How to use Micro-Manager Software with Teledyne Photometrics Cameras

Dr. Matthew Köse-Dunn, Teledyne Photometrics – Version A4, 2023

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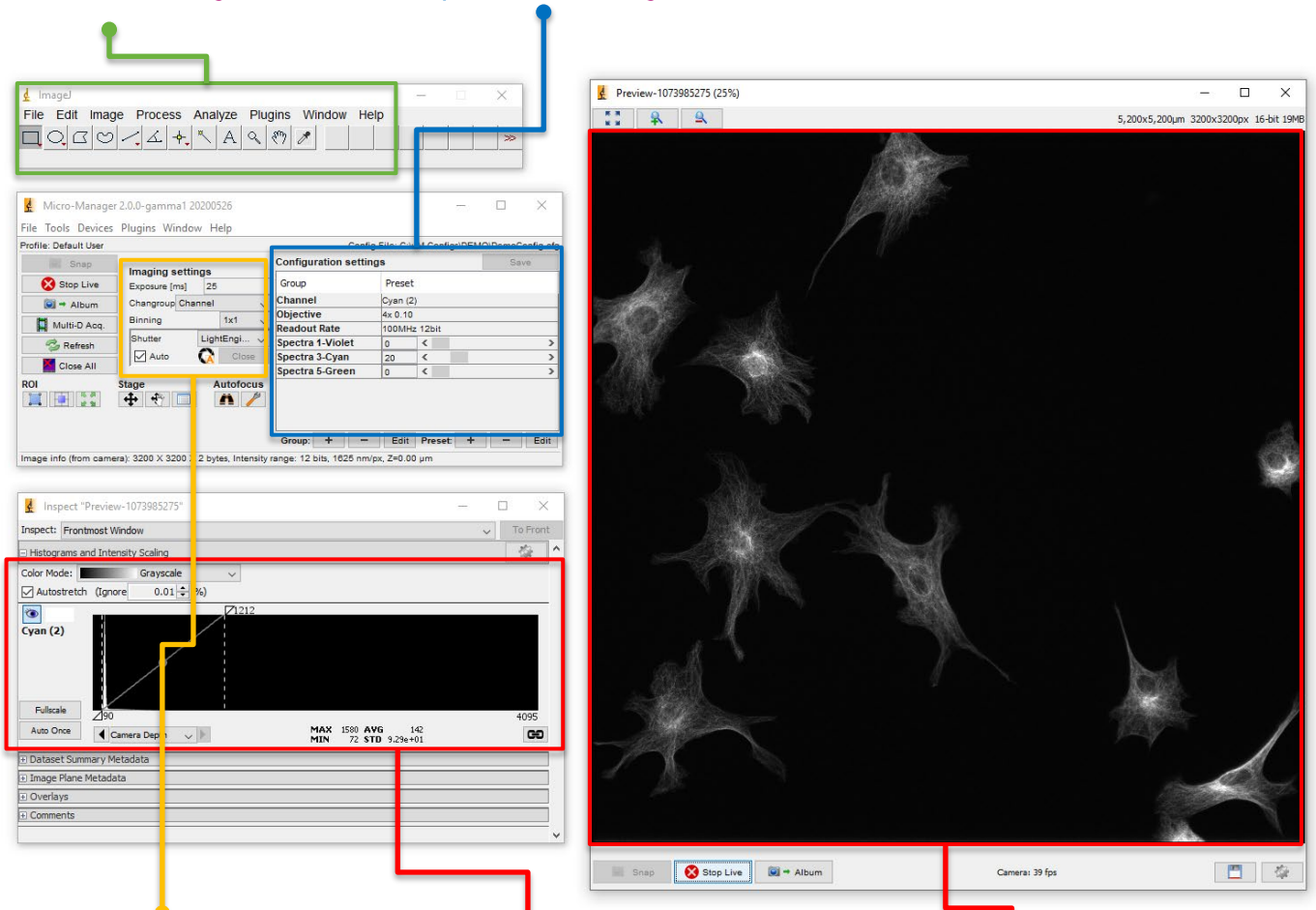
Feature Overview

Micro-Manager is a free, open-source microscope control software plugin based inside ImageJ.

- Microscopes supported from every major brand
- Stages, Piezos, Filter Wheels, Shutters, and more specialist hardware also supported
- A vast library of ImageJ and Micro-Manager image analysis and acquisition plugins developed and maintained by a strong and diverse community of imaging users
- Built-in macro support and ability to write custom plugins
- Teledyne Photometrics Micro-Manager camera drivers are developed in-house so are always up to date with camera features well supported

ImageJ Analysis tools and plugins available easily
ImageJ Functions, Page 37

Easy access to hardware controls & custom shortcuts
Groups & Presets, Page 17



Easy Camera & Light Source Controls

Histogram & Image Scaling Controls (LUTs)
Image Handling, Page 33

Live Preview, before and during acquisition
Image Handling, Page 29

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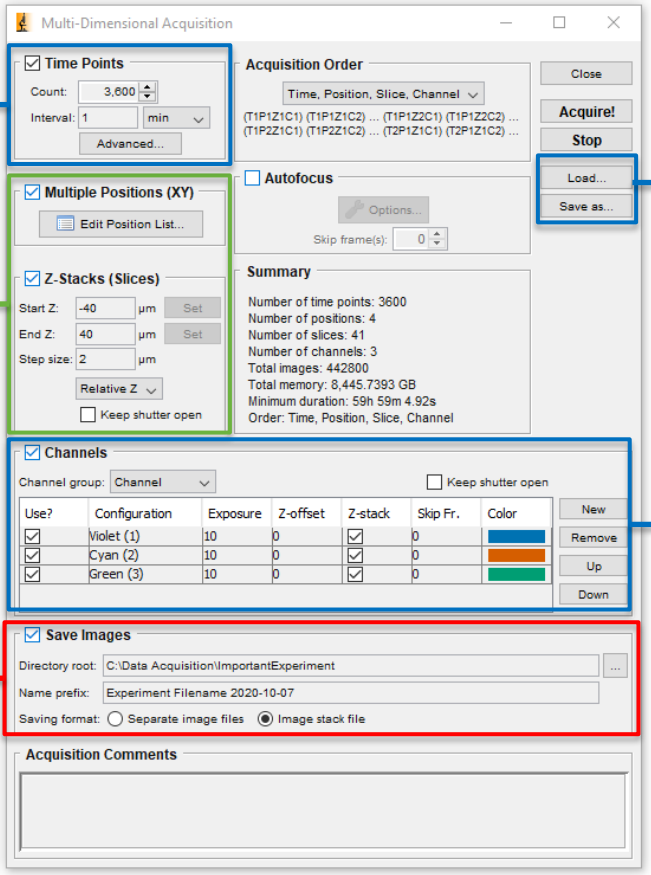


Streaming Acquisition and Timelapse Movies & Timelapse, Page 31

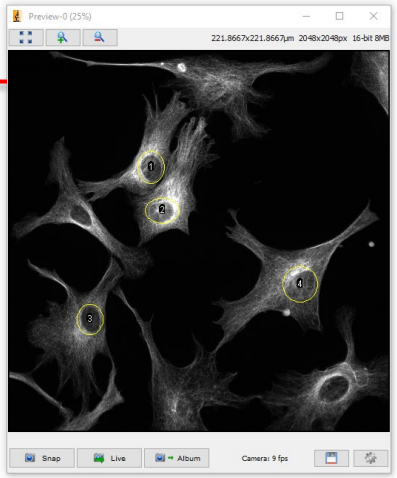
Support for XY Stages and Tiling, and for Z / Piezo Stages

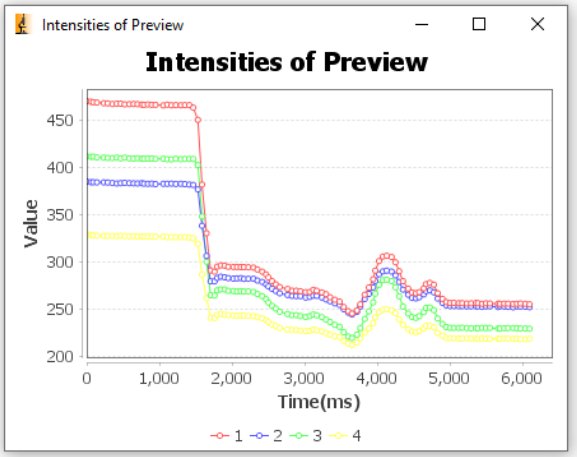
Streaming to RAM or to disk

Save & Load Experimental Configurations

Multi-Channel Acquisition, Multi-Channel, Page 31

Real time intensity graphs & ratiometric intensities, Real Time Intensity, Page 38





...with many additional features regularly added!

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How to Get Started

Welcome to this guide on how to use Micro-Manager software with Teledyne Photometrics cameras! Below are our suggested steps for how to begin using Micro-Manager to make the most of your camera.

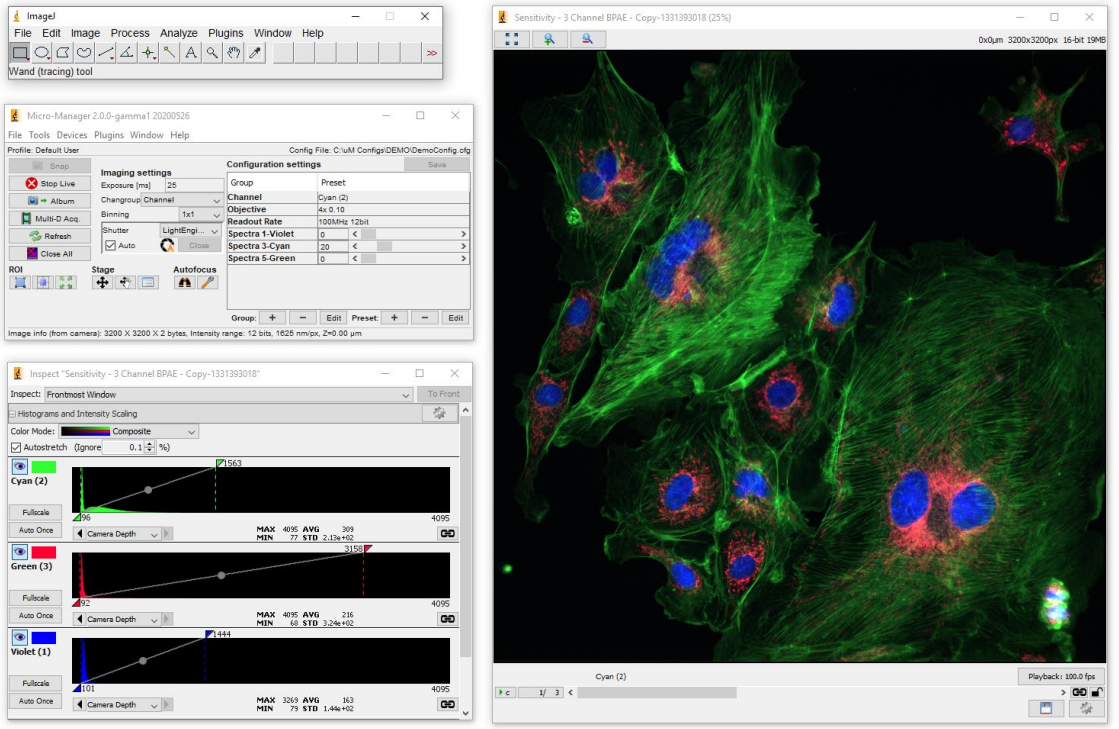
1. Install your camera onto the microscope and connect it, and any other hardware, to the computer.

2. Download and install PVCAM Drivers (see Page 6)

3. Download and install Micro-Manager (see Page 7)

4. Download or create a Hardware Configuration File to tell Micro-Manager what hardware you'll be using (see Page 9)

5. Go 'Live' and start imaging!



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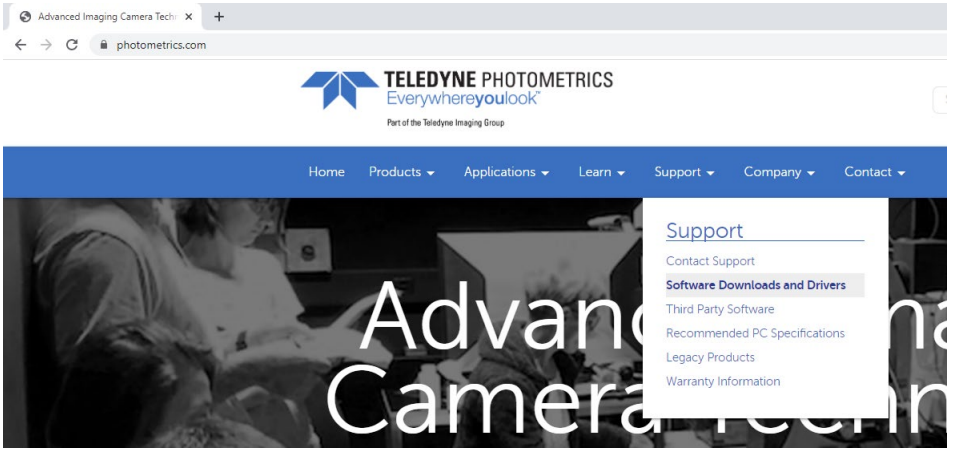
Installation

Download and Install PVCAM Drivers

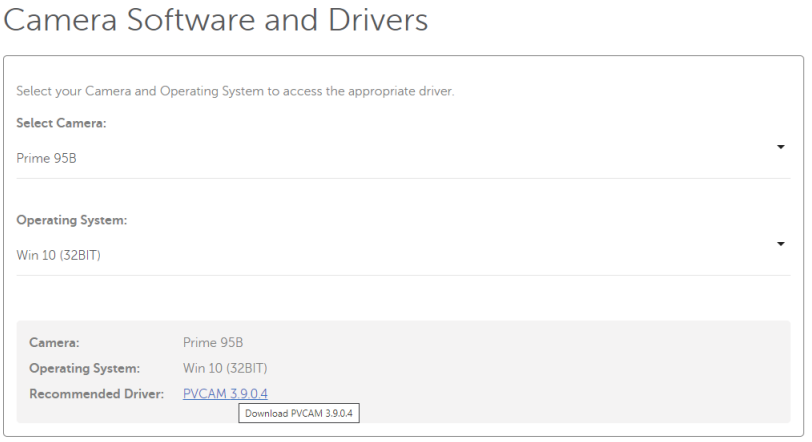
The first step to installing your Teledyne Photometrics camera is to download the latest PVCAM drivers from our website. Here is a link:

1. Visit: https://www.photometrics.com/support/software-and-drivers

   Or alternatively, visit www.photometrics.com and click '**Support**', then '**Software Downloads and Drivers**'.



2. Specify which camera you are using and which operating system. The website will then recommend the appropriate driver version. Click the '**Recommended Driver**' link.



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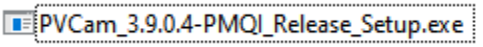
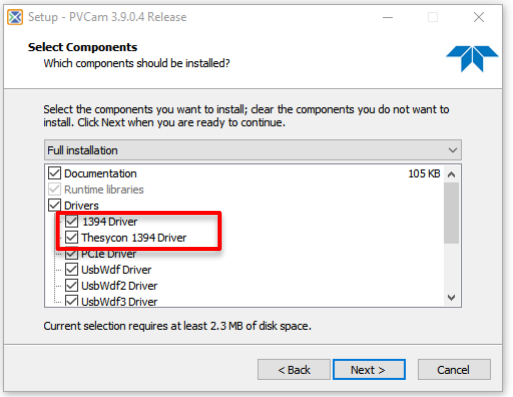
3. You will be invited to fill out a form to immediately receive a link via email to download our driver set in a .zip file. Download and extract this, and run the .exe file contained inside:



4. Run the installer.

   Note: If your camera has a USB or PCIe interface and does not run on the older Firewire interface, **but** you do have other firewire hardware such as older microscopes or devices, uncheck the two drivers containing '**1394**', as these may conflict with other firewire devices. If you have no firewire devices, you do not need to change anything.



5. Connect your camera power and data cables as directed in the camera manual.

Download and Install Micro-Manager

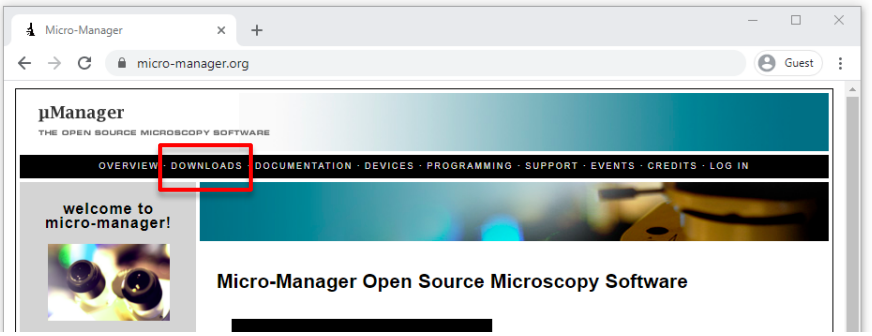
Micro-Manager has an enormous array of versions available. However, at any one time there is likely only one recommended version, that being the **latest nightly build** of **Micro-Manager 2.0.1** (the current version at the time of writing).

For some hardware and plugins other versions may be required; please see the support pages for your hardware.

To download Micro-Manager:

1. Open the Micro-Manager website at https://micro-manager.org/, or skip to Step 4 by following the link below:

   https://download.micro-manager.org/nightly/2.0/Windows/



2. Click 'Downloads' (https://micro-manager.org/Download_Micro-Manager_Latest_Release)

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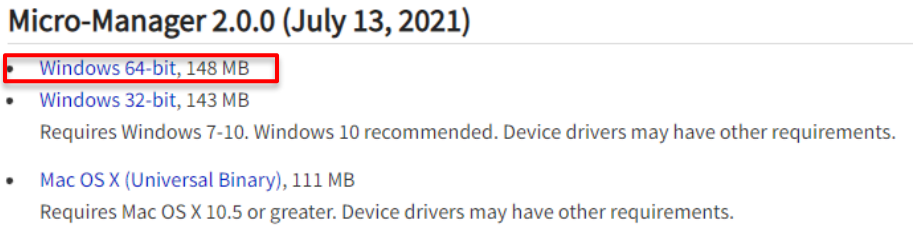
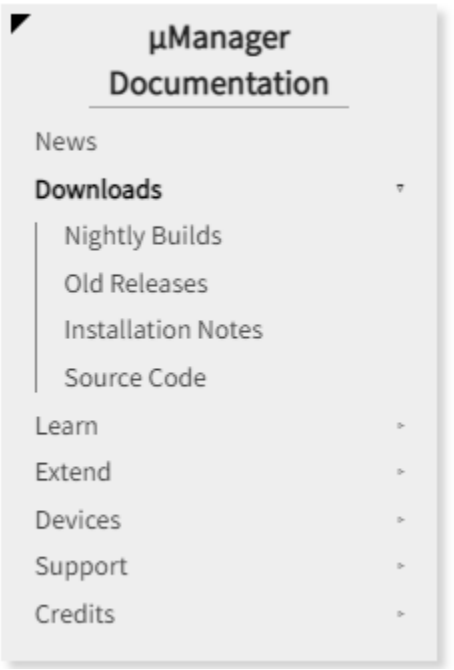
3. Scroll down to and click the link for **Micro-Manager 2.0.0 (Windows 64-bit).** The software will start to download. This version is the latest stable version of Micro-Manger as of July 2021.



4. Alternatively, if you wish to use the latest nightly build, access the menu to the right of the page and select '**Nightly Builds**', then select '**Version 2.0 (Windows)**'**.** The topmost link will be the latest released version for 64-bit computers. Click this to download, and run the installer.



As of 2022-02-26, nightly builds are generally skipped when no changes have been made to the source code since the previous nightly build.

MMSetup_64bit_2.0.1_20230120.exe (160.2 MiB) 2023-01-21T06:20:46.893Z
MMSetup_64bit_2.0.1_20230118.exe (160.2 MiB) 2023-01-19T06:21:57.838Z
MMSetup_64bit_2.0.1_20230117.exe (160.2 MiB) 2023-01-18T06:21:24.075Z
MMSetup_64bit_2.0.1_20230116.exe (160.2 MiB) 2023-01-17T06:20:00.566Z
MMSetup_64bit_2.0.1_20230110.exe (160.2 MiB) 2023-01-11T06:22:06.039Z
MMSetup_64bit_2.0.1_20230106.exe (160.2 MiB) 2023-01-07T06:20:05.192Z
MMSetup_64bit_2.0.1_20230103.exe (160.2 MiB) 2023-01-04T06:21:40.172Z

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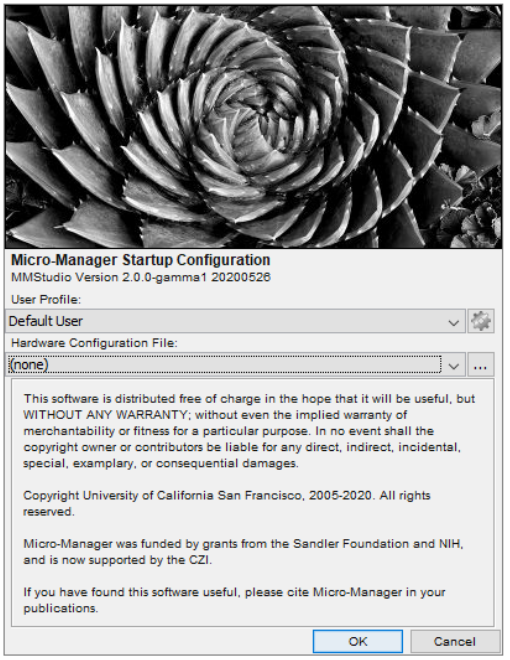
Creating A Hardware Configuration File

Micro-Manager can be configured to control a very wide array of microscopy and other imaging hardware. When you first open Micro-Manger you will need to create a 'Hardware Configuration File' where you tell Micro-Manager what imaging hardware you are using (camera, microscope, motorized stages, light sources, etc.) so you can control it all through Micro-Manager.

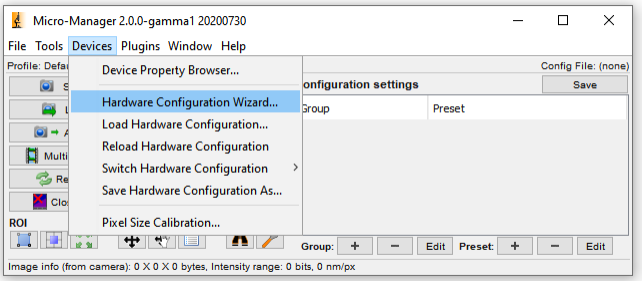
Hardware Configuration Files are created and edited via the Hardware Configuration Wizard. On your first time opening Micro-Manager you'll need to create the config file, but every subsequent time you use the software this file will be pre-loaded and ready to go. You can also modify existing files, such as adding a new piece of hardware.

Creating a new Hardware Configuration File

1. Make sure your camera and any additional imaging hardware is powered on, turned on, and successfully connected to the computer.
2. Open Micro-Manager, and when prompted for a Hardware Configuration File, select '(none)'.



3. When the Micro-Manager window opens, go to 'Devices', choose 'Hardware Configuration Wizard'.



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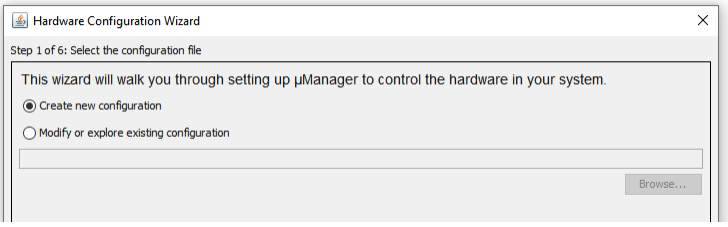
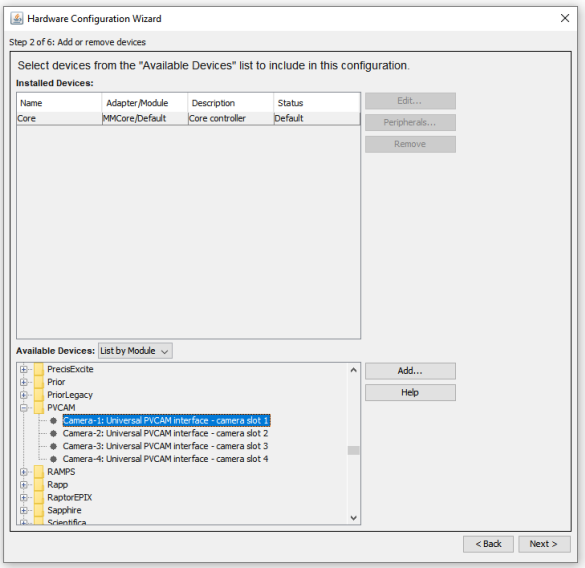
4. Select '**Create new configuration**'.



5. On the following page you can add or remove imaging hardware. In order to control the camera, light source etc., they will need to be added to this list for Micro-Manager to recognize.
    a. Under '**Available Devices**' is an alphabetical list of imaging hardware vendors. Find your device folders and select '**Add**', where you will be prompted to name the device, at which point it will be added to Micro-Manager.
    b. Most devices have an online help and installation guide, which can be found through selecting the device and clicking '**Help**'.
    c. Teledyne Photometrics cameras are listed under the driver set '**PVCAM**'. There are up to four camera slots that you can use. If you have only one camera connected, this will always be '**Camera-1**'.



6. Once you have added all your necessary imaging hardware, advance the wizard. Some hardware may have further configuration steps; for PVCAM Cameras, no further steps are necessary!
7. The final step will invite you to **name and save** your Hardware Configuration File. This will be saved by default to the Micro-Manager installation folder within C:\Program Files, and will be pre-selected every time you use Micro-Manager. You can make a number of different config files for different imaging setups, just make sure you know where they are saved.

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Installing other microscope hardware

Light Sources

Micro-Manager can control light sources from many manufacturers. Some example installation guides are linked below:

CoolLED pE300, pE340 and pE4000 Light Sources

Please see the guide linked below for instructions on driver installation. This also discusses the creation of shortcuts to necessary settings ('Groups') and the use of the light source for multi-channel acquisition.

https://www.coolled.com/technical-resources/videos/micro-manager-for-pe-300-series-and-pe-340fura/#pe-400016122586602461612263176353

Lumencor SPECTRA, SPECTRA X, AURA and CELESTA Light Engines

Instructions to connect these light sources can be found here:

https://micro-manager.org/wiki/LumencorSpectra

Other Hardware

For a list of supported hardware in Micro-Manager, complete with installation guides for devices, please see the following web page:

https://micro-manager.org/wiki/Device%20Support

For some hardware, additional files and drivers may be needed from the device manufacturer. Please see the website for your device manufacturer or contact their support teams.

Which Hardware Is On Which Port?

Please note, whenever adding any hardware to Micro-Manager using USB interfaces, you will need to check which COM port each device is assigned to in order for them to function as intended. To find out which device is connected to which COM port please refer to the '**Device Manager**' in Windows:

1. In Windows, right click the start button and select '**Device Manager**'
2. Look at '**Ports (COM & LPT)**' in the Device Manager to see your connected hardware (if connecting them via USB)
3. In order to determine which device is on which port you may need to unplug the device and then go to '**Action**' and '**Scan for hardware changes**' to see which device is no longer present.
4. Once you know which device is on which COM port, select this port when adding the device to Micro-Manager

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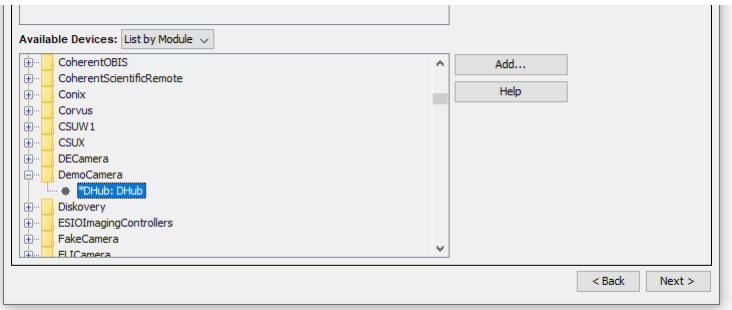
Adding a Manual Objective Turret

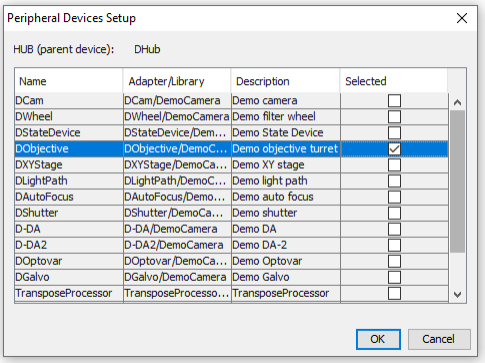
Micro-Manager supports automated objective turrets for many microscopes. However, for manual microscopes, a manually changed objective or magnification indicator can be added in, which can be used to record which objective was used, and provide automatically updating pixel scaling information.

Adding a Manual objective magnification indicator

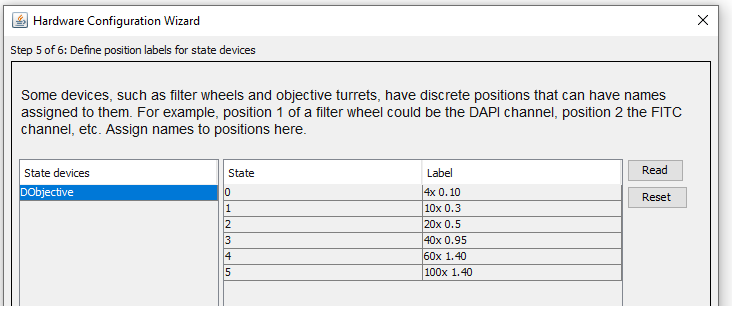
1. In the Available Devices list on the Hardware Configuration Wizard, locate the 'DemoCamera' folder and add 'DHub'.



2. A Peripheral Devices Setup window will then appear – within this, select 'DObjective'. This will add a dummy/demo objective to the device configuration. The same can be done for the other components in the peripheral list.



3. Advance to Step 5 of 6, where you can set the label for each of your objectives according to their magnification, NA etc.



4. Finish the wizard and add a group for 'Objective' as described in Groups and Presets on Page 17.
5. You can now set up Pixel Scaling Information following the instructions on Page 21.

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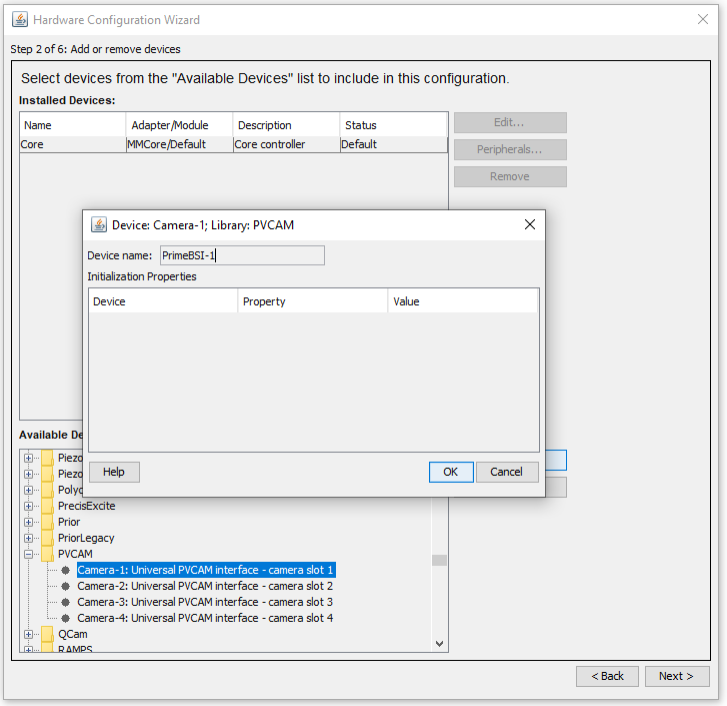
Running two or more PVCAM Cameras Simultaneously

Micro-Manager is capable of running up to 4 PVCAM cameras simultaneously. The only requirement is that the cameras have the same sensor size or region of interest applied, and the same bit depth of resulting images.

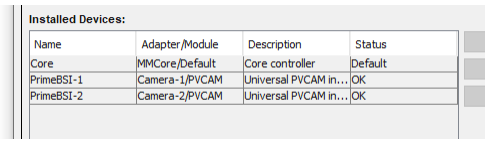
The below guide is for the specific case where the cameras will be run simultaneously; if you want to install and access multiple cameras without simultaneous use, follow only steps 1, 2 and 6.

How to set up multiple cameras in Micro-Manager

1. Creating a new configuration file or editing an existing one, and add in each of the Camera-1, Camera-2 etc. PVCAM camera slots that you are using and name them appropriately.



2. These should all appear in the Installed Devices list with a status of 'OK'



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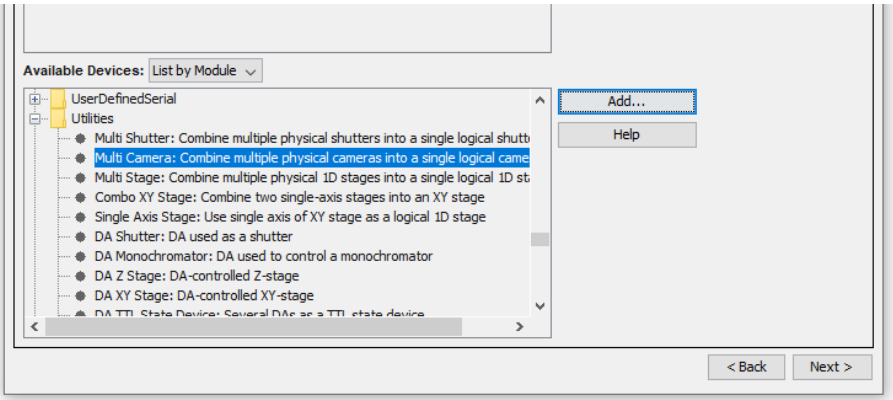
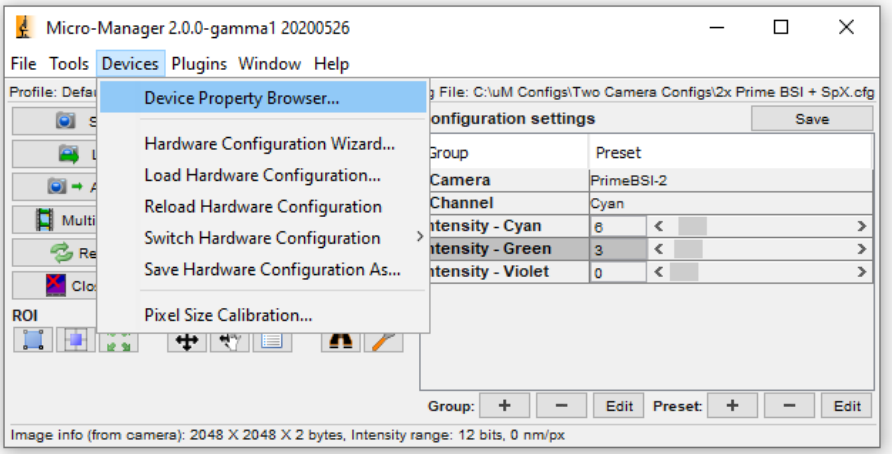
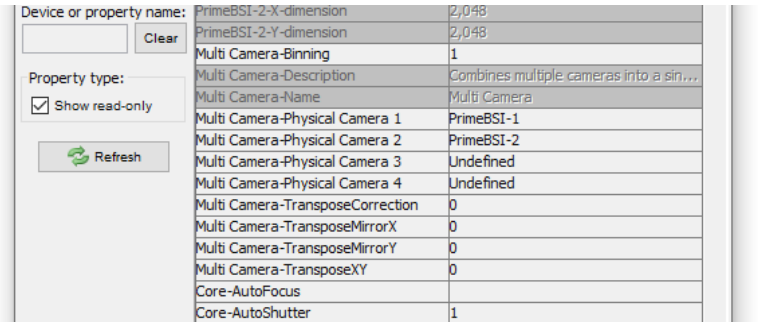
3. Now we add in a special '**Multi Camera**' Utility to treat multiple cameras as one multichannel camera device for simultaneous acquisition. Go to the '**Utilities**' folder and add '**Multi Camera**'.



4. Now finish off your Hardware Configuration File.
5. Once your file is completed and loaded, you may see a '**Circular buffer can't be started**' error, ignore this, as we need to inform the Multi Camera utility which devices to use. Go to '**Devices**', open '**Device Property Browser**' and find '**Multi Camera-Physical Camera 1**'. Choose the appropriate camera for each of these labelled 1 through 4.





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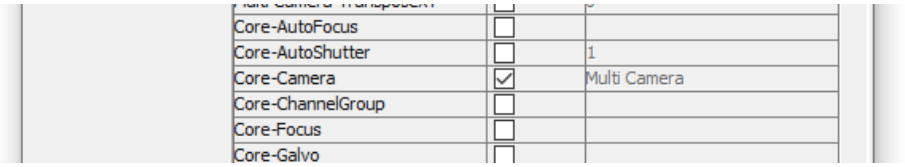
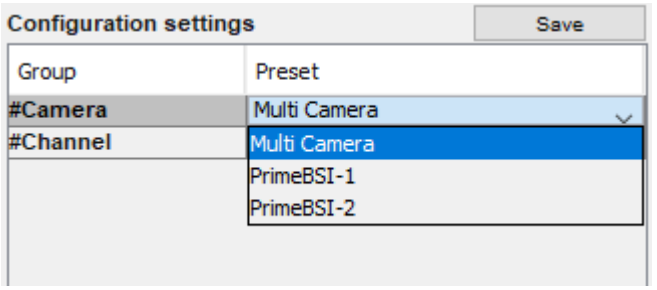
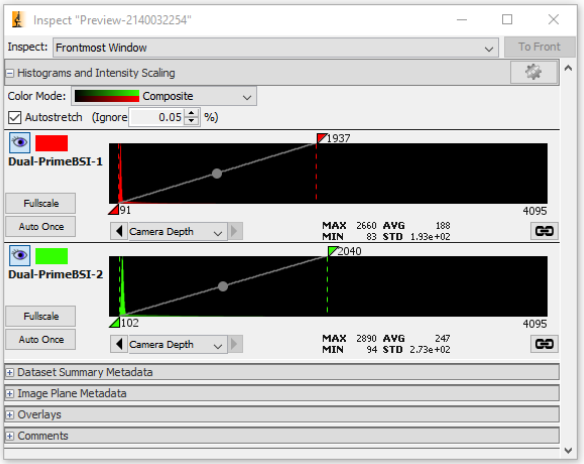
6. Now create a Group for Core-Camera as described in 'Groups and Presets' on Page 17.

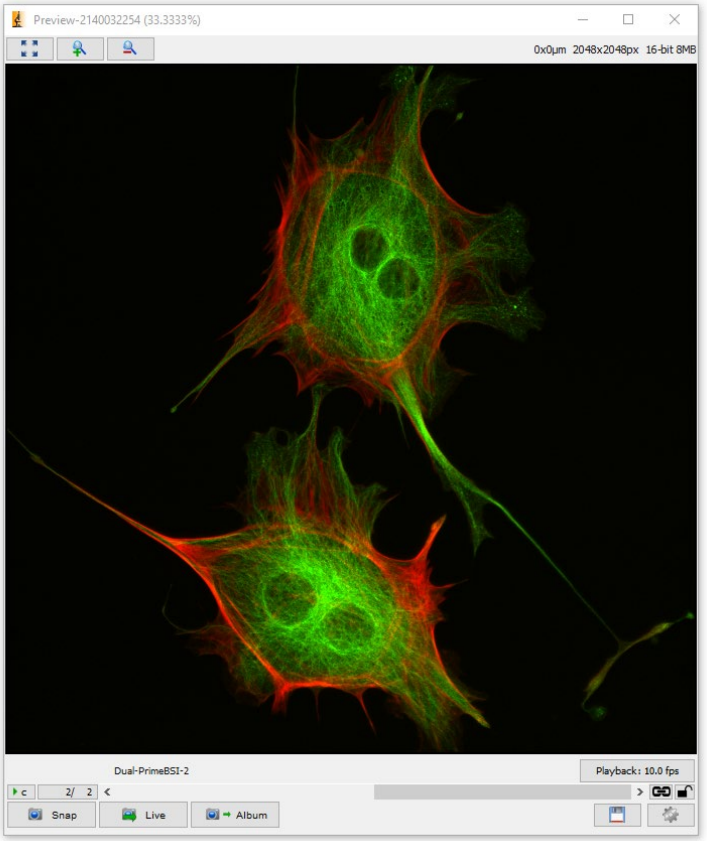


7. Select 'Multi Camera' in your Camera group to view simultaneous output.



8. Hit 'Live' to image in multiple colors simultaneously. You may need to set up your image display settings to view a composite image. In the Inspect window under 'Histograms and Intensity Scaling', choose 'Color Mode: Composite'.
9. Choose the appropriate color for each channel by clicking the rectangle next to the eye symbol for each histogram, shown below in green and red. The eye symbol will toggle that channel's visibility.





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Using a Single-Camera Splitter for Simultaneous Multichannel

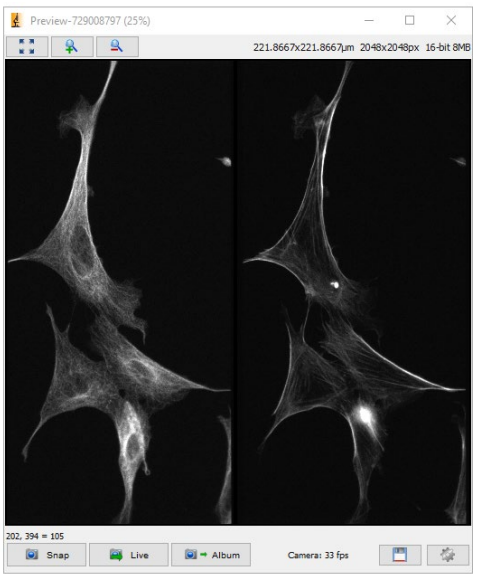
Micro-Manager also supports using single camera splitters, where separate wavelengths are directed onto separate portions of a single camera sensor. Any combination of horizontal and vertical splits is possible, splitting each dimension 2, 3 or 4 ways. Commonly a 2-way or 3-way vertical split, or a combined 2-way vertical and 2-way horizontal split is used.

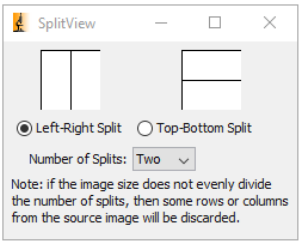
For advice on how to align splitting devices, please see manufacturers' instructions or our guide:

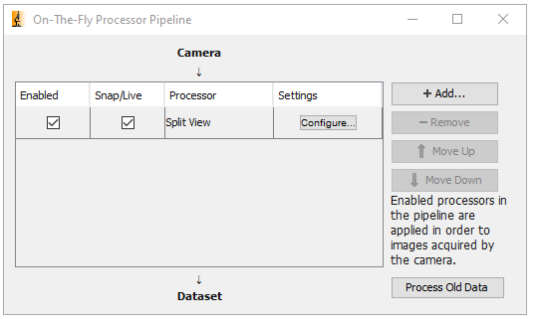
https://www.photometrics.com/products/imaging-splitters/how-to-align-a-splitter

Setting up single-camera splitters

1. Align your splitter according to manufacturer's instructions. In the case of a 2-way or 3-way single direction split, for rolling shutter cameras it is most advantageous to align the channels vertically as shown below (left).
2. In Micro-Manager, open '**Plugins → On-The-Fly Image Processing → Split View**' to add one direction of split, specifying how many times the view is split in this dimension, as shown below (center, top).
3. To add further splits in the opposing direction, in the '**On-The-Fly Processor Pipeline**' Window, click '**Add**' and select '**Split View**'.
4. Hit '**Live**' to image in multiple colors simultaneously. You may need to set up your image display settings to view a composite image. In the Inspect window under '**Histograms and Intensity Scaling**', choose '**Color Mode: Composite**'.
5. Choose the appropriate color for each channel by clicking the rectangle next to the eye symbol for each histogram, shown below in green and red. The eye symbol will toggle that channel's visibility.







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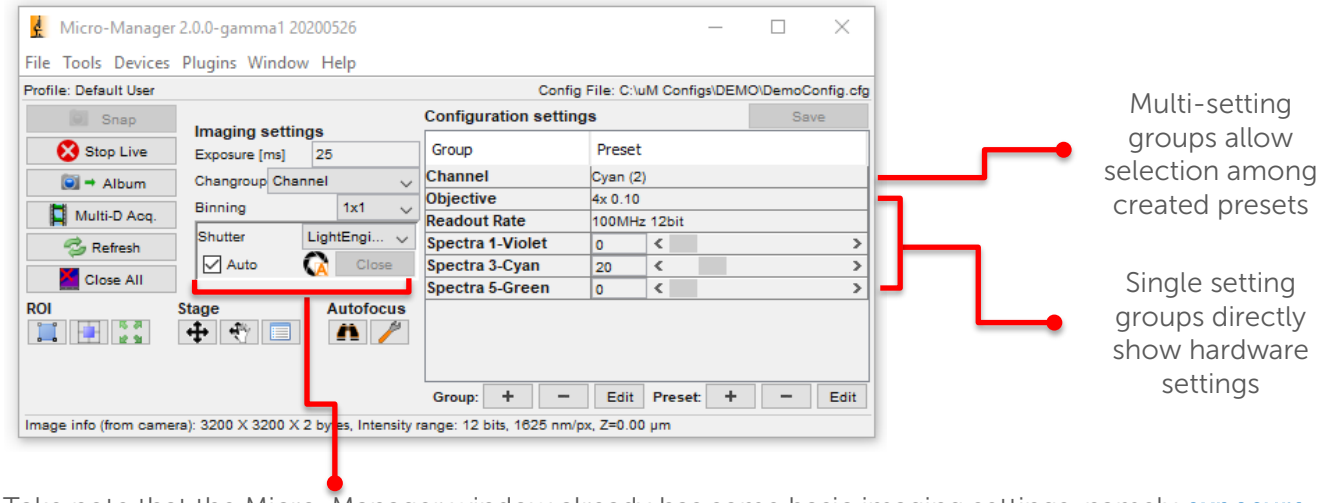
Hardware Setup

Groups and Presets: Shortcuts to key settings

In Micro-Manager, we can make shortcuts to any hardware controls that will be frequently used. These are called '**Groups**' and drastically increase your imaging efficiency, as everything you need to control about your imaging setup can be accessed all in one place. Each Group can control a single setting (such as camera gain) or multiple (which filter wheel is in the light path).



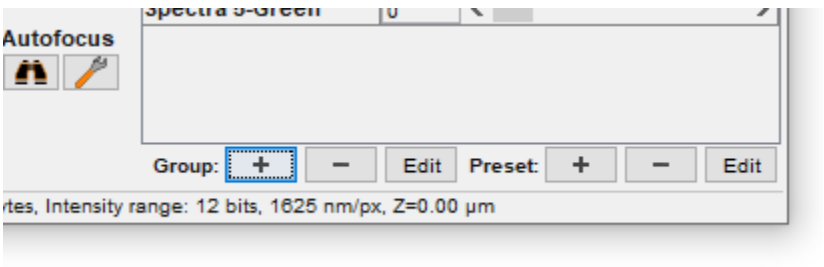
Take note that the Micro-Manager window already has some basic imaging settings, namely **exposure time** (in milliseconds), **binning**, **light source shutter**, and establishing a **region of interest (ROI)**.

Single Setting Groups

Single setting groups will provide direct access to a hardware control, such as a camera readout mode, light source intensity, or microscope objective turret position. Most groups you make will likely be single setting groups.

Creating a Single Setting Group

1. Click the **plus button** next to '**Group**' in order to add a new group. If you want to remove or edit a group, use the minus and edit buttons respectively (but not the buttons to the right in the Preset section)



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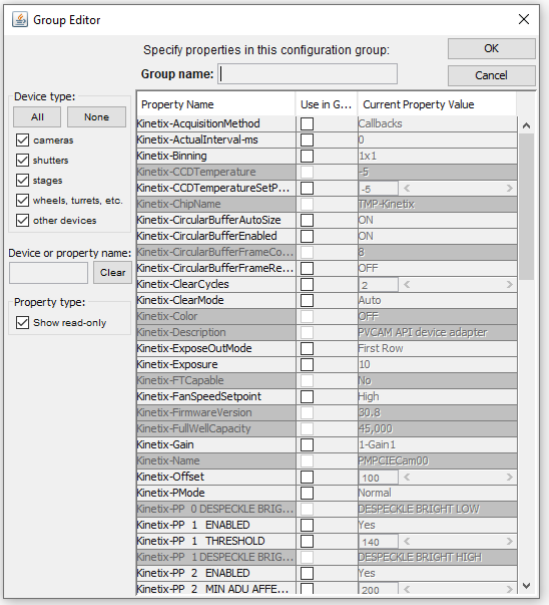
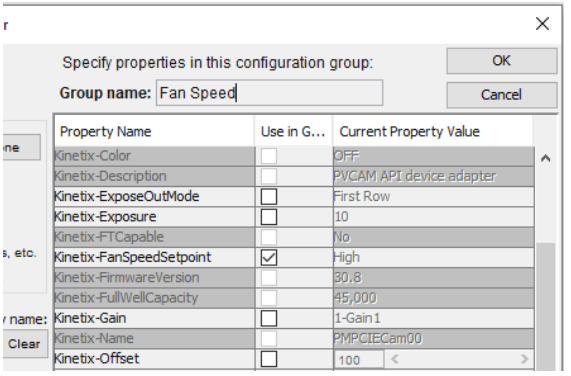
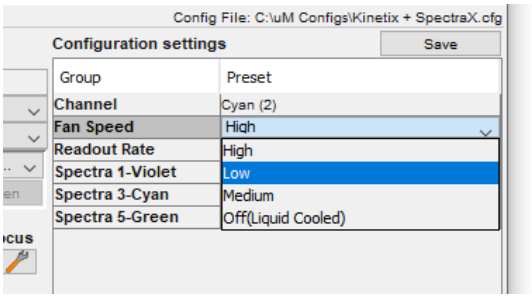
2. The Group Editor menu will appear, showing a list of every setting for all available hardware, listing each piece of hardware in alphabetical order (depending on how you named the hardware when using the Hardware Configuration Wizard).



3. Find the setting that you want and check the box next to it. Be sure to only select one setting at a time.
4. Give the group a name that makes sense to you ('Fan speed' for a group that controls camera fan speed, for example), and click OK.



5. You will now have a group that allows you to directly change that hardware setting.



6. Add a Group for every setting you want to be able to change when imaging.

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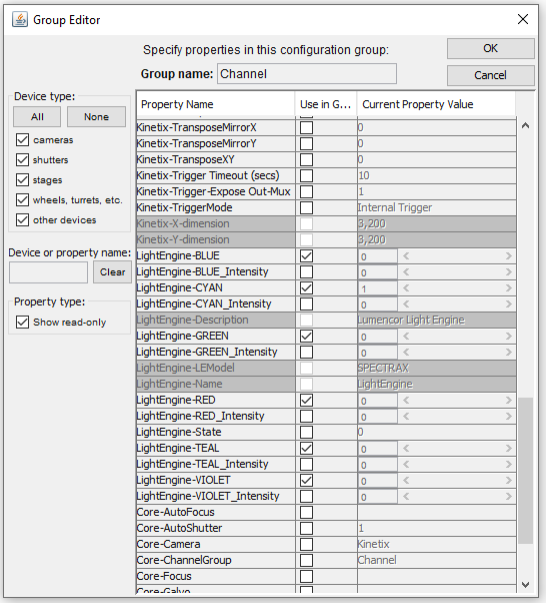
Multi-Setting Groups (Example: Channels Group)

Sometimes changing a hardware state in the system requires multiple individual components to be changed. For example, to change the illumination channels on your light source you'll need to select multiple settings such as the 'Violet' and 'Cyan' channels in order to switch between them, and not have both active at the same time. This is where multiple-setting groups come in.

In Micro-Manager, we can create groups that control multiple settings, and then create presets that specify the value of each setting. In the case of the Channel group, we would create one preset per wavelength channel – Brightfield can also be included if your microscope has computer-controllable brightfield lamp and shutters.

Creating a multi-setting group

1. Click the plus button next to 'Group' in order to add a new group.
2. Identify the settings you want to change in the Group Editor menu, and then select every box next to all the hardware settings that need to change. In this case, each different wavelength channel you want to use from your light source.



3. Give this group a name that makes sense and click OK. This multi-setting group will require several presets in order for you to control each setting within the group. When you click OK, the 'Preset Editor' window will appear.
    a. Each preset is a setting within the group, for example if your group contains multiple illumination channels, each preset would have one channel on and all the other channels off. Switching between these presets would then cycle between the illumination channels.

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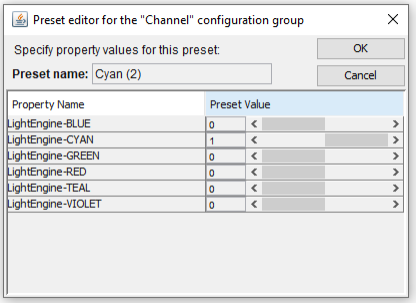
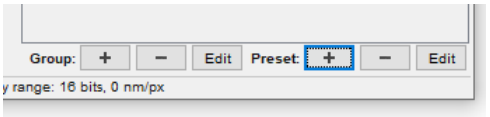
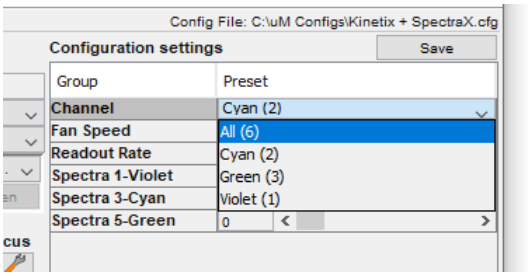
4. Make the first preset for this group in the preset editor window. – note, changing settings here will change the physical hardware state, so be aware parts may move or activate.



5. Once your first preset is created, you'll be back at the Micro-Manager window. Select the multi-setting group you created, and then click the plus button in the Preset controls (next to the group controls) to add a new Preset for all remaining options required.





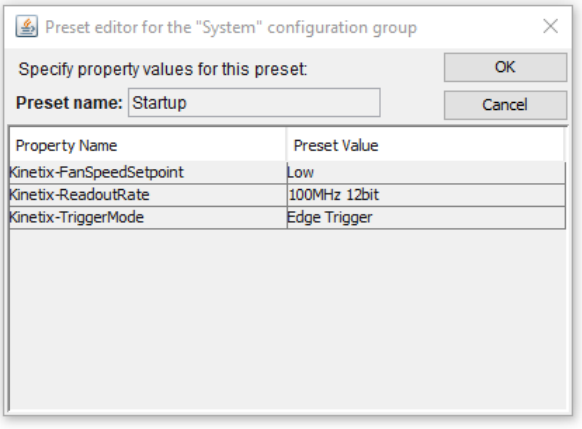
Startup Configuration Settings

When Micro-Manager is closed, the values in the Groups list and in the Device Property Browser are not stored, they will go to their default values the next time Micro-Manager is loaded.

To avoid this and specify which values your settings should be on startup, or even on shutdown, Micro-Manager has a special Group called 'System'.

How to set default startup parameters for hardware

1. Create a new Group as described above, called 'System'. Select every hardware option that you would like to set a Startup or Shutdown value for.
2. In the preset editor, for Startup values name the preset 'Startup', and set the values.
3. To add a Shutdown preset, create a new preset as described above, naming it 'Shutdown', and set the values appropriately.



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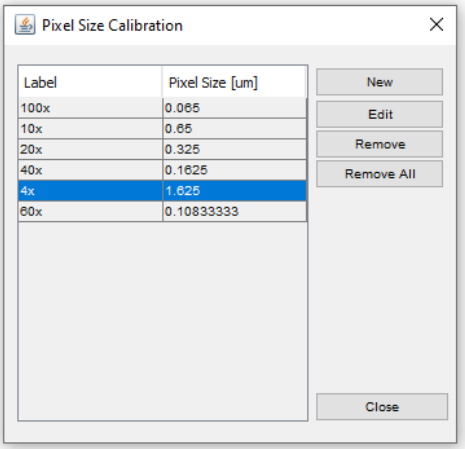
Pixel Scaling Calibration

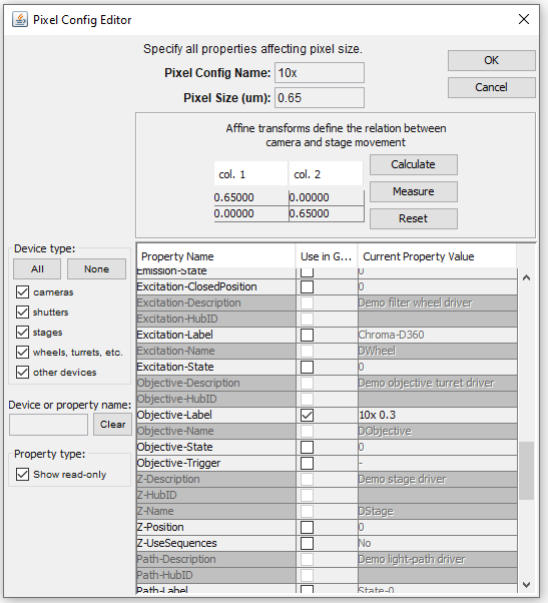
To allow XY scaling information in images and the inclusion of scale bars, you must set up your Pixel Size Calibration. This requires either a manual objective turret to be included in your Hardware Configuration File as described on Page 12, or an automated objective turret controlled in Micro-Manager.

How to set up Pixel Scaling Calibration

1. Under 'Devices', select 'Pixel Size Calibration'. In the window that appears, select 'New'.



2. The Pixel Size Preset Editor shown below will then appear, where all hardware that affect magnification (objectives, optivars, multiple cameras) can be selected though ticking the 'Use in Group' checkbox.



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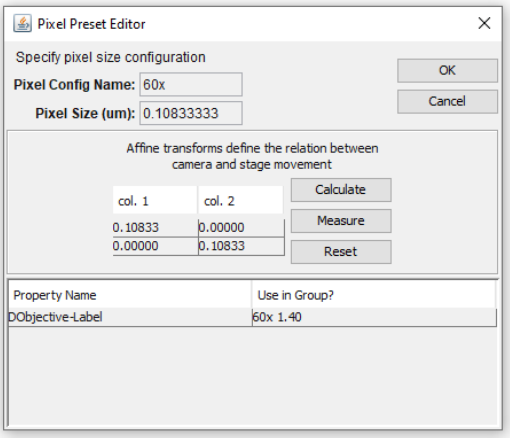
3. Set an initial value for a pixel configuration (for example, setting your Objective turret to '10x'), and give this pixel configuration a corresponding name "10x".
4. Calculate and enter the resulting image pixel size in micrometers based on the following formula, including any C-mount / optical tube magnification in the system:

$$Image\ Pixel\ Size = \frac{Camera\ Pixel\ Size}{Objective\ Magnification \times Additional\ Magnification}$$

You can find your camera pixel size on the Camera Specification Sheet, or in the following table:

Camera	Pixel Size
Kinetix	6.5 µm
Prime 95B	11.0 µm
Prime BSI / BSI Express	6.5 µm
Iris	4.25 µm
Retiga E7	4.5 µm
Retiga E20	
Moment	4.5 µm
Evolve 10	10 µm
Evolve 13	13 µm
Evolve 16	16 µm
Retiga R6/LUMO/ELECTRO	4.54 µm

5. Once you have inserted your pixel size, click 'Calculate'. Alternatively, if you have a correctly calibrated, motorized XY stage installed into Micro-Manager you can click 'Measure' for an automated measurement of pixel size.
6. Click 'OK', and then repeat the process for all remaining objectives, or permutations of different magnification-adjusting hardware.



7. Once you have finished adding these, click 'Close' and you will be prompted to save the changes you have made into the Hardware Configuration File.

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Controlling Teledyne Photometrics Cameras

Important Groups & Optimal Camera Settings

Kinetix Family

	Camera Setting	Setting Name in list	Values
Essential	Mode – Change between the four imaging modes of the Kinetix. This changes both the readout and gain, which are optimized for each mode.	Port	'Speed' – 8-bit, 498 fps across full sensor 'Sensitivity'– 12-bit, 88 fps across full sensor, low read noise mode at speed 'Dynamic Range' – 16-bit, 83 fps across full sensor, large full well capacity for imaging intense signals 'Sub-Electron' – 16-bit, 5 fps across full sensor, ultra-low read noise of 0.7 e^-
Recommended	Metadata – Additional hardware metadata including Timestamps	MetadataEnabled	'Yes' – Add additional hardware metadata including a 10ns precision timestamp for the beginning and end of each frame – see Page 28.
	Fan Speed – Control over air cooling fan speed	FanSpeedSetpoint	'Low' – For air cooling for typical (< 2 seconds) exposure times and room temperatures, camera airflow unobstructed. 'High' – Air cooling with long exposures or in confined or warm environments 'Off' – Use only when water cooling is in use
Triggering	Trigger In – Hardware trigger to control camera acquisition	TriggerMode	'Internal' – Camera runs on own internal timing as defined by software. Other modes allow external control of camera. Please see camera manual for explanation of other trigger modes.
	Expose Out Trigger Behavior	ExposeOutMode	Used when synchronizing external hardware through hardware triggering. Please see camera manual for explanation of the options.
	Number of Expose Out Cables Used	Trigger - ExposeOutMux	If using Multi Expose Outs to trigger multiple light sources etc., how many cables to use
Advanced	SMART Streaming Enable – Use for faster sequential multi-channel acquisitions with channel-dependent exposure times	SMARTStreamingEnabled	'No' – Don't use SMART Streaming 'Yes' – Use SMART Streaming. Please see camera manual for instructions on SMART Streaming and when to use it.
	SMART Streaming Exposure Times – list of exposure times to use for above feature	SMARTStreamingValues	List of exposure times to use, separated by semicolons ;

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Prime 95B

	Camera Setting	Setting Name in list	Values
Essential	Readout Mode – Main camera mode setting	ReadoutRate	'200MHz 12 Bit' - Low light / high speed imaging '100MHz 16 Bit' - High dynamic range imaging
	Gain – Conversion factor between photoelectrons signal and grey levels displayed	Gain – Create this group **after** setting 'Readout Rate' to '200MHz 12 Bit'	For practically all low-light or fluorescence imaging: '3-Sensitivity' For brightfield imaging: '1-Full Well' or '2-Balanced'
	Clearing – Antiquated setting regarding charge clearance	ClearMode	'Never' – For all imaging. Other settings may introduce framerate and timing delays. This may require setting on startup.
Recommended	Metadata – Additional hardware metadata including Timestamps	MetadataEnabled	'Yes' – Add additional hardware metadata including a 10ns precision timestamp for the beginning and end of each frame – see Page 28.
	Fan Speed – Control over air cooling fan speed	FanSpeedSetpoint	'Low' – For air cooling for typical (< 2 seconds) exposure times and room temperatures, camera airflow unobstructed. 'High' – Air cooling with long exposures or in confined or warm environments 'Off' – Use only when water cooling is in use
Triggering	Trigger In – Hardware trigger to control camera acquisition	TriggerMode	'Internal' – Camera runs on own internal timing as defined by software. Other modes allow external control of camera. Please see camera manual for explanation of other trigger modes.
	Expose Out Trigger Behavior	ExposeOutMode	Used when synchronizing external hardware through hardware triggering. Please see camera manual for explanation of the options.
	Number of Expose Out Cables Used	Trigger - ExposeOutMux	If using Multi Expose Outs to trigger multiple light sources etc., how many cables to use
Advanced	SMART Streaming Enable – Use for faster sequential multi-channel acquisitions with channel-dependent exposure times	SMARTStreamingEnabled	'No' – Don't use SMART Streaming 'Yes' – Use SMART Streaming. Please see camera manual for instructions on SMART Streaming and when to use it.
	SMART Streaming Exposure Times – list of exposure times to use for above feature	SMARTStreamingValues	List of exposure times to use, separated by semicolons ;

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Prime BSI & Prime BSI-Express

	Camera Setting	Setting Name in list	Values
Essential	**Readout Mode** – Main camera mode setting	ReadoutRate	'**200MHz 12 Bit**' - Low light / high speed imaging '**100MHz 16 Bit**' - High dynamic range imaging
	Gain 100MHz – Conversion factor between photoelectrons signal and grey levels displayed	Gain – Create this group **after** setting 'Readout Rate' to '100MHz 16 Bit' (both modes required)	'**CMS**' – Recommended for all low light imaging. Low read noise mode. '**HDR**' – For imaging bright and dark things simultaneously. Maximum dynamic range.
	Gain 200MHz – Conversion factor between photoelectrons signal and grey levels displayed	Gain – Create this group **after** setting 'Readout Rate' to '200MHz 11 Bit' (both modes required)	For practically all low-light or fluorescence imaging: '**3-Sensitivity**' For brightfield imaging: '**1-Full Well**' or '**2-Balanced**'
Recommended	**Metadata** – Additional hardware metadata including Timestamps	MetadataEnabled	'**Yes**' – Add additional hardware metadata including a 10ns precision timestamp for the beginning and end of each frame – see Page 28.
	Fan Speed – Control over air cooling fan speed	FanSpeedSetpoint	'**Low**' – For air cooling for typical (< 2 seconds) exposure times and room temperatures, camera airflow unobstructed. '**High**' – Air cooling with long exposures or in confined or warm environments '**Off**' – Use only when water cooling is in use
Triggering	**Trigger In** – Hardware trigger to control camera acquisition	TriggerMode	'**Internal**' – Camera runs on own internal timing as defined by software. Other modes allow external control of camera. Please see camera manual for explanation of other trigger modes.
	Expose Out Trigger Behavior	ExposeOutMode	Used when synchronizing external hardware through hardware triggering. Please see camera manual for explanation of the options.
	Prime BSI Only Number of Expose Out Cables Used	Trigger - ExposeOutMux	If using Multi Expose Outs to trigger multiple light sources etc., how many cables to use
Advanced	**Prime BSI Only SMART Streaming Enable** – Use for faster sequential multi-channel acquisitions with channel-dependent exposure times	SMARTStreamingEnabled	'**No**' – Don't use SMART Streaming '**Yes**' – Use SMART Streaming. Please see camera manual for instructions on SMART Streaming and when to use it.
	Prime BSI Only SMART Streaming Exposure Times – list of exposure times to use for above feature	SMARTStreamingValues	List of exposure times to use, separated by semicolons ;

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Iris Family

	Camera Setting	Setting Name in list	Recommended Value
Rec.	Metadata – Additional hardware metadata including Timestamps	MetadataEnabled	'Yes' – Add additional hardware metadata including a 10ns precision timestamp for the beginning and end of each frame – see Page 28.
Triggering	Trigger In – Hardware trigger to control camera acquisition	TriggerMode	'Internal' – Camera runs on own internal timing as defined by software. Other modes allow external control of camera. Please see camera manual for explanation of other trigger modes.
	Expose Out Trigger Behavior	ExposeOutMode	Used when synchronizing external hardware through hardware triggering. Please see camera manual for explanation of the options.
Advanced	SMART Streaming Enable – Use for faster sequential multi-channel acquisitions with channel-dependent exposure times	SMARTStreamingEnabled	'No' – Don't use SMART Streaming 'Yes' – Use SMART Streaming. Please see camera manual for instructions on SMART Streaming and when to use it.
	SMART Streaming Exposure Times – list of exposure times to use for above feature	SMARTStreamingValues	List of exposure times to use, separated by semicolons ;

Retiga E Family

	Camera Setting	Setting Name in list	Recommended Value
Ess	Readout Mode – Main camera mode setting	ReadoutRate	'Speed' – High-speed imaging 'Long Exposure' – Ultra-low dark current mode, allowing for exposures of up to 60 minutes 'Long Exposure EDR' – Ultra-low dark current mode with Extended Dynamic Range (EDR) to increase full-well capacity
Rec.	Metadata – Additional hardware metadata including Timestamps	MetadataEnabled	'Yes' – Add additional hardware metadata including a 10ns precision timestamp for the beginning and end of each frame – see Page 28.
Triggering	Trigger In – Hardware trigger to control camera acquisition	TriggerMode	'Internal' – Camera runs on own internal timing as defined by software. Other modes allow external control of camera. Please see camera manual for explanation of other trigger modes.
	Expose Out Trigger Behavior	ExposeOutMode	Used when synchronizing external hardware through hardware triggering. Please see camera manual for explanation of the options.

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Moment CMOS

	Camera Setting	Setting Name in list	Recommended Value
Rec.	Metadata – Additional hardware metadata including Timestamps	MetadataEnabled	'Yes' – Add additional hardware metadata including a 10ns precision timestamp for the beginning and end of each frame – see Page 28.
Trig.	Trigger In – Hardware trigger to control camera acquisition	TriggerMode	'Internal' – Camera runs on own internal timing as defined by software. Other modes allow external control of camera. Please see camera manual for explanation of other trigger modes.

CCD Family: Retiga R6, Retiga ELECTRO, Retiga LUMO, MicroPublisher 6

	Camera Setting	Setting Name in list	Recommended Value
Essential	Clearing – Antiquated setting regarding charge clearance	ClearMode	'Clear Pre-Sequence' – Achieve full speed of acquisition, for streaming / snap applications 'Clear Pre-Exposure' – Remove excess charge between frames, for time-lapse imaging
Trigger	Trigger In – Hardware trigger to control camera acquisition	TriggerMode	'Internal' – Camera runs on own internal timing as defined by software. Other modes allow external control of camera. Please see camera manual for explanation of other trigger modes.

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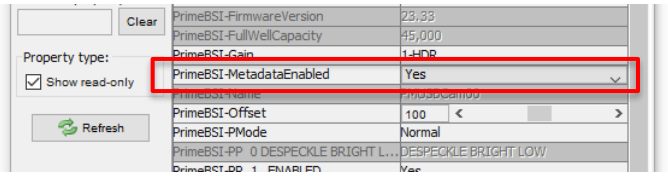
Advanced Features

Hardware Timestamps

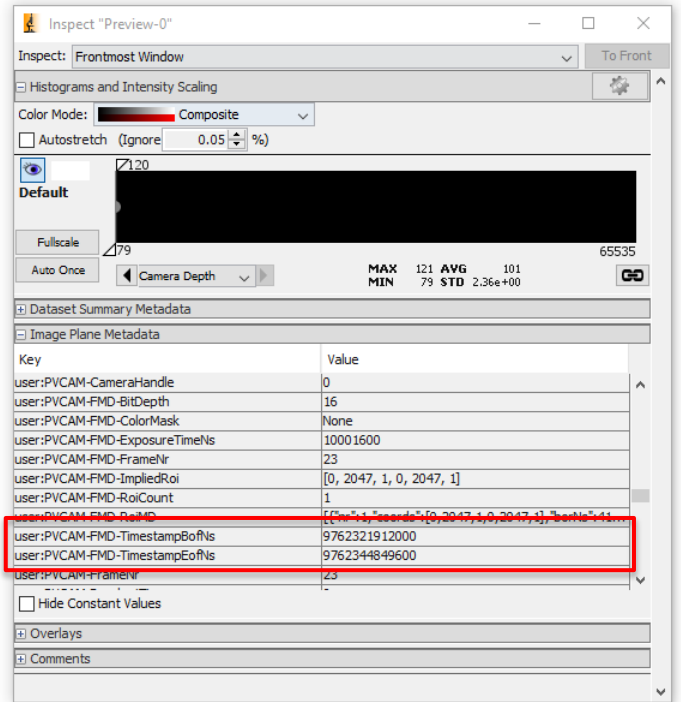
Many cameras from Teledyne Photometrics are able to provide highly accurate and precise hardware timestamps, generated on the camera's onboard intelligence. This will be the most precise timestamp available for when an image was taken. These can be enabled within Micro-Manager, and will then be included in all streaming (i.e., anything but pressing 'Snap') acquisitions.

How to enable and view Hardware Timestamps

1. In Device Property Browser or by creating a group, find the property '[Camera Name]-MetadataEnabled', and select 'Yes'.



2. Now each acquired image will show additional metadata under the 'Image Plane Metadata' tab of the Inspect window, starting with "PVCAM-FMD" ("Frame Meta Data").
3. This metadata includes many additional items, the hardware timestamps are under the name "PVCAM-FMD-TimestampBofNs" and "...EofNs", meaning "Beginning of frame, Nanoseconds" and "End of frame" respectively. These two times will differ by the frame roll time for rolling shutter cameras, and are in units of 1 ns, with 100 ns precision and 1 line time accuracy (10-20 µs, depending on camera speed).



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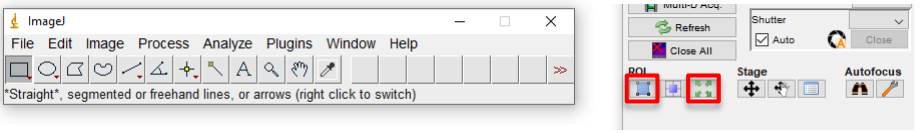
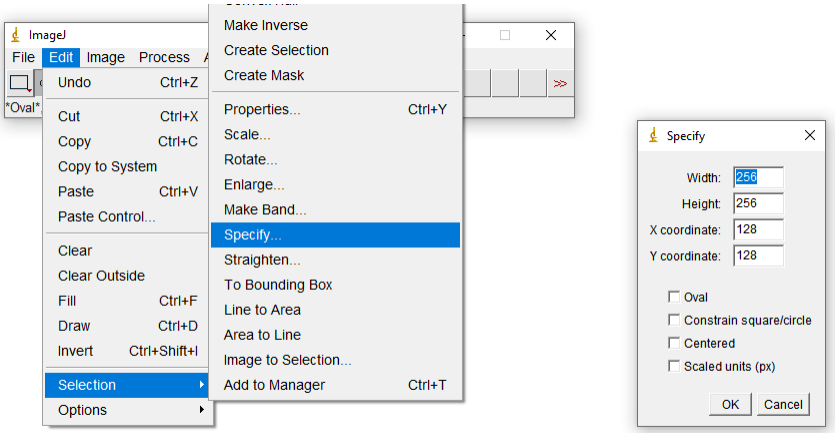
Regions of Interest

To create a region of interest in Micro-Manager, use the ImageJ rectangle tool to draw on a live or snapped image, and click the blue 'ROI' button outlined below (left red box). To return to the full field of view, click the four green arrows button (right red box).



Specifying ROI sizes & positions

To specify an exact size and position of region of interest, use Edit → Selection → Specify... within ImageJ to specify Width, Height and X and Y co-ordinate of the top left corner. The 'Centered' option would instead make the reference point for the X and Y co-ordinate the center of the ROI.



You can also save selections to ImageJ's ROI Manager to save them to a file and load them by dragging and dropping the file onto ImageJ, or to select between multiple ROIs.

Once you have drawn up your chosen ROI, click the blue ROI button outlined above to apply it to the camera.

Multi-ROI

Some Teledyne Photometrics cameras support Multi-ROI, allowing up to 16 regions to be imaged simultaneously. The overall camera readout and image size will then be determined by the bounding box containing all ROIs – the camera will not skip rows not contained within an ROI. The rest of the image will be set to a greyscale value of '0', which when used with lossless compression can significantly reduce file sizes, and lead to easier segmentation.

Note: Multi-ROI support requires a compatible camera and Micro-Manager version 2.0 – Micro-Manager version 1.4 is not compatible with this feature.

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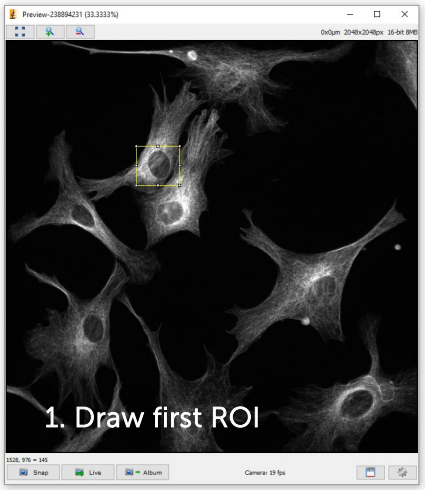
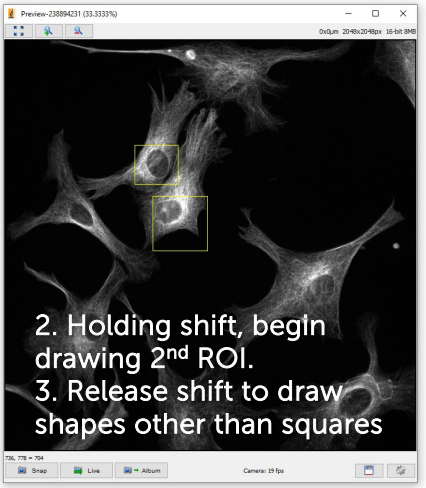
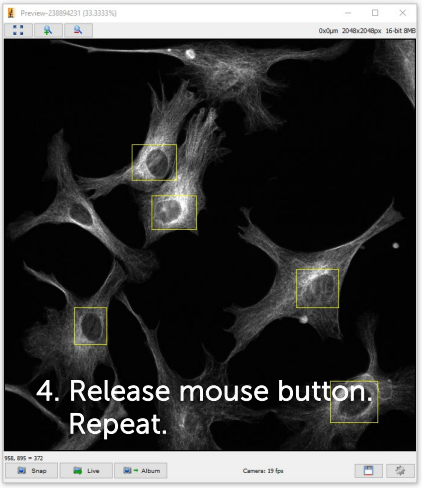
How to set up Multiple Regions of Interest

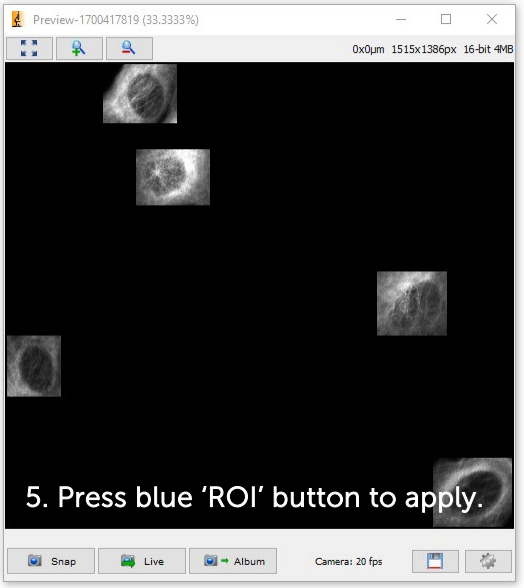
1. Draw your first region of interest as normal
2. Hold shift, and begin drawing the second ROI from the top left corner. With shift held, the ROI will be constrained to a square.
3. Release shift **without** releasing the mouse button to draw rectangular ROIs.
4. Release the mouse button, and repeat for all further required ROIs. Note: it is permitted for ROIs to overlap.







5. Should you wish to store the ROI locations for future acquisitions, at this stage, you are able to press '**Ctrl+T**' to add this multi-part ROI to the ROI Manager, where it can then be recalled if lost, or saved for future use.
6. Press the blue 'ROI' button to apply the regions.



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Multi-Dimensional Acquisitions: Movies, Multi-Channel, Z-stacks, XY Positions

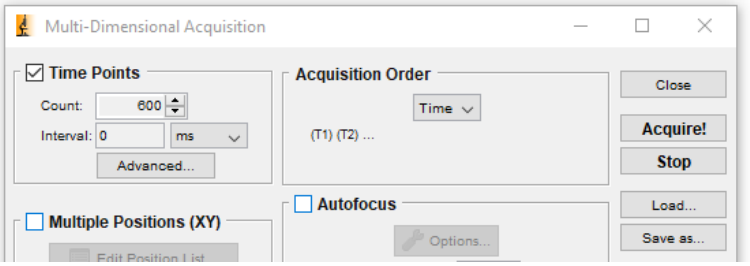
Acquiring more than one frame in Micromanager is handled via the Multi-Dimensional Acquisition window ('Multi-D Acq.'). This is where you can design experiments with whatever hardware you have connected – multi XY positions, Z positions, multi-channel, and of course time points for both streaming and timelapse acquisition. There is also the option for autofocus at designated times.

The Acquisition Order dropdown provides control over what order changing hardware parameters happens. For example, whether each wavelength channel is imaged sequentially on each z-slice (desirable for best co-localization), or whether the entire z-stack is acquired before changing between channels (desirable if changing wavelength channels involves time-consuming filter wheel or dichroic changes).

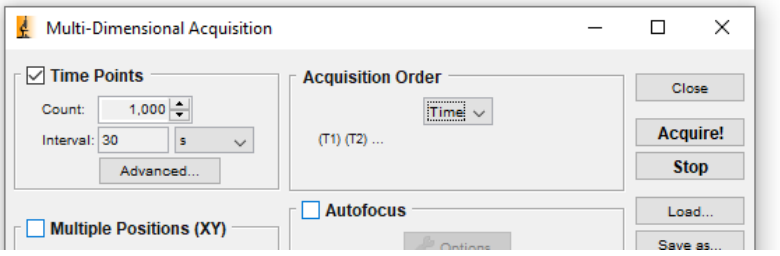
Setting up multiple XY Positions and Z stacks is intuitive and straight forward, but not covered in this guide. Information can be found on the Micro-Manager website here:
https://micro-manager.org/wiki/Micro-Manager_User%27s_Guide#Recording_Images

Movies & Timelapse

To acquire a movie/streaming acquisition where the camera runs at full speed (or according to external triggering control), simply set the number of frames you wish to acquire under 'Time Points → Count', and leave 'Interval' at 0.



To acquire a timelapse where there is a delay between frames/stacks (including where appropriate multiple channels, xy & z positions), the 'Interval' corresponds to the target time between acquisitions, not to any additional added time between frames. If this interval is set shorter than the acquisition time per time point, it is ignored.



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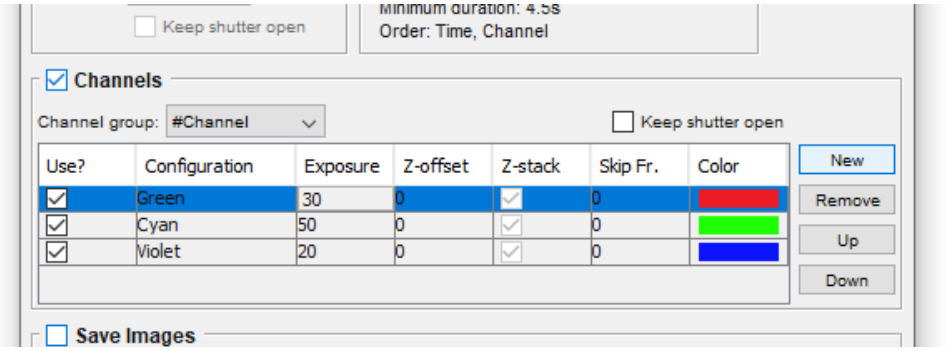
Multi-Channel

To set up a multi-channel acquisition, ensure you have set up your Channel group as outlined on Page 19.

How to set up a Multi-Channel Acquisition

1. Specify your channel group that controls channel hardware in the 'Channel Group' box.
2. Click 'New' to add a channel to the list to be acquired.
3. Under 'Configuration' specify which preset value of the Channel group this channel should have. You can set the exposure time for this channel, whether to include a z positioning offset correction, whether to include this channel in Z-stacks, whether to skip a certain number of time points before acquiring this channel, and finally the color the channel should appear.



4. Repeat this for as many channels as you intend to acquire.

Saving Multi-Dimensional Acquisitions

By default, with 'Save Images' unchecked, images are acquired into RAM as usual and can be saved after acquisition.

To save to hard disk/SSD as images are acquired, check the 'Save Images' checkbox and specify a folder and filename. As with saving after acquisition a choice between Separate Image Files and Image Stack File is provided – Image Stack Files save faster, and hence are highly recommended for streaming acquisitions.

You will need to make sure that your computer has high enough hard disk / SSD write speed to keep up with the data rate of the camera for high-speed streaming to disk.

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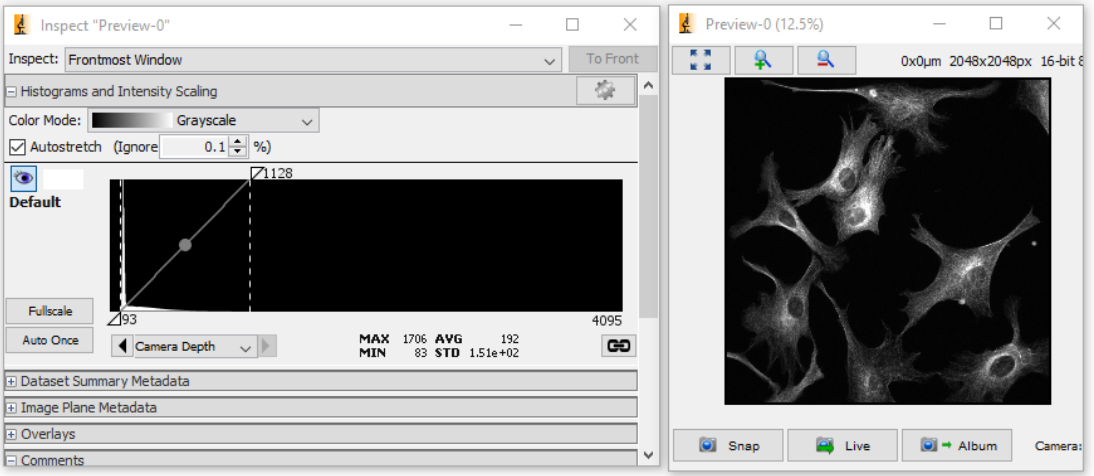
Image Handling

Histogram Control: Changing your image display scaling settings

After you go 'Live' with a camera or acquire some images, the 'Inspect' window will appear in Micro-Manager. This window allows you to inspect your images, both as they stream live and after you've acquired them. The main part of the inspection window is the histogram, which gives a quick overview of the intensity information in the image. The x-axis are the possible signal intensity values, from 0 to the maximum value (255 for 8-bit, 4095 for 12-bit, and 65535 for 16-bit)., and the y-axis shows how many pixels are at each intensity level.

It should be noted that for fluorescence or low-light imaging of sensitive samples, the signal intensity from the sample will be far lower than the maximum value the camera can display. In the example histogram above the camera can display intensities of up to 4095, but the highest signal from the sample is at 1128. The large peak at the left of the histogram (red square) represents all the black background pixels where there is no sample present, the area in the green square represents the pixels of the sample, displaying a range of intensities.

By changing the histogram and scaling controls you can determine how your acquired data is displayed on your screen. Please note that this doesn't change the signal intensity from your sample, just how this data appears on your screen.



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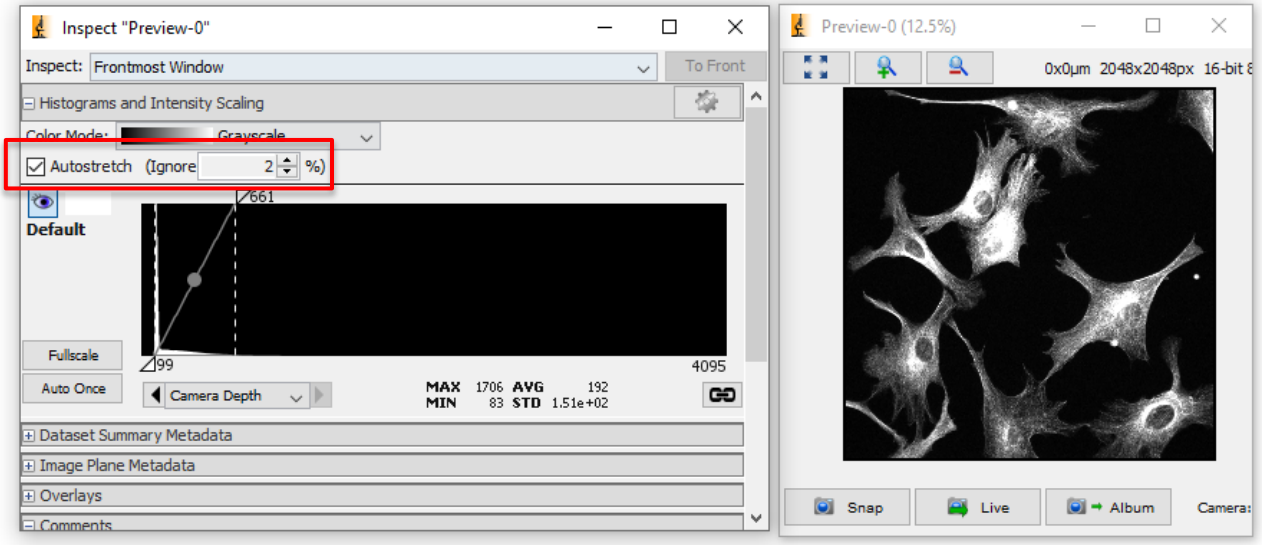
How to set up scaling in Micro-Manager

By default, automatic image scaling is used to set the maximum and minimum displayed intensity values based on image data. In Micro-Manager this is called '**Autostretch**', and is enabled by default, as seen by the tick box above the histogram (red square on below image). When autostretch is enabled, the scaling will automatically match to the max and min values, such as 93 and 1128 as seen on the previous histogram.

If autostretch is disabled, the maximum and minimum will need to be manually set for each acquisition. If the light level or exposure time are changed, the signal intensity will also change and the histogram may go beyond the manual scaling points, so if deactivating autostretch please check your histogram and image each time you change anything that may affect signal level.

As values of the very brightest and very darkest pixels can change rapidly, it is typical to ignore some percentage of the brightest and darkest pixels in determining image scaling. This is shown in Micro-Manager as the '**Ignore %**' and typically defaults to 0.1%. This can be manually changed, but bear in mind a higher **Ignore %** as shown below (of 2% in this case), will result in a higher contrast image, but with more saturated pixels. The most important thing is to get the image that is most representative of your sample while also answering your scientific questions.



It is possible to manually set the max and min display values by dragging the triangles on the top and bottom of the histogram respectively. Alternatively, double click on the min or max displayed value to type in a new value. For PVCAM cameras it is important to ensure that the lowest value is **~100**, as this is the base level of offset in the camera settings.

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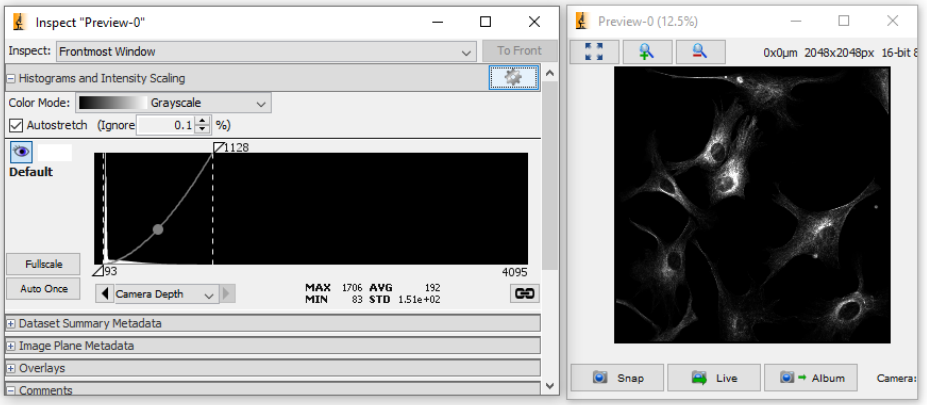
Gamma

The typical way to display image values is linearly, with min scaling value mapped to 0 display intensity, and a linear increase up to max scaling value mapping to 255. However, for some images such as thick samples in brightfield imaging, this can result in either poor contrast in dark areas or completely saturated bright areas.

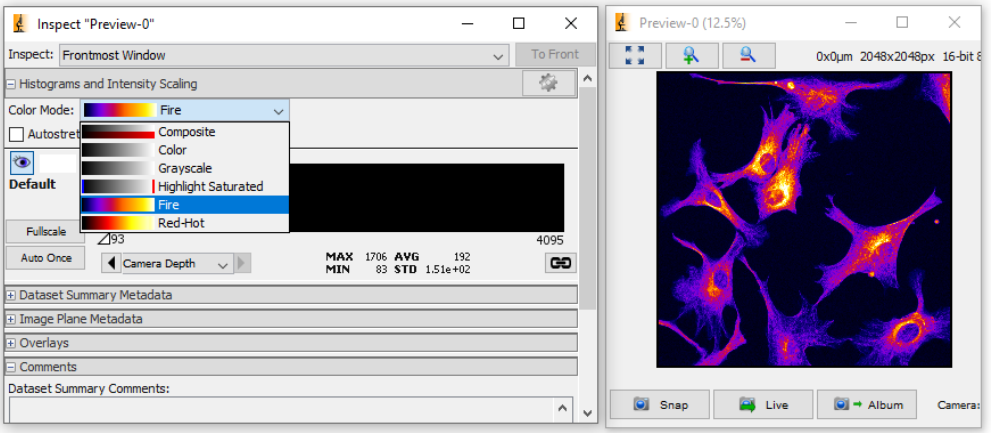
Gamma is a non-linear mapping of intensity values, meaning more display values can be used to give contrast to dark areas of the sample without saturating bright areas, and vice versa. To change gamma, click and drag the circle in the center of the line that connects the max and min display values on the histogram.



Lookup tables (LUTs)

Lookup-tables or LUTs apply false colors to image channels. The vast majority of scientific cameras are monochrome, meaning colors such as those seen in fluorescence are false colors applied after acquisition in order to differentiate between channels (such as DAPI being blue, etc.).

Micro-manager comes with several LUT options for an alternative to the typical monochrome/greyscale appearance. In the above figure the image has a 'Greyscale' LUT, in the below figure 'Fire' is used. Different LUTs can help highlight small intensity differences in your image.



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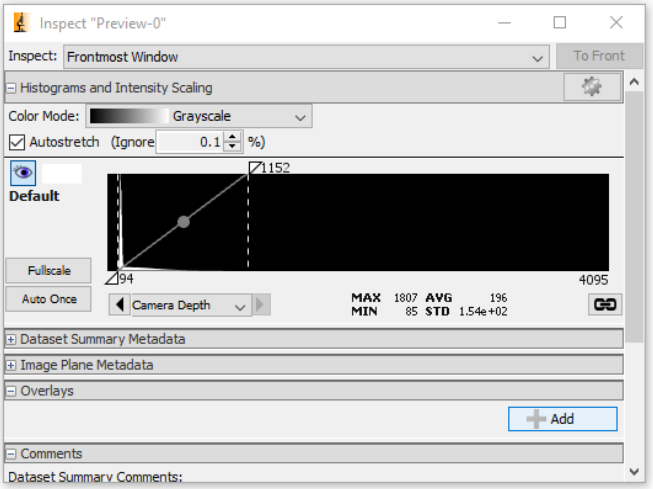
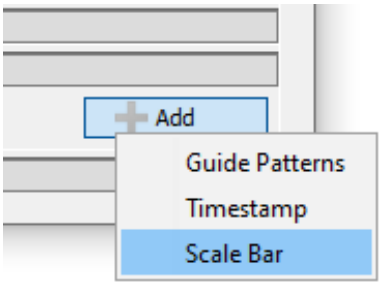
Scale Bars

To display a scale bar in Micro-Manager, you will first need to set your Pixel Scaling Calibration, according to the instructions on Page 21.

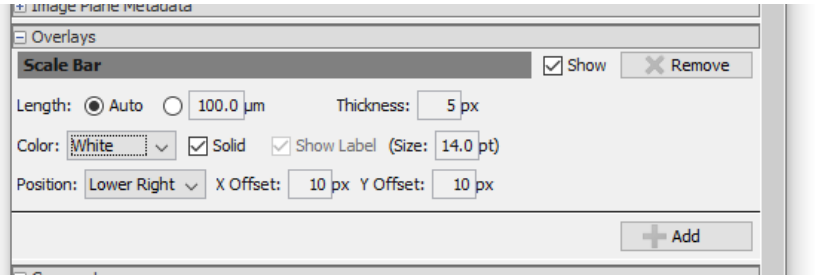
How to set up Scale Bars

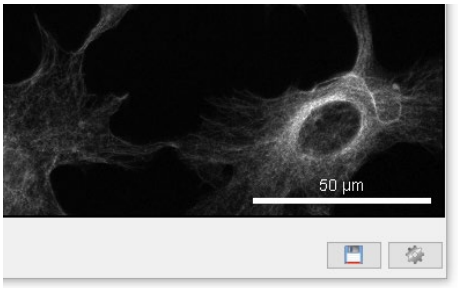
1. In the Inspector window under the Overlays tab, click the '+ Add' button, and choose 'Scale Bar'.





2. Multiple options for length, thickness, color, label and position are then available.





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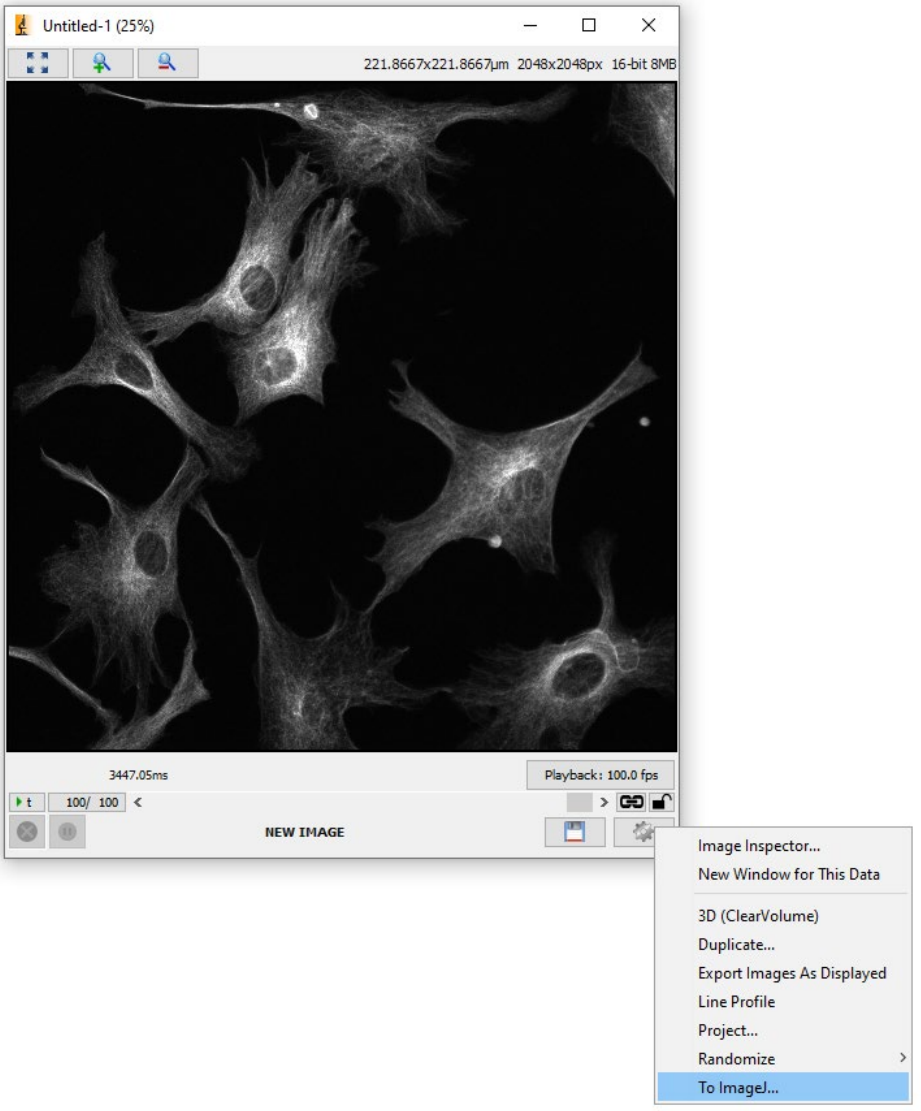
Using ImageJ Functions & Plugins

Some ImageJ analysis functions can be used on Micro-Manager images; however, some require the image to be brought explicitly across to a new instance of ImageJ. While Micro-Manager opens with an ImageJ window, this has limited functionality and lacks typical ImageJ Functions such as:

- Plot Z Axis Profiles
- Image summation & averaging
- Use of ImageJ Plugins

In order to access these and use the full suite of ImageJ analysis tools, you can open ImageJ from within Micro-Manager. This can be done by clicking the following option on the image window:



Alternatively, you can manually open a new instance of ImageJ or Fiji, then open the image.

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Live Analysis

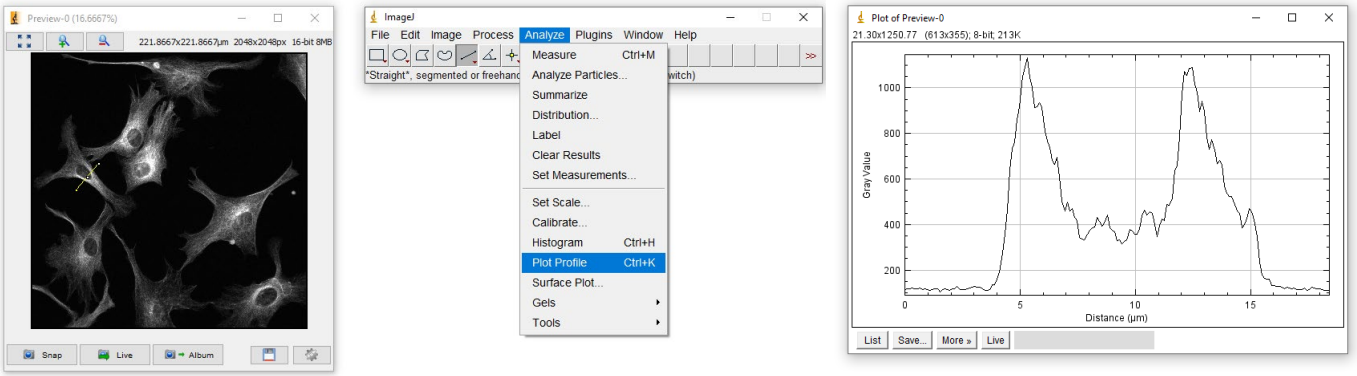
Micro-Manager and ImageJ has multiple plugins and functions for live, on-the-fly analysis as well as post-acquisition analysis.

Line Profiles

To draw a line profile in Micro-Manager, use the ImageJ Line tool and then in ImageJ choose 'Analyze → Plot Profile'. You can also use the keyboard shortcut 'Ctrl + K', although this shortcut will not work on a live updating image.

You can click the 'Live' button on the graph window that appears to have the graph live update for either an updating image or for moving the line.

You additionally have the option to thicken the line to take an average intensity measurement across the thickness of the line at each point along its length. To do this, double click the ImageJ line tool to specify line thickness.

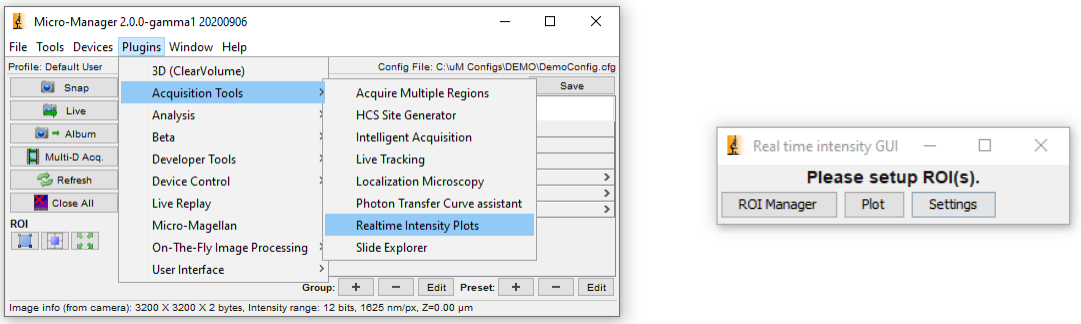


Intensity over time and ratiometric charts

Micro-Manager can display live intensity charts for multiple regions of interest, on either a live view or an acquiring sequence, and display a ratiometric intensity of two ROIs, for applications such as Calcium imaging.

Setting up Realtime Intensity Plots

1. In Micro-Manager, choose 'Plugins → Acquisition Tools → Realtime Intensity Plots'.



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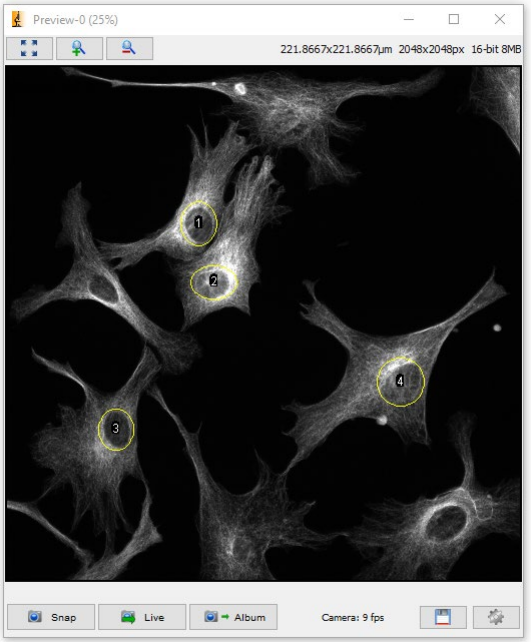
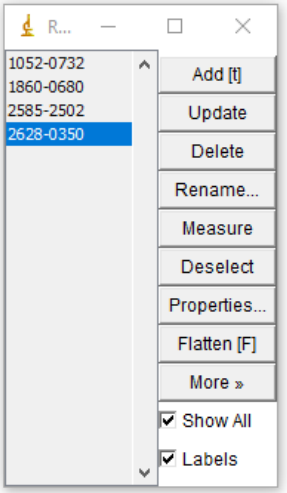
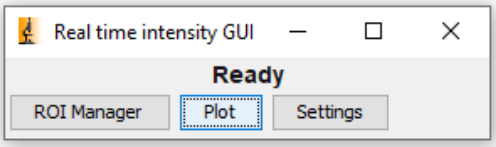
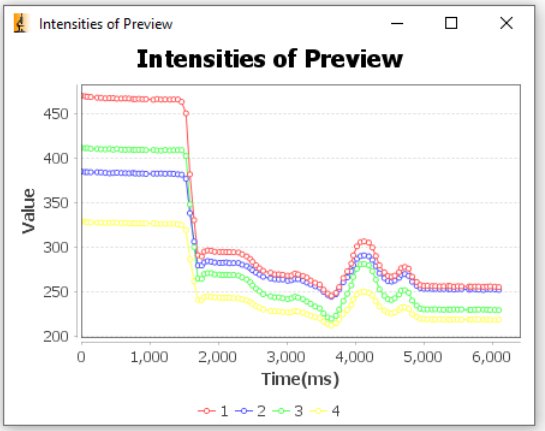
2. A window will appear asking you to set up ROI(s). You can do this using the ImageJ ROI Manager.
3. To add an ROI to the manager, select your tool of choice (ROIs can be any shape) and draw an ROI, then press 'Ctrl + T' to add this to the Manager. You can repeat this for multiple ROIs.





4. Once all of your ROIs are listed in the ROI Manager, the 'Real time intensity GUI' window should show 'Ready'. Once you hit 'Plot', a live updating intensity window will appear once your display is updating with live data.
5. To make a Ratiometric plot between ROIs numbered 1 and 2 in the ROI Manager, click 'Settings' in the 'Real time intensity GUI' and choose 'Ratiometric image'.





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Saving and Opening Acquired Data

Files saved by Micro-Manager use the ome.tiff format, and can be universally opened in other imaging software, complete with metadata. However, Micro-Manager itself can only open files that were acquired using Micro-Manager.

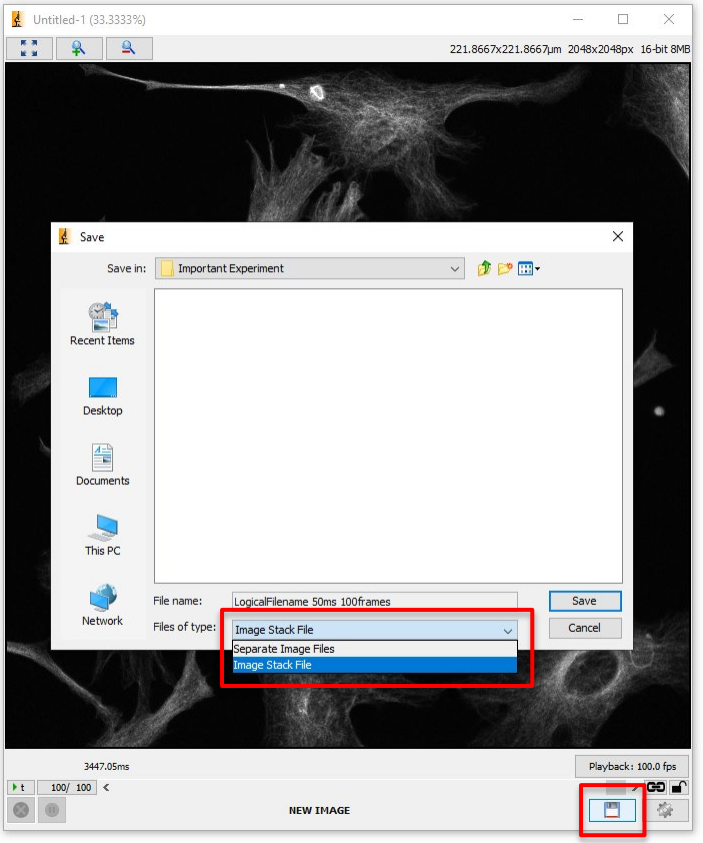
Whenever you 'Snap', 'Album' or use 'Multi-Dimensional Acquisition (MDA)' you can acquire an image, but this image needs to be saved to your storage drive if you want to open it later on. The method to save Micro-Manager files is given below. The alternative of saving via ImageJ's 'File → Save' menus may not retain all metadata, and these files cannot be re-opened in Micro-Manager. Only by saving through the Micro-Manager window can you get the ome.tiff files for universal analysis.

Saving acquired images to disk in MM

1. To save an acquisition, click the Save icon to the bottom right of the image (red square).
2. You will be prompted to choose between two saving methods: Image Stack Files or Separate Image Files. Image Stack Files are faster to work with and can be much more convenient than separate image files for large stacks.

   Note: Very large Image Stack Files are split into 4GB chunks according to the maximum size of a multi-page TIFF file. These will open as if they were one file.
3. Files will save in a folder according to your file name.

   See below for some options relating to how files are saved.



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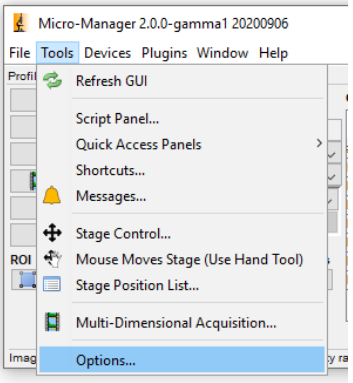
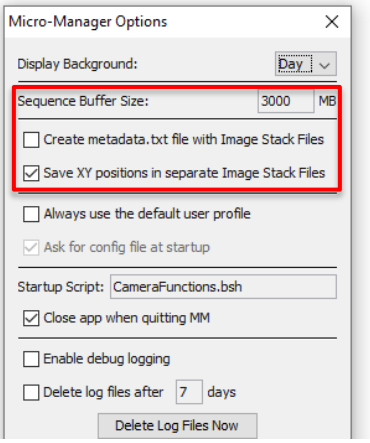
Saving Options

The 'Tools → Options' menu in Micro-Manager provides access to two saving-related settings:

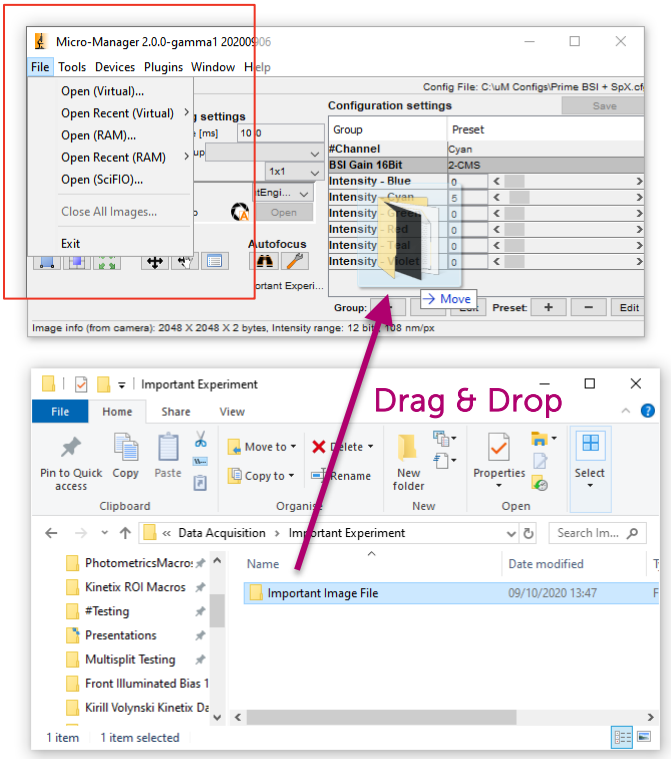




- 'Sequence Buffer Size' – determines how much RAM is allocated to store images before saving. If Micro-Manager is crashing during large acquisitions (1000s of frames) you may need to increase this. The value is MB so the 3000 MB in the example is only 3 GB of RAM.
- 'Create metadata.txt file with Image Stack Files' –stores the image metadata separately, which provides easier access to post-acquisition analysis pipelines
- 'Save XY positions in separate Image Stack Files' –separates XY stage positions in an acquisition into separate stack files. Note: Irrespective of this setting, when saving Separate Image Files, these files will be separated into folders according to XY stage position.

Loading images saved from Micro-Manager

1. Loading images can be done via Micro-Manager's File menu.
2. Either choose Virtual access where each frame/stack slice is loaded on demand from the hard drive, or RAM access where the entire image stack is pre-loaded into the RAM. RAM loading leads to faster image access, but large stacks will require large amounts of RAM.
3. Alternatively, you can open files by dragging either the ome.tiff file or the folder containing it onto the Micro-Manager window.
4. By default, this will load images into RAM, unless there is insufficient RAM in which case you will be prompted to open virtually instead.



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Streaming to RAM or Disk for High-Speed Imaging

Acquiring and saving individual files or small stacks is easily done through Micro-Manager, whether using RAM or Disk. However, with larger and faster cameras continually in development, it is important to know the limitations of Micro-Manager when streaming data to RAM or Disk at high-speeds, working with large fields of view, or doing high-throughput imaging in general.

This section mostly applies to our Kinetix sCMOS camera, which has a far higher speed and larger field of view than other scientific cameras, and consequently has a much higher data rate.

The Kinetix has a 3200x3200 pixel array, meaning every acquired frame has a file size of 10 or 20 MB when using 8-bit or 12-/16-bit modes respectively. When using the 8-bit speed mode, the Kinetix can acquire 500 frames a second, and with each frame being 10 MB this results in a data rate of ~5 GB/s. When imaging close to or at this data rate, Micro-Manager can struggle. This section outlines how to stream to RAM or Disk at these high speeds when using Micro-Manager.

Streaming to Disk

12-bit 'Sensitivity' and 16-bit 'Dynamic Range' modes are able to stream at full speed to disk, allowing for constant acquisition as long as the disk still has capacity to store each frame.

But due to limitations in the internal architecture of Micro-Manager, it currently isn't able to match the full speed of the Kinetix in 8-bit 'Speed' mode while streaming to disk. When testing computers (that met our specifications) for full-speed streaming to disk, our engineers have found a data rate limit of ~1.5-2 GB/s when writing to disk (the full data rate of the Kinetix in 'Speed' mode is 5 GB/s). In order to successfully stream to disk with a Kinetix at full speed when using Micro-Manager the data rate must therefore be limited, either by using regions of interest to reduce the frame size, or by reducing the frame rate by increasing exposure or using external hardware triggers.

We are currently working on a solution to enable full-speed streaming to disk in Micro-Manager. In the meantime, for streaming to disk with a Kinetix in 8-bit 'Speed' mode, please contact us for an alternative free solution developed by Teledyne Photometrics.

Streaming to RAM

On a computer that meets our recommended specifications, Micro-Manager is capable of streaming to RAM (memory) at the full speed of the Kinetix in all modes. However, not all of your computer's RAM is available to Micro-Manager for acquisitions, typically ~40-50% of the available RAM is accessible.

If using a computer with 64 GB of RAM, the Kinetix may be able to stream ~32 GB of data uninterrupted. If imaging at the full 500 fps speed of the Kinetix, at 10 MB per frame, this 32 GB of RAM represents ~7 seconds of constant acquisition. If using the slower 12-bit 'Sensitivity' or 16-bit 'Dynamic Range' modes at 20 MB per frame, the 32 GB of RAM represents ~18 seconds of constant acquisition.

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How to Stream to RAM at High-Speed with Micro-Manager

Step 1: Installing the correct version of Micro-Manager

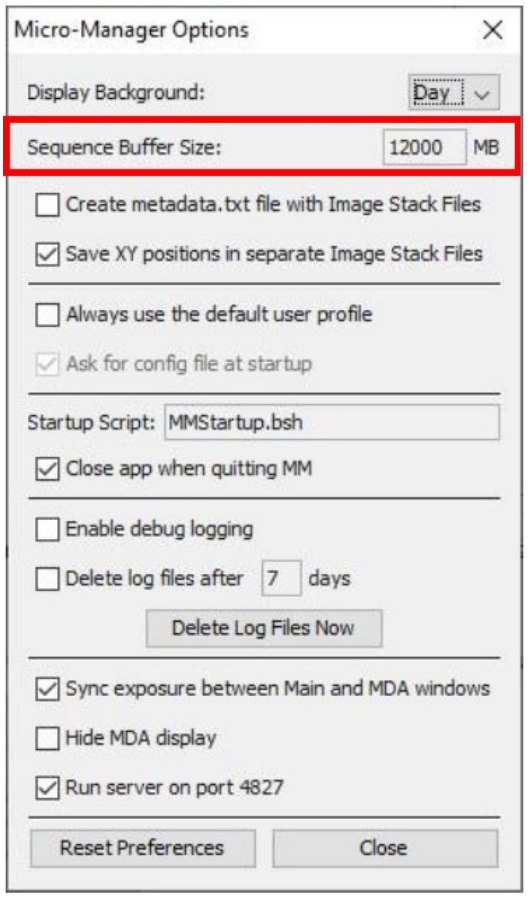
Streaming to RAM will function optimally when using the latest nightly build of Micro-Manager 2.0.0 or 2.0.1, please use a version dated from the 4th of March 2021 onwards. If you are constrained to use particular versions of micro-manager due to limited compatibility with other hardware, please contact us.

Step 2: Installing the Kinetix into Micro-Manager

For instructions on installing the Kinetix (or other high-speed PVCAM cameras) into Micro-Manager, please consult the relevant section of this guide on Page 9.

Step 3: Setting the Sequence Buffer size

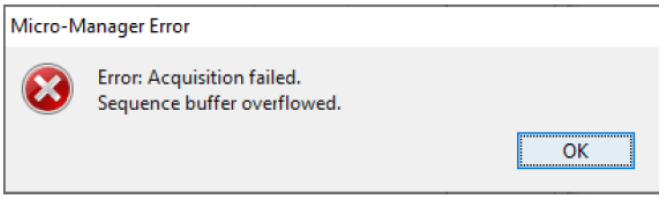
Once you have Micro-Manager installed and your required hardware configured, there are a few settings to enable high speed acquisition to RAM. The first is the Sequence Buffer Size, found within Micro-Manager under Tools -> Options, highlighted in red as seen in the image to the right.



The sequence buffer holds frames that come from the camera before they are copied to and processed by the Java environment (ImageJ). At its full field of view, the Kinetix is delivering frames to the buffer up to every 2 ms (at 500fps), but the copying to Java takes longer than this, so the sequence buffer will fill up while frames wait to be copied.

The capacity of the sequence buffer can be set via Micro-Manager in the image shown. The default size of the buffer is 250 MB, and with single snaps or slower multi-D acquisitions the buffer is not used at all, with images copied to Java directly.

To maximize the possible acquisition data size. the sequence buffer should be set to ~40-45% of available computer RAM. For a given acquisition size the sequence buffer should be set to a similar size. If your available PC RAM isn't sufficient, the buffer can overflow and acquisition may fail, as seen in the error below.



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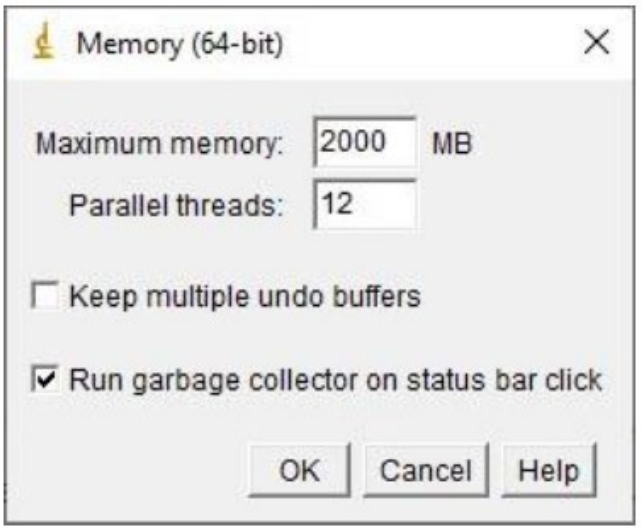
Step 4: Setting the ImageJ Memory size

After being stored in the sequence buffer within Micro-Manager, your images are copied to ImageJ for processing, displaying, or saving to disk. This means you also need to consider the memory size allocated to ImageJ itself.

The memory size can be set via ImageJ -> Edit -> Options -> Memory & Threads, which opens the menu seen to the right.



To maximize the possible data acquisition size. the ImageJ maximum memory should be set to ~45-50% of the computer's RAM, similar to the amount allocated to the sequence buffer.

With ~40% RAM allocated to the Micro-Manager sequence buffer, and ~40% RAM allocated to the ImageJ memory, your PC has 20% of its total RAM left for the operating system and any other open programs to run.

To see how much RAM your computer is using, and what it is being allocated to, right-click the windows button and select 'Task Manager'. There will be columns for CPU, Memory, Disk and Network. The 'memory' column is your computers RAM, and the percentage shows how much of it is in use. If more than 20% of your RAM is being used before you even open Micro-Manager, you may need to close some programs, especially RAM-hungry programs like Google Chrome.

If you don't know how much RAM your computer has, click the 'Performance' tab within the Task Manager and go to 'Memory', this will show you how much memory you have, and what is currently in use.

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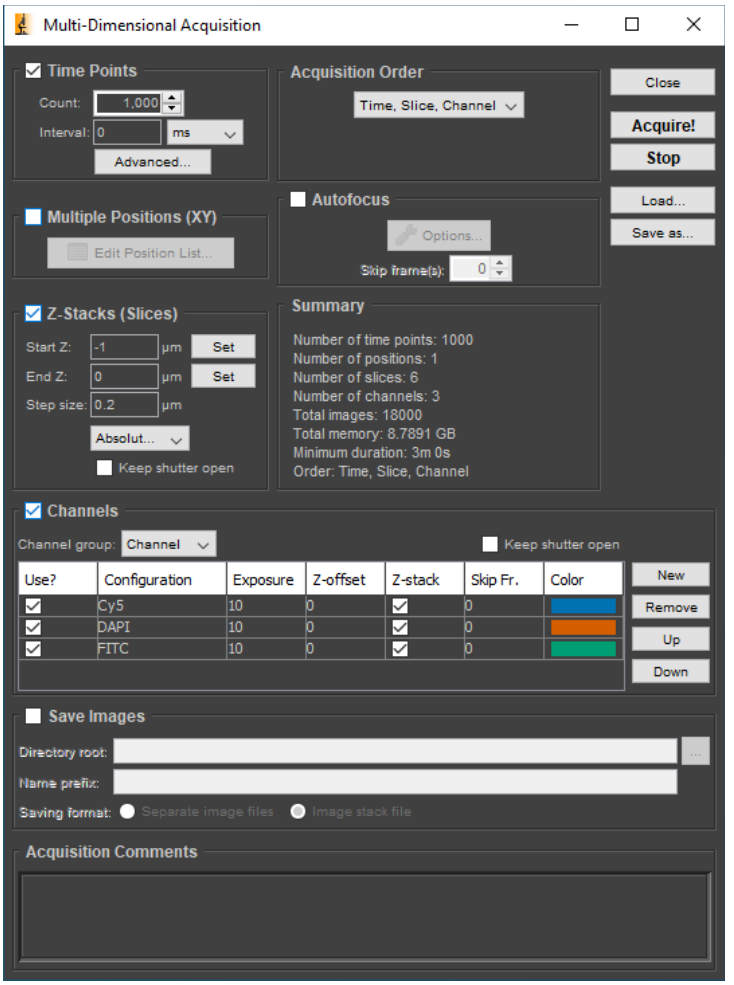
Step 5: Running an Acquisition

Set up your experiment in Micro-Manager as normal. When setting up your large acquisition through the Multi-Dimensional Acquisition window you will see the '**total memory**' for the acquisition updating as you fill in the fields.

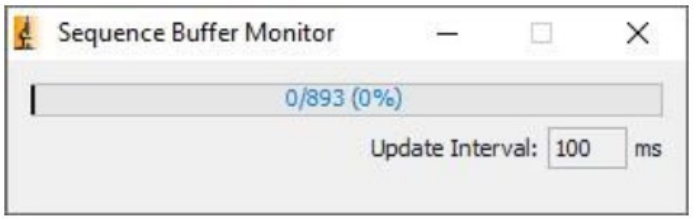
For example, in the right image, this acquisition will require **~8.8 GB** of memory. This should be less than the memory given to ImageJ in step 4, and roughly the same as the sequence buffer size given to Micro-Manager in step 3.



Monitoring Image Acquisition

If you are acquiring at high speed, the sequence buffer is expected to fill up very quickly. This can be monitored via **Micro-Manager -> Plugins -> Developer Tools -> Sequence Buffer Monitor**, as shown Below in the left-hand image. This should not reach 100% during acquisition.

Java memory utilization can also be monitored: **ImageJ -> Plugins -> Utilities -> Monitor Memory**, shown below in the right-hand image. Similarly, this should stay below 100%.



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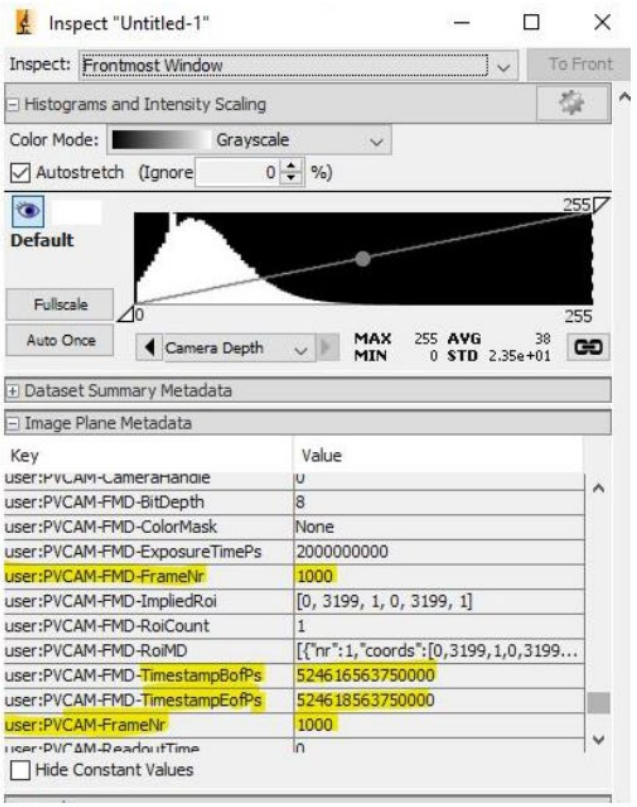
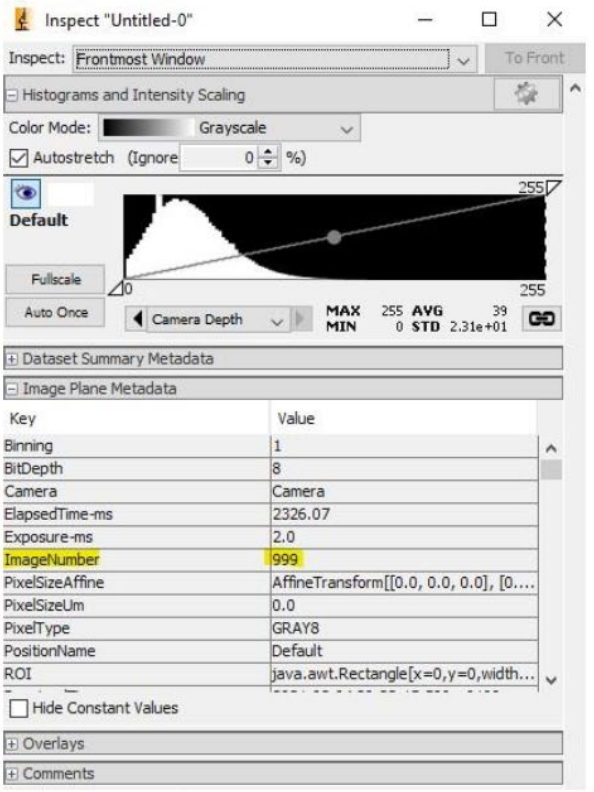
Checking that the acquisition completed successfully

Once an acquisition has completed, select the Inspect window in Micro-Manager and open the '**Image Plane Metadata**' section, as shown below.

The total frame values in '**PVCAM-FMD-FrameNr**' and '**PVCAM-FrameNr**' should be the same. This should also be one greater than the '**ImageNumber**', as the first frame has a ImageNumber of 0.

If the metadata frame numbers are larger than your requested number of frames, frames were acquired by the camera that the computer was not able to record, and those frames are lost. To avoid frame losses, you may need to reduce the total acquisition size or reduce the acquisition speed or data rate.





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