – Controls parameter settings for movie acquisition
- **Exposure Settings** – Replicates **Live View** tools
- **Playback** – Basic navigation tools



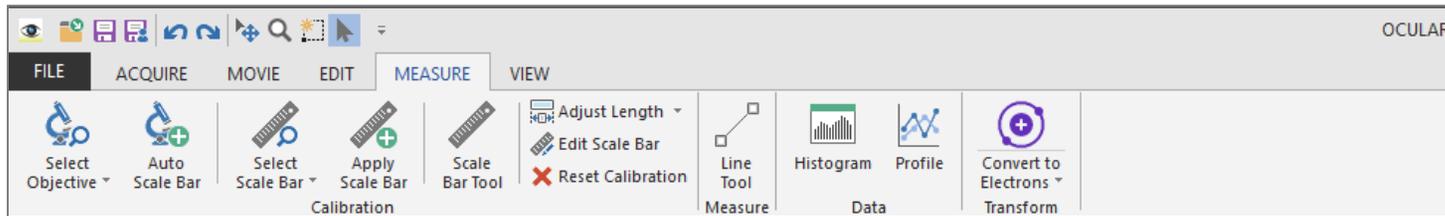
## Edit Tab



- **Clipboard** – Cut/Copy/Paste images or regions
- **Picture – Publish** – Converts an image to a 24-bit color image
- **Picture – Crop/Extract/Delete** – Basic cropping tools
- **Manage – Convert** – Allows conversion to differing bit-depths
- **Manage – Rename** – Allows image to be re-named pre-save
- **Color – Merge Color** – Merge multiple greyscale images into a single multi-color image
- **Color – Extract Color** – Allows for Red/Green/Blue images to be extracted
- **Colour – Color to Mono** – Converts color images to monochrome
- **Colour – Tint Image** – Replicates **Live View** tools
- **Geometry – Rotate & Flip** – Correct image orientation & mirroring



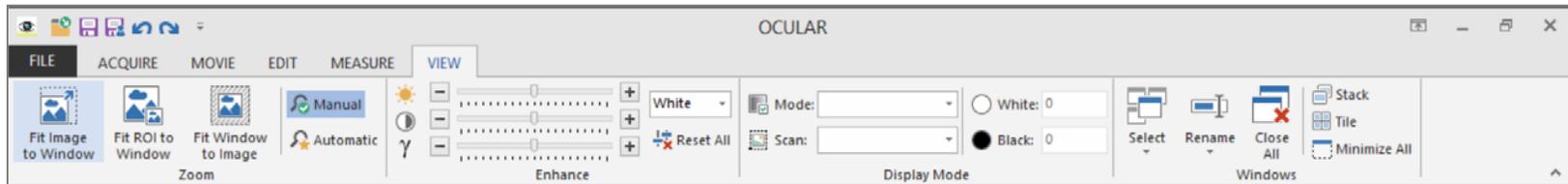
## Measure Tab



- **Select Objective** – Allows users to select preselected Objectives or create new ones
- **Auto Scale Bar** – Places a Toolbar on the image, (20% of the image width) and applies calibration settings.
- **Select Scale Bar** – Allows users to select or setup dedicated Scale Bars width
- **Apply Scale Bar** – Applies to the selected Scale Bar
- **Adjust Length** – Allows users to adjust the length of the **Auto Scale Bar**
- **Edit Scale Bar** – Allows users to **Edit Scale Bar** (length/position etc.)
- **Reset Calibration** – Removes **Calibration** from the image
- **Line Tool** – Draw a line on the image to measure distance or plot a **line profile**.
- **Histogram** – View and adjust the histogram for the image
- **Profile** – Display a line profile for selected line (drawn with the Line Tool)
- **Convert to Electrons** – Display brightness in units of electrons measured in each pixel



## View Tab



- **Zoom Fit** – Allows users to select different auto fit/zoom preferences
- **Zoom Manual** – Allows users to zoom as needed with no override
- **Zoom Automatic** – Sets display to one image pixel to one screen pixel
- **Enhance** – Post Acquisition Brightness/Contrast and Gamma tools
- **Display Range** – Allows users to adjust display range of acquired images
- **Windows** – Allows users differing lay-out styles for multiple images in a workspace and **Close All** tool.



**PHOTOMETRICS**

CMOS, EMCCD & CCD CAMERAS FOR LIFE SCIENCES

# Contact Photometrics for Additional Information



**OCULAR**<sup>®</sup>

Scientific Image Acquisition Software

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