

# Use of the HSW5 Spinning-Disk Confocal Microscope

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## Getting Started:

1. Turn Lasers 'On'.

**#1** 405nm: Turn key to "On" to power up laser.

**#2** Argon (488nm/514nm) : Turn key on laser to "On" to power up laser. When you see blue emission in the laser tube, flip the switch on the circuit board out of 'standby' mode. Note: Start with laser on standby. If power insufficient for imaging application switch out of standby.

**#3** 561nm: Turn key to "On" to power up laser.

**#4** 640nm: Press toggle to "I" to power up laser.

2. Turn the key on the front of the spinning disk all the way to 'Laser On'.

3. Turn on the following in any order

- A. The power strip to the right of the scope.
- B. The AOTF controller. On shelf above scope (to the right of the 488/514 laser controller)
- C. The stage heat controller (silver box, green switch)
- D. The camera chiller
- E. The computer

4. The scope will now be 'setup' with the output of the sample going to the eyepiece.—remember that for actual collection, you will rotate the knob on the left to redirect light to the camera.

5. Drop a very small drop of oil onto the objective and mount your sample in the appropriate sample carrier. (The 40x objective is a default and is oil immersion—other lenses are available and see Omar/Pete to use these). Open micromanager and choose 'Contrast' from the 'Wavelength' Group and click 'Live' to open this shutter and find your sample (see 'The Micromanager GUI', 'Wavelength' below). Note about objectives: DO NOT add oil to any objective with a black top (generally 10x/20x). This means "air objective" for these particular objectives.

**6. Turn on the camera temperature controller/power supply (black box above camera, on shelf) and Stanford Photonics camera controller (black box to the left of the scope) only AFTER camera chiller is down to temperature (about 5-10mins after chiller has been turned on). If the monitor starts to alarm, do not turn on the camera. Turn off the temperature monitor/power supply, wait 5 more mins to let the chiller get down to temperature then turn on the monitor again. MAKE SURE THE STANFORD PHOTONICS CAMERA CONTROLLER IS SET TO STANDBY PRIOR TO TURING TOGGLE SWITCH TO "I" (ON).**

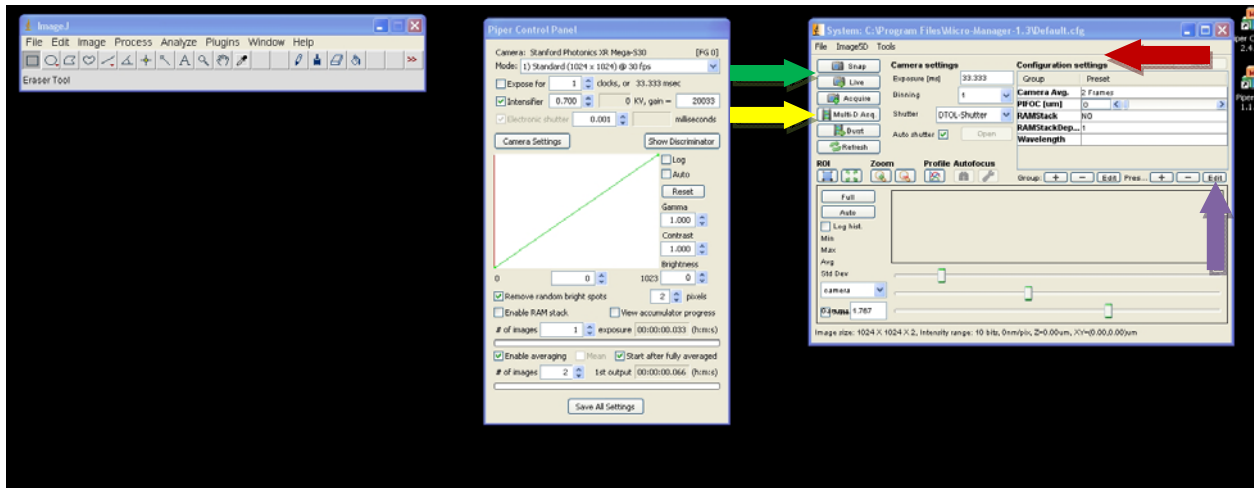
# Starting Micromanager and Loading a Configuration

1. Double-click 'Micromanager' on the Desktop to open the application. This will also open Image J toolbar which is open throughout but is not necessary for any part of an acquisition.
2. A splashscreen will appear. Choose 'Default.cfg' if you are a new user OR choose your configuration for established users (Note: 'Default.cfg' will be located in the current version micromanager folder on the C: drive)

## The Main Micromanager GUI

Opening the Micromanager GUI yields the "Configuration Settings" Screen as well as the Piper Control panel. Please do not adjust any of the settings in the Piper control panel unless you would like to use the "bin" controls.

The Main GUI should look as follows:



### **Configuration Settings (red arrow)**

Under 'Configuration Settings' you should find several 'groups'. They represent settings etc. of various hardware that you can access to define acquisition.

### *Wavelength*

This is the definition of a wavelength combination—excitation laser and its power, and emission filter choice.

### *PIFOC Z (um)*

This always represents the current position of the PIFOC z-motor in microns. It is a slider and will both change the 'current position' and can be used during 'Live' to set your z-movement in acquisitions (aka 'Set' in Multi-D Acq.)

### *Camera Averaging*

This defines the number of frames averaged (or none) for the ICCD. This is important, particularly at high-gain since the camera gets noisy.

### *Ram Stacking*

This option allows the user to use frame addition to increase overall signal. Noise is also amplified with this setting. Users can turn this option On/Off and select how many frames to add.

### *System*

The option houses the standard startup settings. Please do not adjust unless you are an advanced user.

### ***Live Button (green arrow)***

This opens the current shutter for the wavelength chosen in the Configuration Setting/Wavelength group (see above, when autoshutter is checked). It also opens a live window to view what that wavelength would look like in its current acquisition settings.

### ***Multi-D Acq (yellow arrow)***

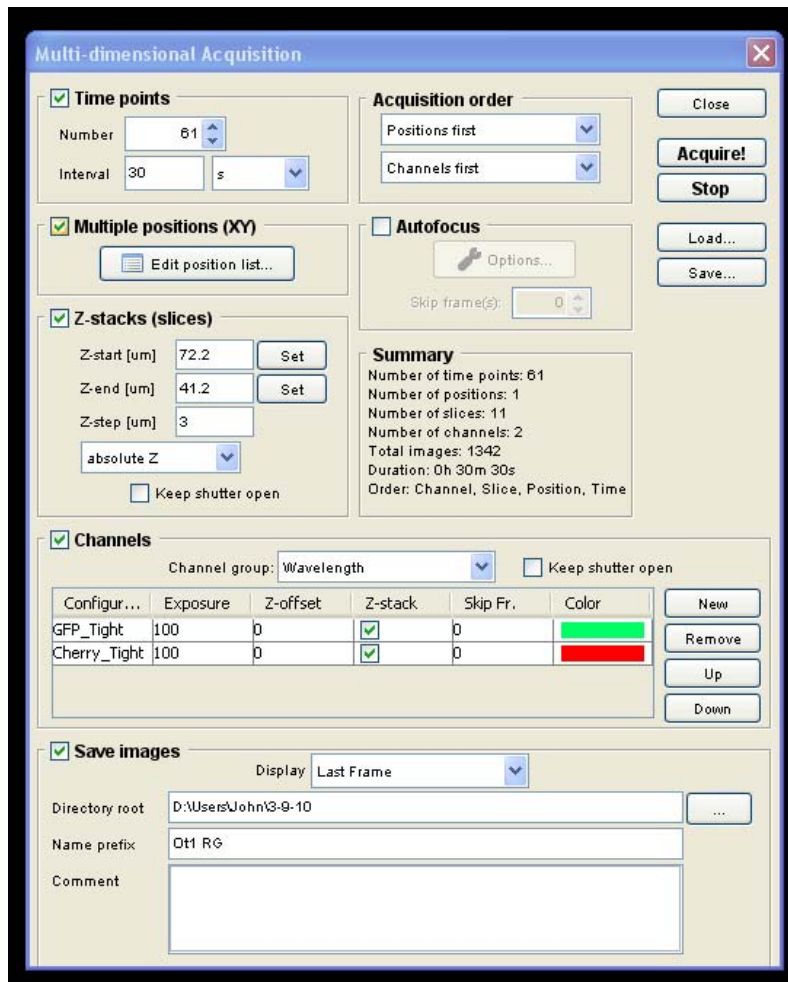
This is what you will click to setup multidimensional acquisitions.

### **Modifying settings for a given Wavelength**

1. Select the wavelength you plan to modify by clicking on the dropdown list that appears within the 'Wavelength' row in Configuration Settings'.
2. Click 'Edit' /'preset' (purple arrow) (NOTE: Basic users should NOT edit 'groups')
3. A window will appear showing you the details of that wavelength (see below).
4. For basic users, you will only edit the 'Laser Power' setting. Typically you will do this in 'Live' mode in order to get the intensity of illumination correct. Move the slider to the appropriate position.
5. To have variable taken, you MUST click outside of the box once to have the button 'set'. To finish, click 'OK'.

## Setting up a Timelapse in Micromanager Using ‘Multi-D Acq.’

Click on the Multi-D Acq button on the main micromanager page to open the Multi-D Acq interface. It will look like this:



There are subregions of the window that allow you to define a timelapse sequence. Each parameter defines a dimension of the acquisition. Use the settings defined below to set up the details of a timelapse. For Z Series and Channels, you probably want to click ‘LIVE’ in the Micromanager’ main window to see the effects of your changes. Note, for changing illumination power specifically, you also will open the appropriate wavelength using ‘Wavelength’ in the ‘Configuration Settings’ area.

### ***Timeseries***

Check this button to enable the use of the timeseries function. This will allow you to define the settings for the acquisition of images over time. Inputs in the “Frames” box allow the user to set the number of individual acquisitions taken (all channels are taken per frame) while inputs in the “Interval” box allow the user to determine the length of time between frame captures. Note: make sure that your interval time accounts for the time required to image your samples according to the exposure/averaging/RAM stacking parameters (i.e. you should see/hear a period of shutter inactivity between intervals). Failure to do so will result in non-linear timings of frame captures.

### ***Multiposition XY (Stage)***

Check this button to enable the function of the automated xy stage. Use the Prior stage controller (joystick) to manually move the stage from location to location in your sample and press the “Mark” button to save the coordinates into the acquisition manager.

### ***Z Series***

Checking this button enables the use of the PIFOC z-axis controller. There are two modes for the operation of this device: Relative and Absolute. These are selectable from the dropdown menu at the bottom of the Z Series box. “Relative” zeroes the PIFOC controller to the current position of the objective (set using coarse and fine focus knobs on the sides of the scope). From the current position the PIFOC will move up the amount (in um) set by the user in the “Bottom” and “Top” input boxes. This is performed by setting the “Bottom” to zero and the “Top” to the desired PIFOC height. Incremental movements can be adjusted using the appropriate input box. “Absolute” mode allows the user to adjust heights using the PIFOC controlling slider in the “Configuration Settings” window. The simplest method to use this function is to adjust the fine focus knob until the region of interest is just above the current focus, clicking the “Set” button adjacent to the “Bottom” input box, then adjusting the PIFOC height using the “Configuration Settings” PIFOC slider until the region of interest is just below the current focus and clicking the “Set” button adjacent to the “Top” input box.

Above the “Acquisition Summary” box there is also a dropdown to select whether “Slices” or “Wavelengths” are acquired first. If “Slices” is selected, the acquisition will illuminate the sample with one wavelength, leaving the shutter open, and move the PIFOC the full range selected by the user after which, the next wavelength will be enabled and the process will repeat. Selecting “Wavelengths” moves the PIFOC only after all wavelengths selected by the user are taken.

### ***‘Current at Start’***

For any channel that z is ‘off’, the acquisition will use the current z position when ‘Acquire’ is pressed (at all subsequent timepoints). This means you should find the coarse focus for this single position and leave the knob and then use the PIFOC (in live mode, for each z channel) to define the bottom and top).

### ***Channels (Wavelengths)***

Press the “New” button to add more wavelengths to the planned acquisition. Each wavelength has a “Z Series” enable/disable function, “Exposure Length” adjustability (in ms), and “Color” setting (this will assign a gradient table based on one color for the wavelength). As a note, when using the 1x1 and 2x2 bin modes the camera acquires in 33.33ms and 16.66ms intervals respectively. That is, if you input a 40ms exposure in a 1x1 bin the program will round to the nearest interval, in this case 33.33ms.

### ***Setting up for Save***

Check this box if you would like to save the files from the planned acquisition. User has the ability to select a save destination as well as name the acquisition files and add comments.

### ***Display***

**IMPORTANT:** This should ALWAYS be set to “Last Frame” from the dropdown menu if the user is setting up a timeseries acquisition. Failure to do so may fill the ImageJ memory buffer and terminate the acquisition prior to its completion.

### ***Acquire***

Press this button to begin acquisition.

### ***Stop Early***

Stop an acquisition by pressing the red “X” button in the image acquisition window or by pressing “Stop” below the “Acquire” button. Micromanager saves images as they are taken, thus if the save option was checked all files will be retained at the selected destination even if the acquisition is prematurely terminated.

## **Using the Stanford Photonics Camera:**

There are essentially only three physical controls the standard user will be concerned with on the camera controller: the Power I/O toggle switch, the Camera On/Standby switch, and the Gain potentiometer.

### **Powering On:**

Make sure the cooling unit (below laser table, to the left of the scope) has been on for at least 5 min prior to turning on temperature monitor/power supply (black box above camera on shelf). Turn on Camera in STANDBY mode after monitor is running. If the monitor starts to alarm, do not turn on the camera. Turn off the temperature monitor, wait 5 more mins to let the chiller get down to temperature then turn on the monitor again. Switch the camera control to “enable” to stream images to the camera.

### **Adjusting Gain:**

Adjust the camera gain using the potentiometer on the left side of the box. Do not adjust the potentiometer on the right or toggle any other switches. Signal to noise ratio is highest between 0.800V-1.350V. DO NOT EXCEED 1.400V.

### **Optimizing image quality:**

This camera/software system's ability to detect images is limited by the following parameters:

1) *Gain set by potentiometer*

Gain is physically controlled and thus applied to every "wavelength" at the selected voltage.

2) *RAM Stacking/Averaging of frames*

Frame modifiers are controlled by the "configuration settings" window and are thus applied to every "wavelength" as well.

The best method to acquire images is to set the Gain potentiometer and RAM Stack/Averaging values to fit the "dimmiest" channel. Then adjust the exposure rates of the other channels and their laser voltages to fit the other channels. Tinkering with these values will be a must for this setup as sometimes a sample will require a combination of additive (RAM) and averaged frames to reduce noise and maximize signal as well as laser power corrections and exposure experimentation to minimize laser illumination at the sample. With that said, please allocate an appropriate amount of time (30mins) to adjust the settings to your liking.

## **When Finished:**

### **Between Users:**

1. Clean oil off the objective, and surrounding surfaces. Failure to do so can damage objective springs.
2. Shut down Micromanager Application and save data to CD/server/hard-drive.
3. Do not shut off lasers if there are users using the scope after you (please check calendar).

### **End of Day:**

1. Clean oil off of the objective, and surrounding surfaces. Failure to do so can damage objective springs.
2. Turn off the electronics in reverse order of turning on—lasers off **LAST**. Laser fan will continue to cool for about five minutes and lights will stay 'on' on the front panel of the laser power supply—these are 'on' all the time.