Olympus CSU W1 Spinning Disk

User Guide

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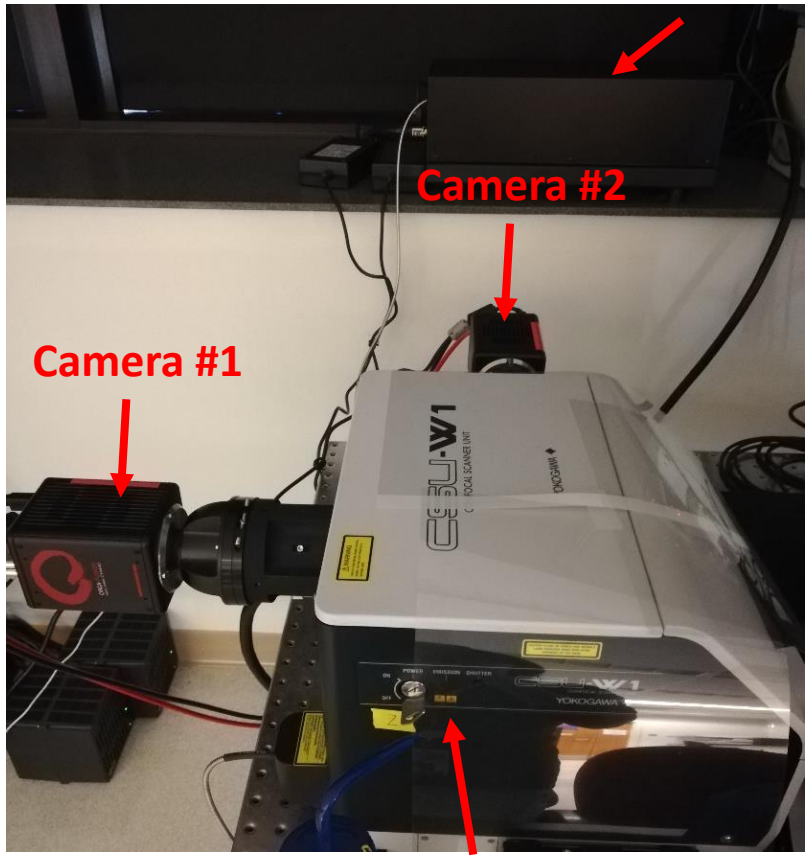
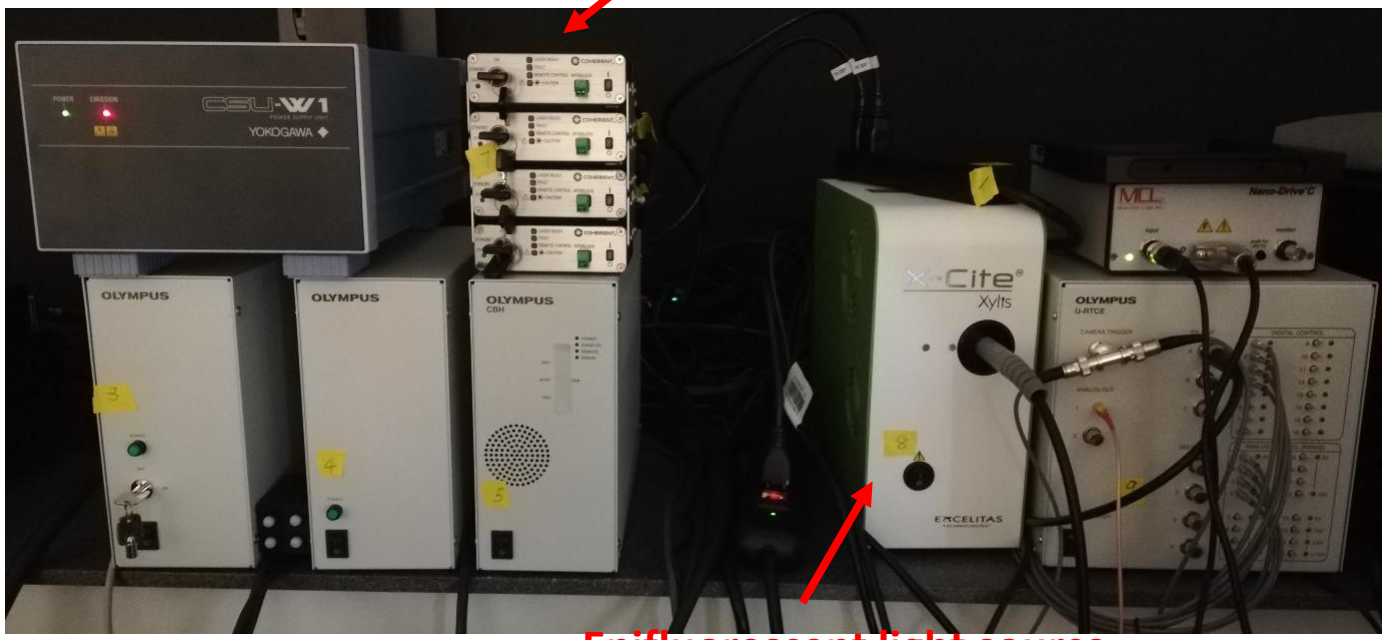
1. Startup the System

Hardware and microscope introduction


Laser unit
Camera #2
Camera #1
Spinning Disk


Laser powers
Epifluorescent light source

Startup the system in the order of 1 to 6.

1. Sign on the login sheet

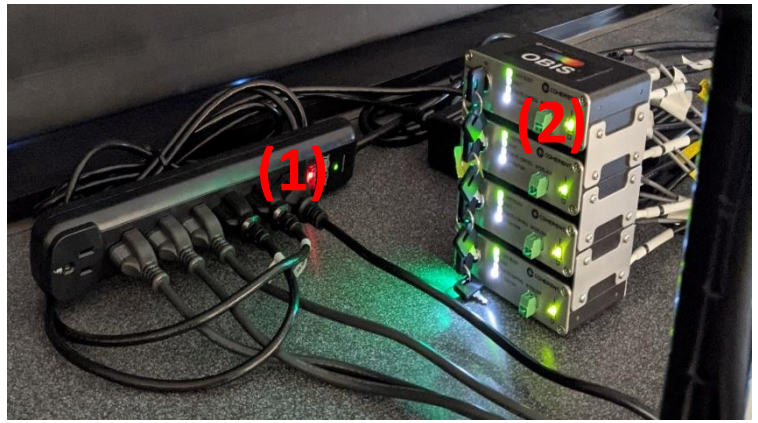
2. The power strip (1) is the power supply for the microscope and controlling units on the shelf.

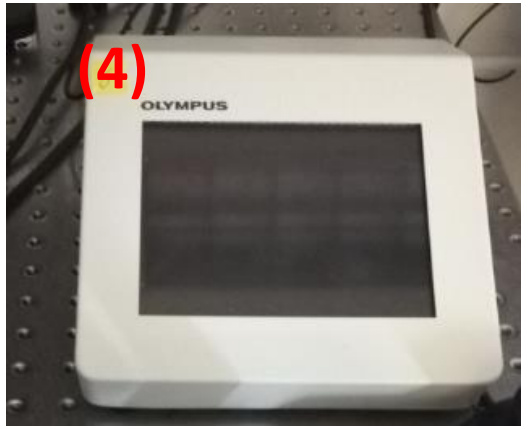
3. Number (2) is power supply for lasers: Turn on the power for the 4 small units first, wait for the lights to turn blue, then turn the keys and the lights will turn green.

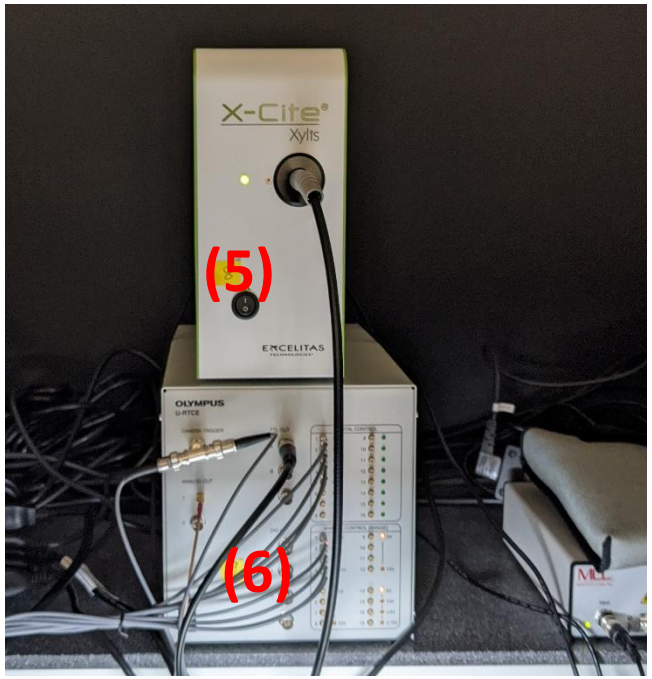
4. Turn Spinning Disk key (3), then turn on the touch pad (4).

5. Turn on unit (5) (epifluorescence light source), followed by unit (6).

6. Last step, log in to the PC and start the software, CellSens.







2. Software

CellSens

1. Log in to the PC with your personal account: **lsumc-master\username** and your personal password (same with your email password).

2. Tilt the flexible head of the microscope to load your sample.

3. Make sure the slide holder is clean and flat before placing the samples. Hold the middle of the slide holder to load it.

4. If using oil objectives, add one drop of oil on top of the objective or the cover slide. **Be cautious to not drip oil on the microscope**.

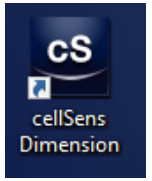
5. Lower the Z-positions of objectives before placing samples.

6. Place the sample on the slide holder with the **<u>cover slip-side down</u>**.

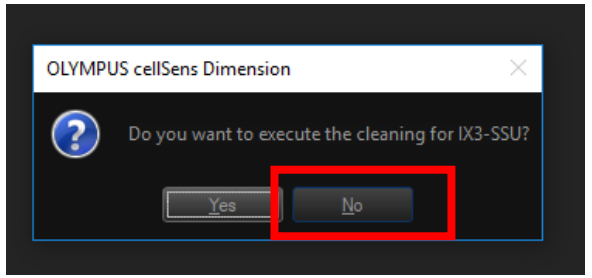
Touchpad

7. You can change the objectives through the touchpad, or you can do it later through the software.

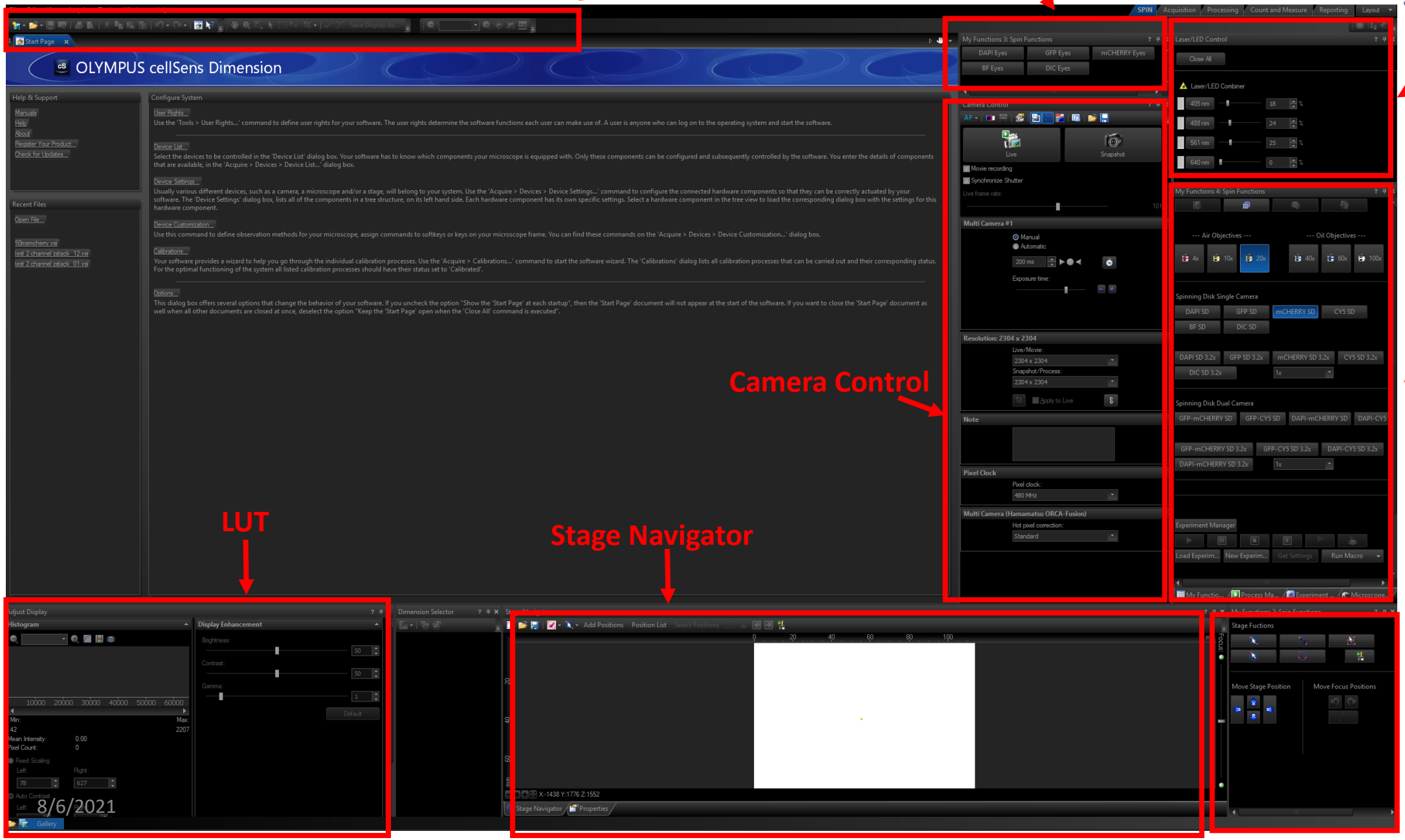
8. Click on the software CellSens Dimention.



9. Click No when asked whether to execute cleaning.

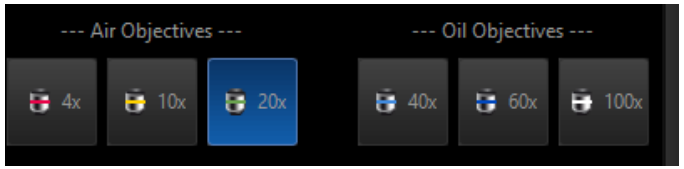


Software layout

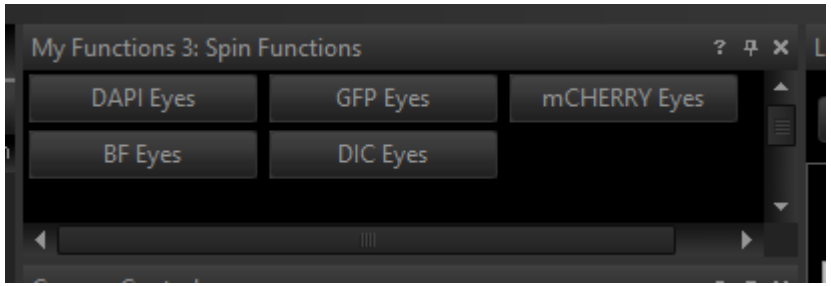


Eyepiece Observation

1. If you did not select objectives from the microscope touchpad, you can select one through the software.

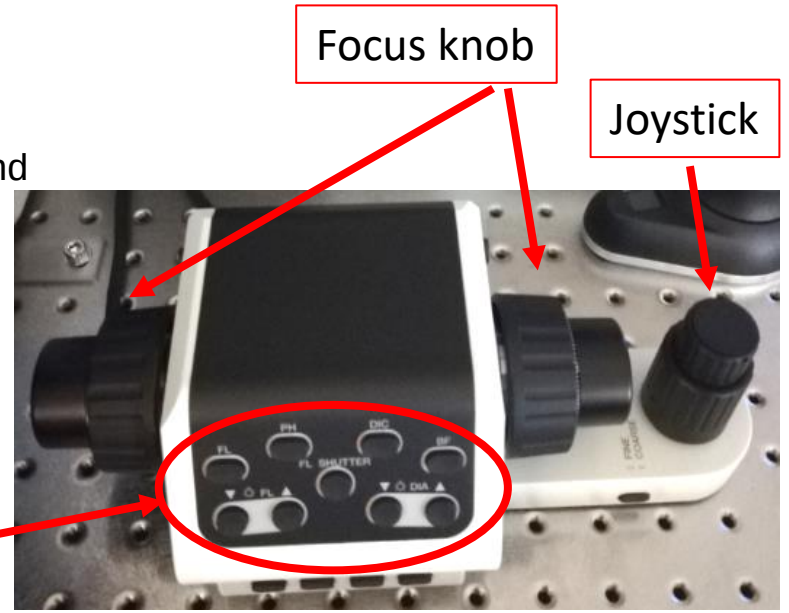


2. To turn on Epi fluorescent light for eyepiece observation, select among **DAPI Eyes, GFP Eyes, mCherry Eyes,** or **BF Eyes and DIC Eyes** for bright field imaging.
There is no Cy-5 option for eyepiece observation, because you cannot see far red through eyes.



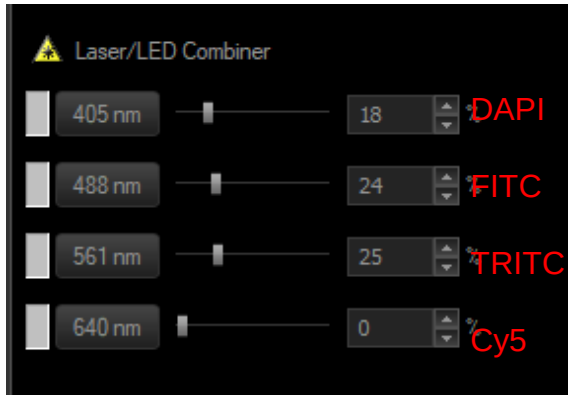
3. Now you should see light turned on in the microscope. Observe through the eyepieces. Find and focus on the samples by adjusting the joystick and focus knobs.

4. **Then press the Epi shutter to turn off the Epi fluorescent light (important)**. This is very important to avoid photo-bleaching your samples.



Single/ Dual camera Imaging

1. Turn on the lasers that you will use, Turn up or type in a starting percentage for each laser.



1. In the My Functions 4: Spin Functions section, there are Single Camera settings and Dual Camera settings.

2. Select the corresponding channel. For instance DAPI SD stands for DAPI Spinning Disk.

3. DAPI SD 3.2x stands for a 3.2x zoom in in the DAPI channel while imaging. This 3.2 is a zoom factor that's built in the camera.

4. Under Dual camera options. You can select a combination of two channels. The longer wavelength light always goes to Camera #1, the shorter wavelength light goes to Camera #2 **(Important when adjusting exposure time for individual cameras)**.

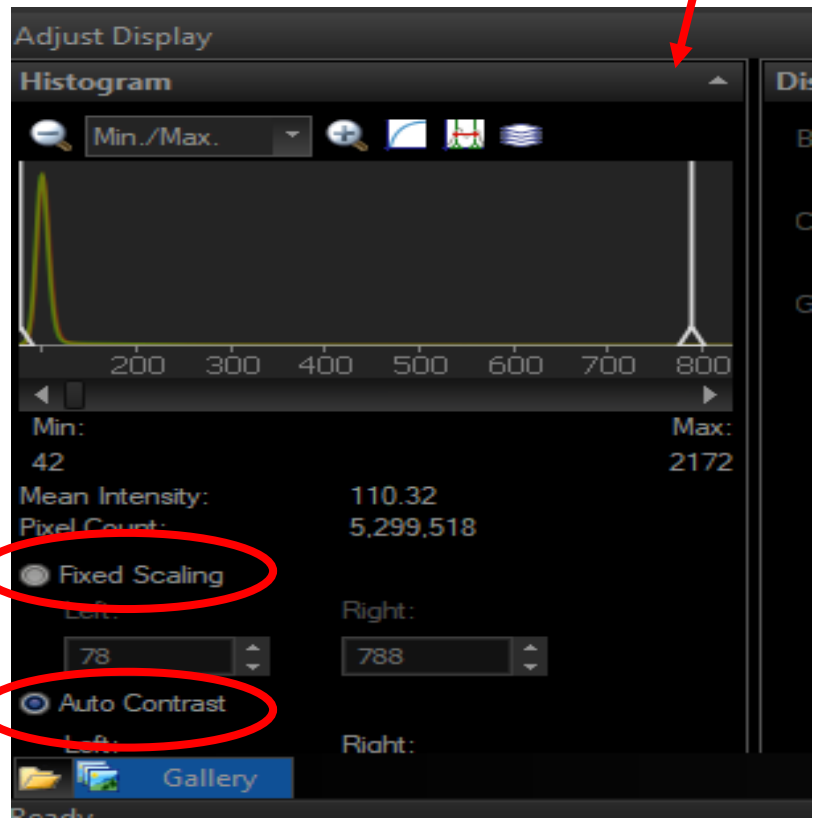
Single/ Dual camera Imaging

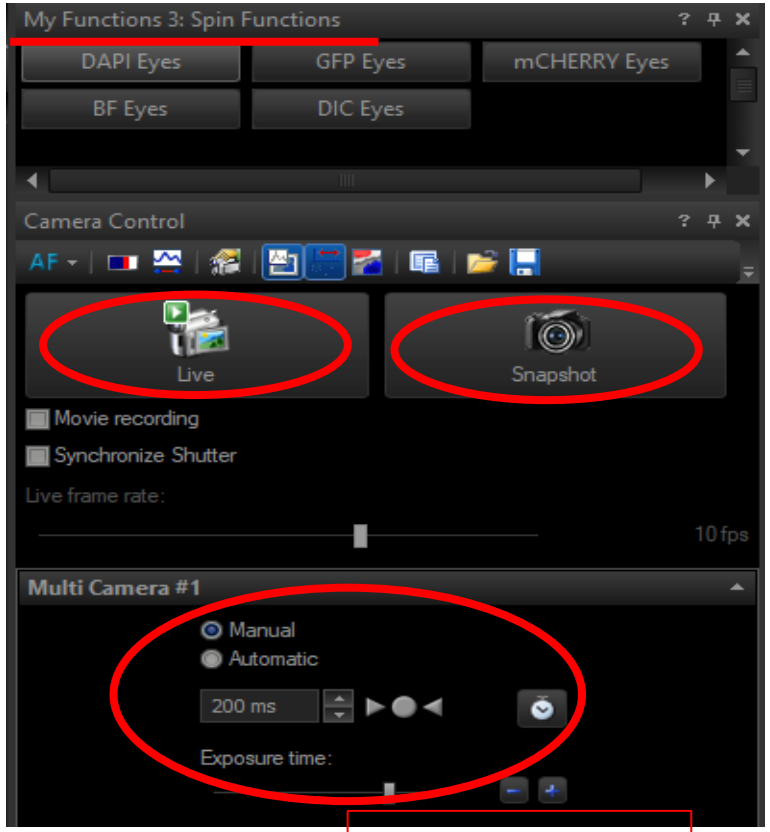
5. In the Camera Control section, click Live to see a live view of your sample.

6. **Then select Auto Contrast in the LUT section if taking fluorescent images. If taking bright field images, stay on Fixed Scaling all the time. (Important! You won't see fluorescent images without selecting Auto Contrast first.)**

7. Once you see an image, **click on Fixed Scaling again** in the LUT section.

8. Then simultaneously press the Ctrl key and use the roller on the mouse to finely adjust the focus. The brighter the image, the better the focus. (**Important) focal plane from the eyepiece is slightly different from focal plane in the software**.





Exposure time

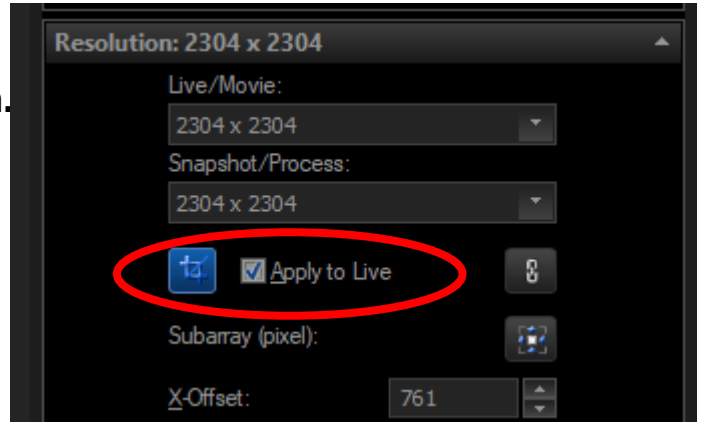
9. After you click Live to see a live view of your sample, you will see the crop session activated.

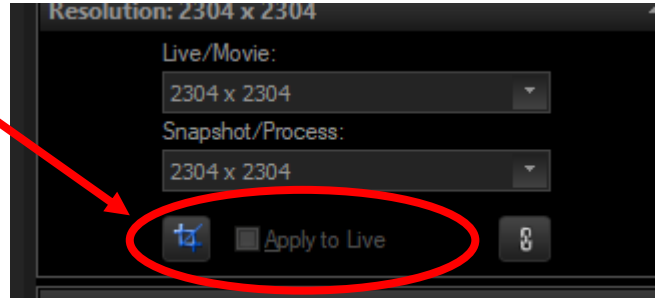
10. Click on  , then a blue sub area is shown in the live window.

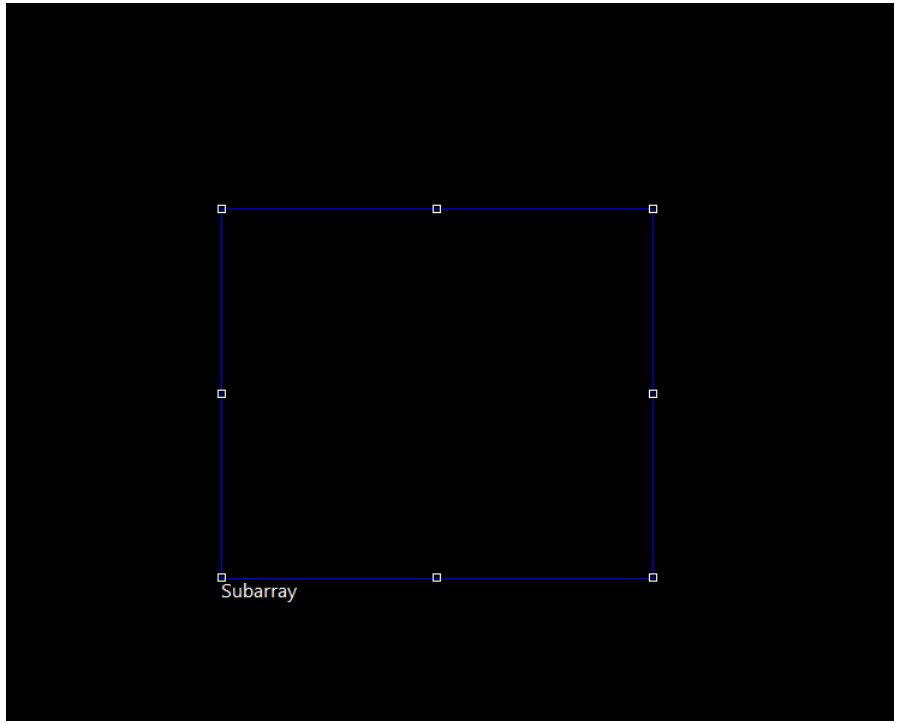
11. You can adjust the size and position of this subarea. Then click Apply to Live to apply it.

12. **This function does not change resolution.**

This function is very useful for large Image scan to minimize the border of each tile image.





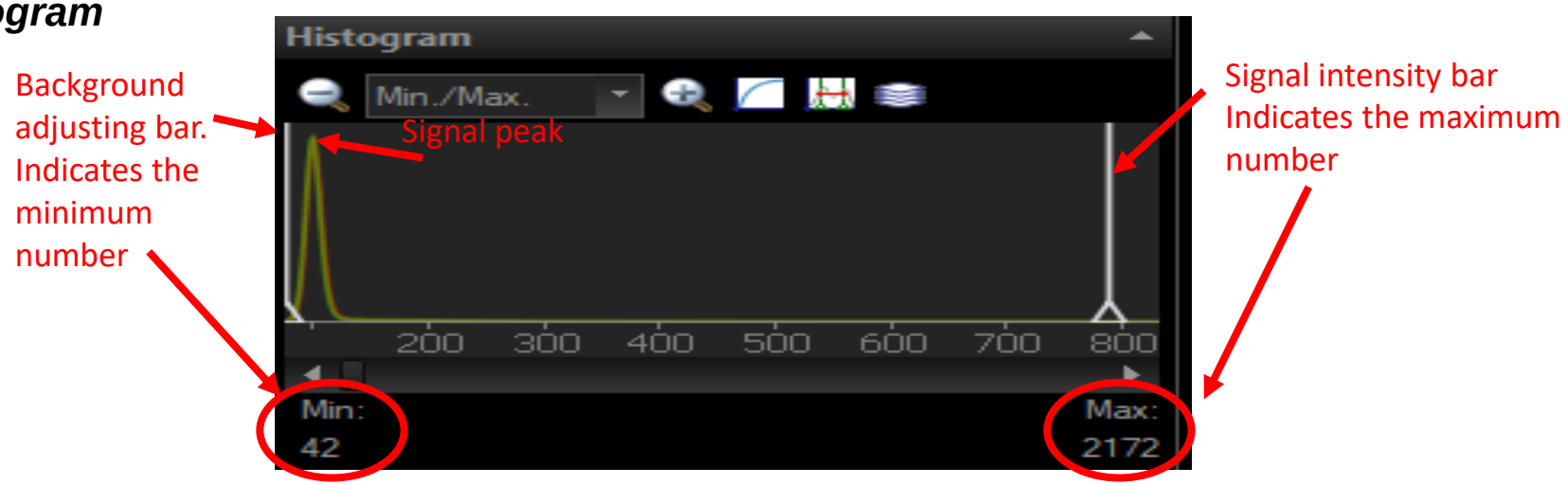


Adjust image brightness

Once you are at the best focal plane, you can adjust the brightness of the image through three parameters:

1. Adjust LUT/Histogram 2. Adjust exposure time 3. Adjust laser power

1. ***Adjust LUT/Histogram***

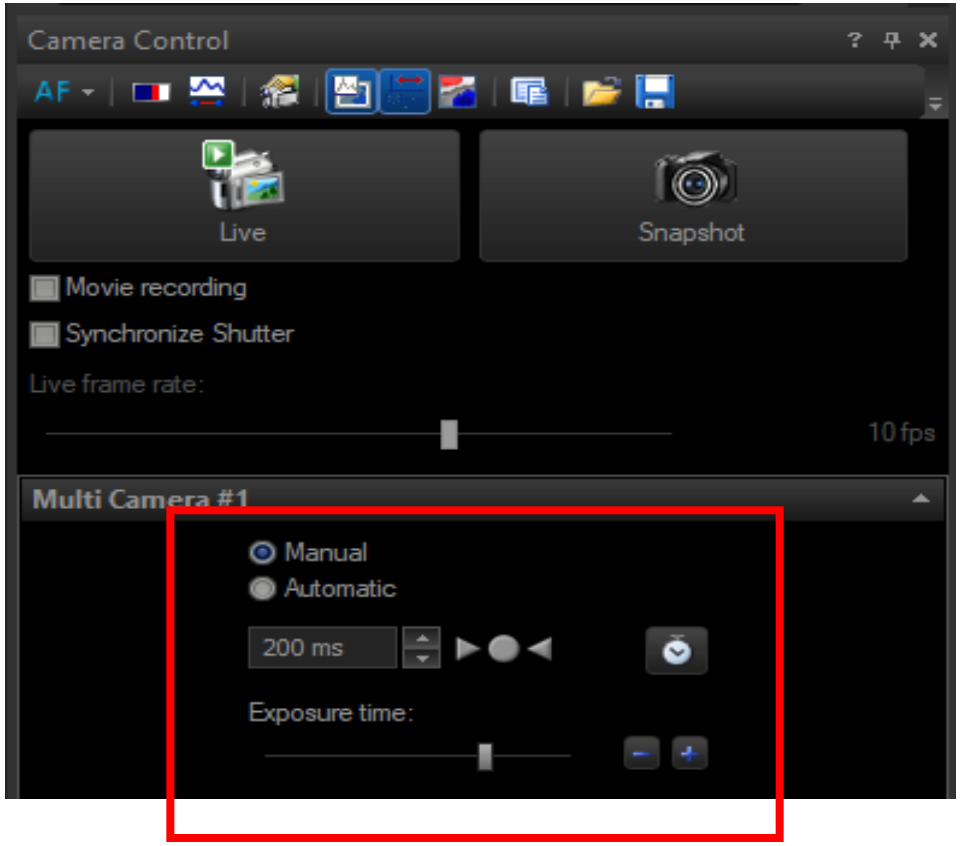


- In the Histogram, the white vertical bar on the left adjusts the background, the white vertical bar on the right adjusts the signal intensity.
- For the signal intensity bar, the more to the left (smaller maximum number), the stronger the signal. The more to the right (bigger maximum number), the dimmer the signal.
- For the background adjusting bar, the more to the left (smaller minimum number), the stronger the background, the more to the right, or the closer to the signal peak, the more black the background. **In most cases, the background bar should not go pass the signal peak.**

Adjust image brightness

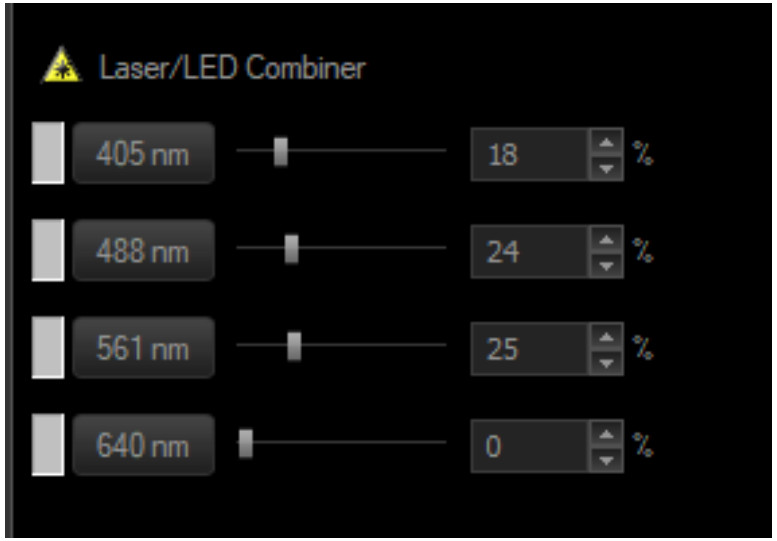
2. Adjust exposure time

Adjust the camera exposure time manually in the Camera Control section. 100 ms or 200 ms are good numbers to start with. You can type in a number or drag the exposure time bar to change it. The longer the exposure time, the stronger the signal. (Avoid pinhole patterns)



3. Adjust Laser Power

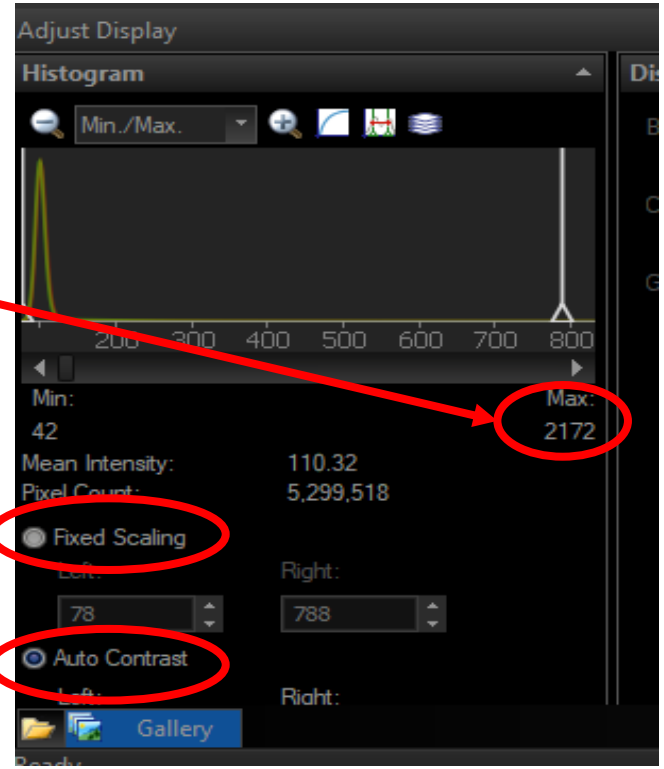
Adjust the laser power by either typing in the number or drag the bar to change it. The stronger the laser power, the brighter the signal, and the more photobleaching. 10-30% are good starting numbers.



Adjust image brightness

How to combine the three adjusting parameters to create a perfect image: LUT, exposure time, laser power.

…Continued from page 11



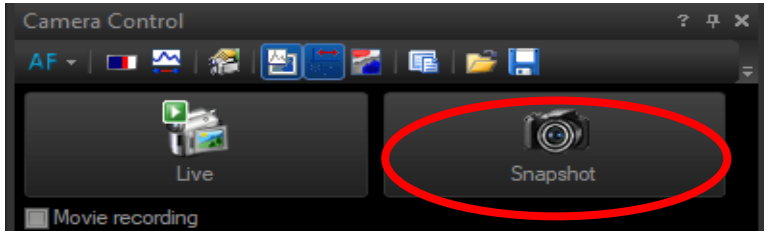
Once you are at your best focal plane (the brightest under fixed scaling). **Switch to Auto contrast** in the histogram, pay attention to the maximum number. The overall range of the camera is up to 60000. **If the maximum number is over 50000, that's considered too high. If your maximum number is less than 1000, that may be is too low.**

If the maximum number is too low, it means the auto contrast is automatically dragging the signal adjusting bar to the left (smaller max number) to increase the brightness of the image. In this case, you can increase the laser power or the exposure time, until the max number is at least above 1000 (Anywhere from 5000 is good).

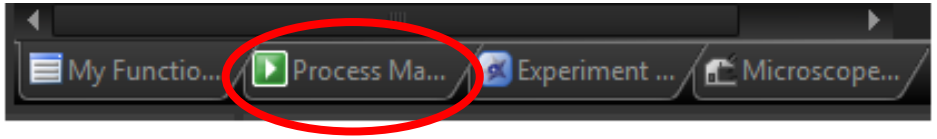
If the maximum number is too high, that means the auto contrast is automatically dragging the adjusting bar to the right (bigger max number) to decrease the brightness. In this case, you need to decrease the laser power or the exposure time, until the max number is at least below 50000. (Anywhere around 5000 is good).

Once your are happy with the outcome, switch back to Fixed Scaling, so your LUT keeps the same through out your imaging.

Then click Snapshot to capture the image.



Process Manager



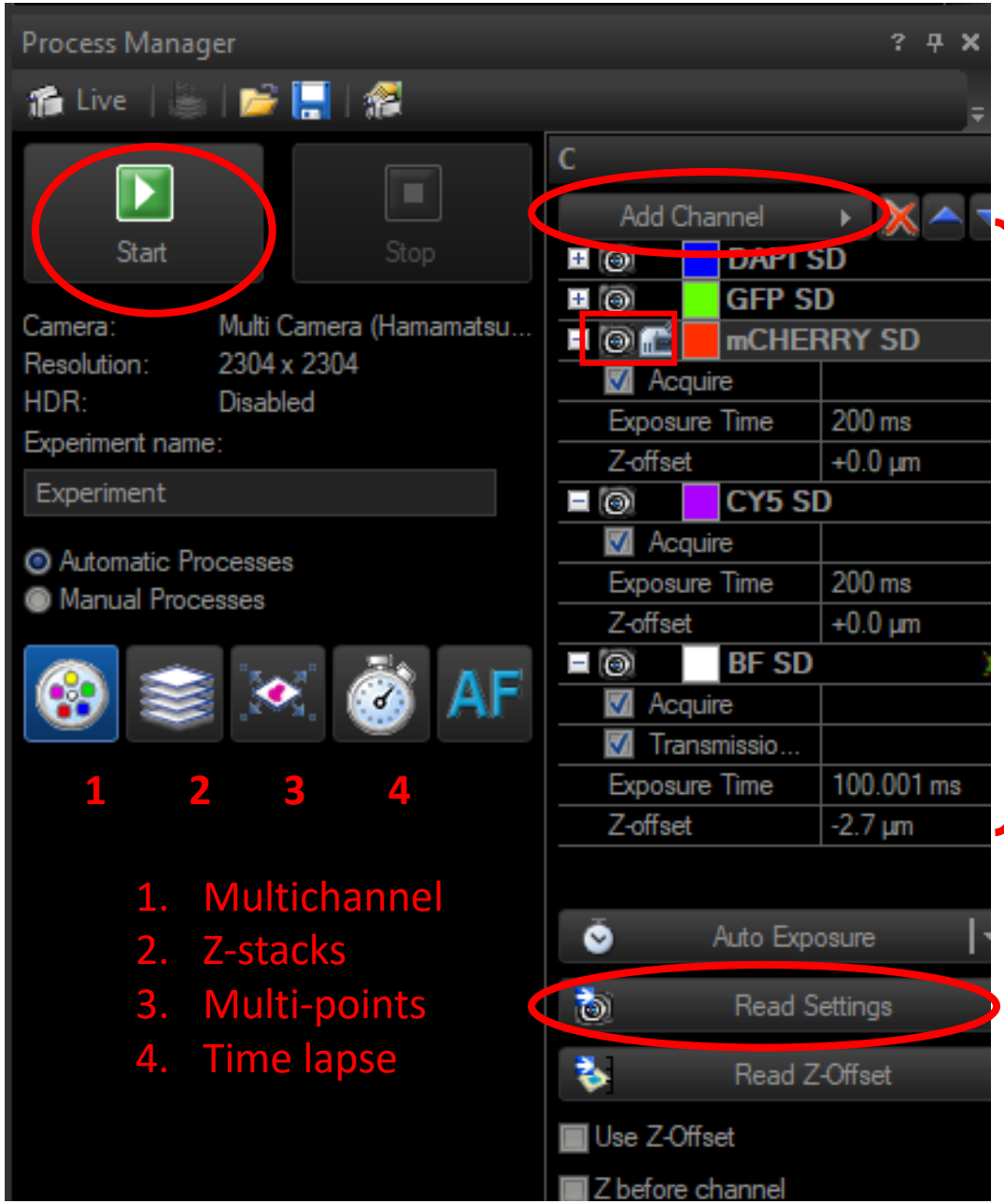
The Process Manager section contains:

1. Multichannel imaging
2. Z-stacks
3. Multi-points imaging/ Large image scan
4. Time lapse imaging

1. Multichannel imaging 

- If you have more than 2 fluorescent channels (a two channel dual camera setting does not meet your requirements), you can set up the experiment in Process Manager-Multichannel imaging.
- Click Add Channel to add channel settings that is not listed.
- **After changing exposure time or laser power, click Read Settings (important!),** to apply the change to the multichannel imaging settings.
- Click Start to snapshot after experiment is set up.

A single camera setting cannot be combined with a dual camera setting in this section. You can only set up a combination of single and dual camera in the Experiment Manager section.



Multichannel Setup

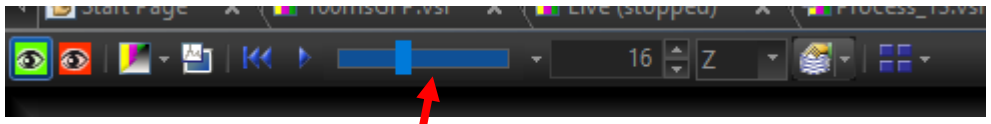
A channel is being selected, will be applied when start acquisition.

A channel is being selected, will be applied when observing through eyepieces.

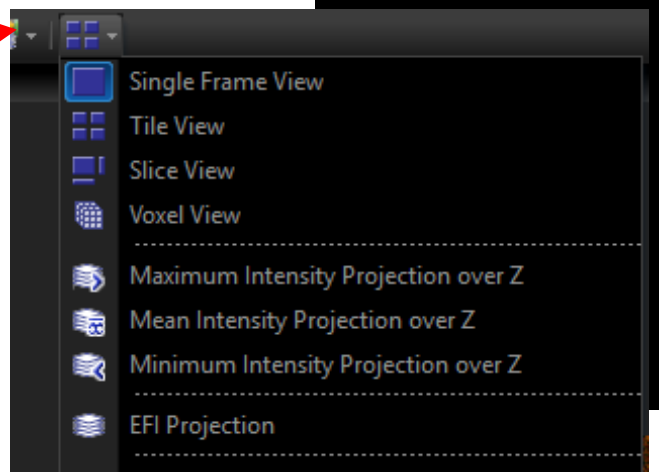
Process Manager

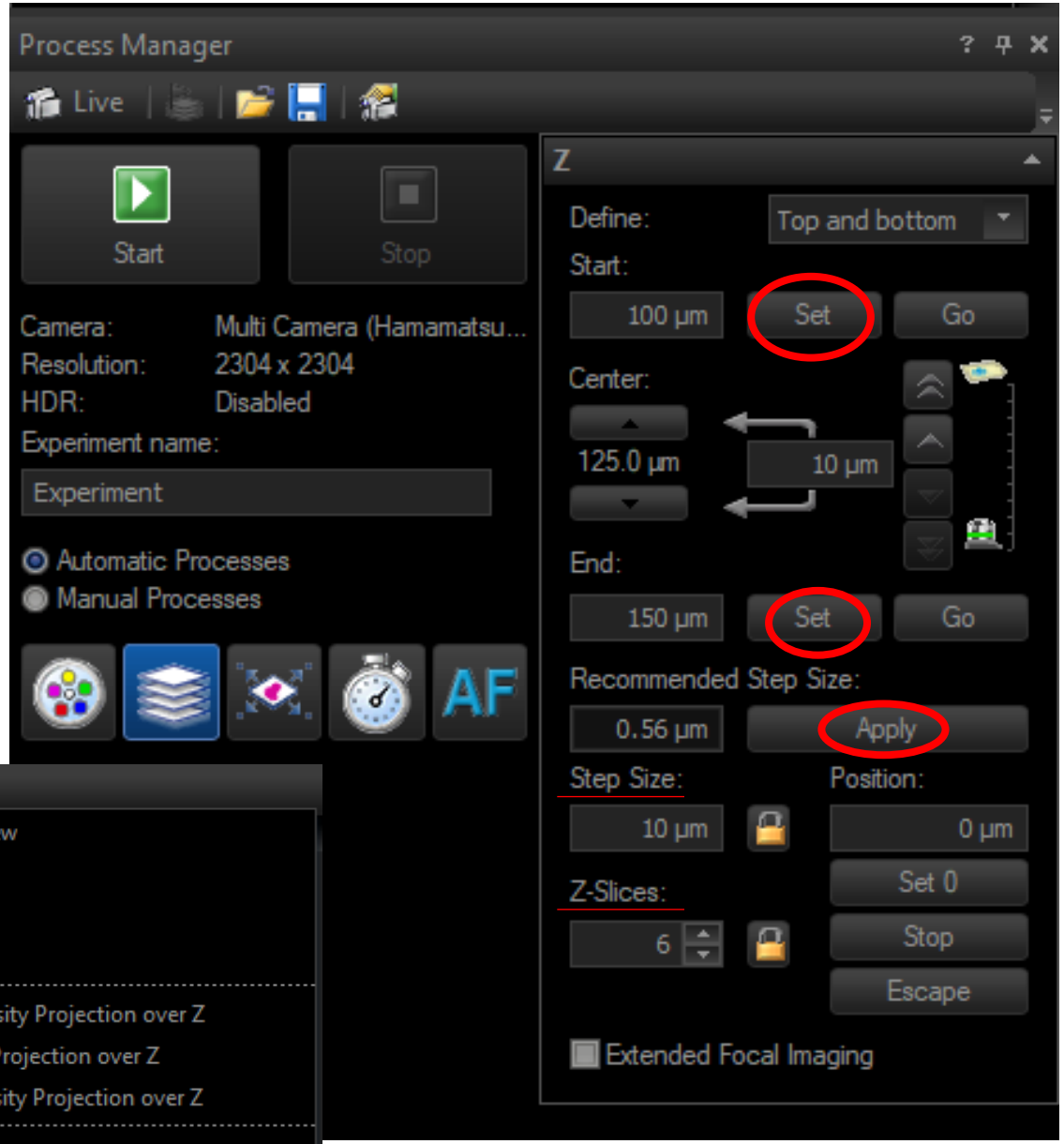
2. Z-stacks:

- After adjusting the signal intensity for the best focal plane, **stay in live view to define the Z range (important!)**.
- Click on the Z stack button. 
- Move the focus knob (or use Ctrl+mouse) to the bottom of the interested z range as the start point, then click Set.
- Move the focus knob to the top of the interested Z range as the end point, then click Set.
- Apply the recommended step size or a your desired step size. if you do not know what step size to use, you can also directly type in the desired Z-slices number.
- Then click Start to capture a Z-stack image.
- You can view the Z-stack image with these options.



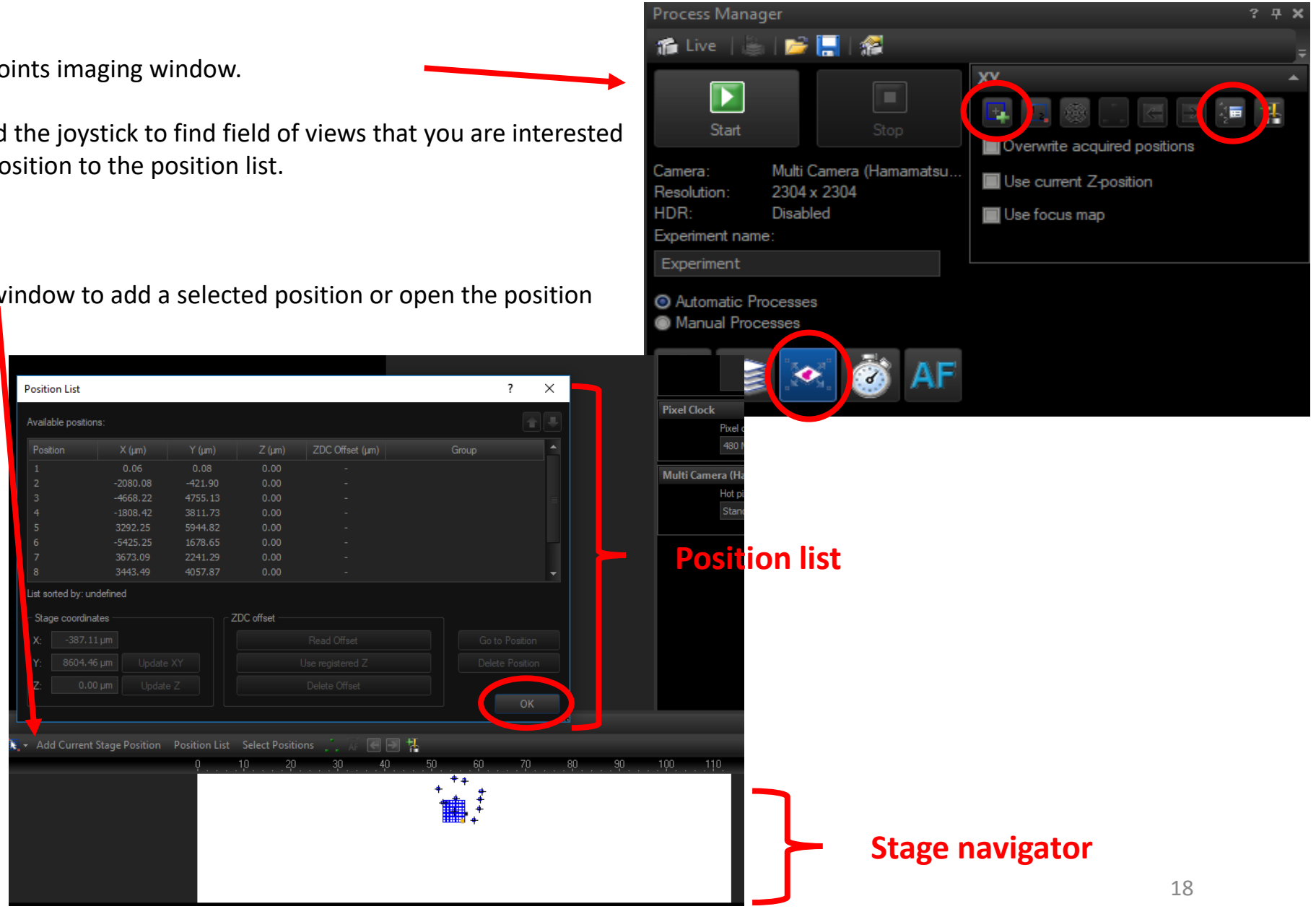
Z slices bar





3. Multi-points imaging:

- Click on  to switch to the multi-points imaging window.
- While in live view mode, move around the joystick to find field of views that you are interested in. Click on  to add the selected position to the position list.
- Click  to open the position list.
- You can also use the stage navigator window to add a selected position or open the position list.
- Click Ok after all positions are added.
- Click Start to capture images.



Process Manager

4. Multi-points imaging:

- Click on  to switch to the large sample imaging. Then click Live to stay in live view mode, and move around with the joystick to define areas.

- Under Stage Functions, there are different ways to define your large field of view.

 Draw a rectangle or square area with your mouse.

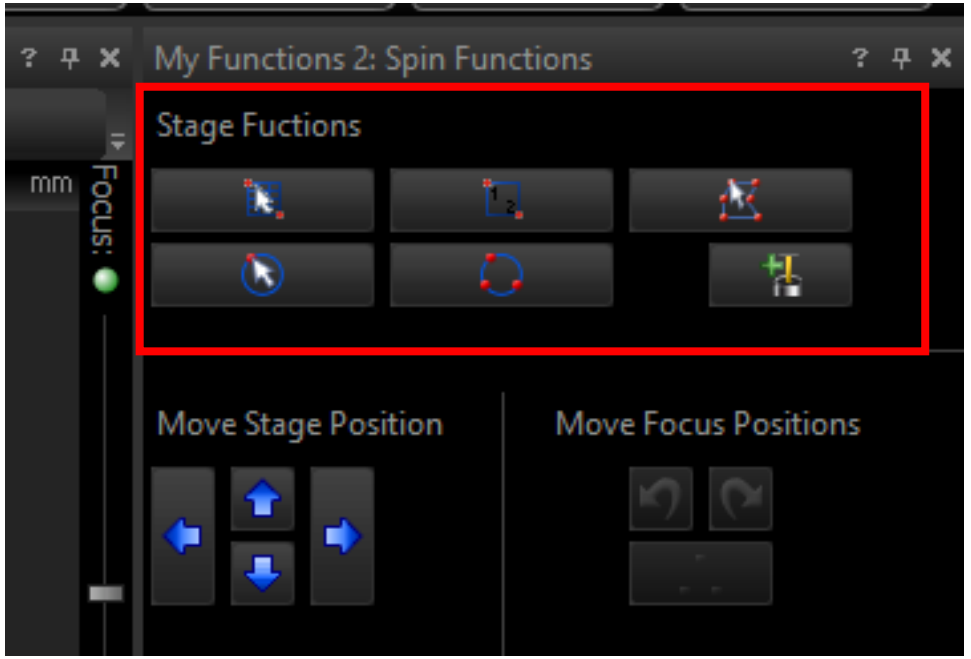
 Draw a relatively round area with your mouse.

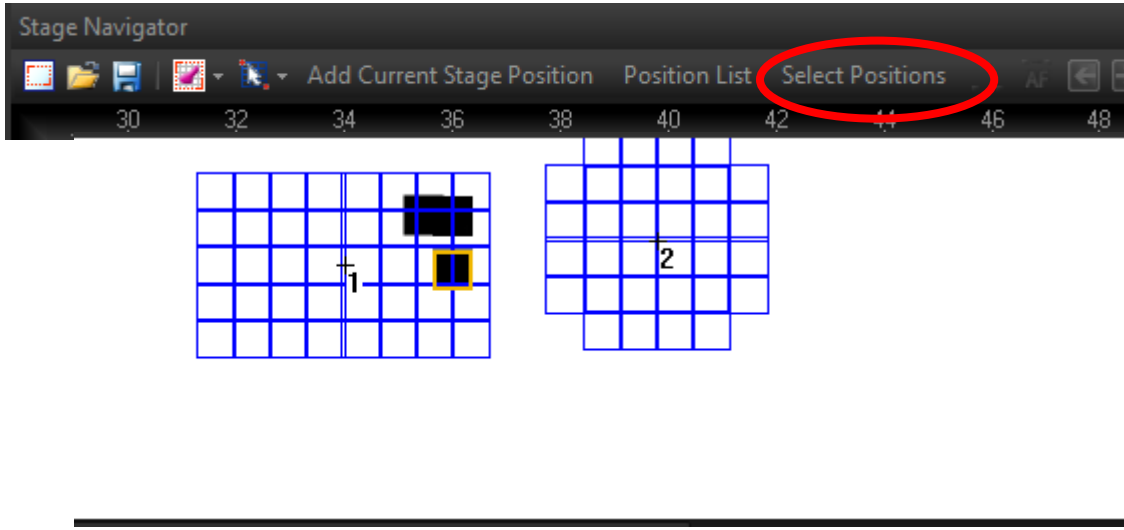
 Draw a polygon area with your mouse.

 Define the upper left and bottom right positions.

- Your defined area will show in the Stage Navigator. Pay attention to the number of areas created. In this illustrated figure, there are 2 areas created. So both areas will be captured during acquisition. You can click on Select Position and delete the one you do not want.

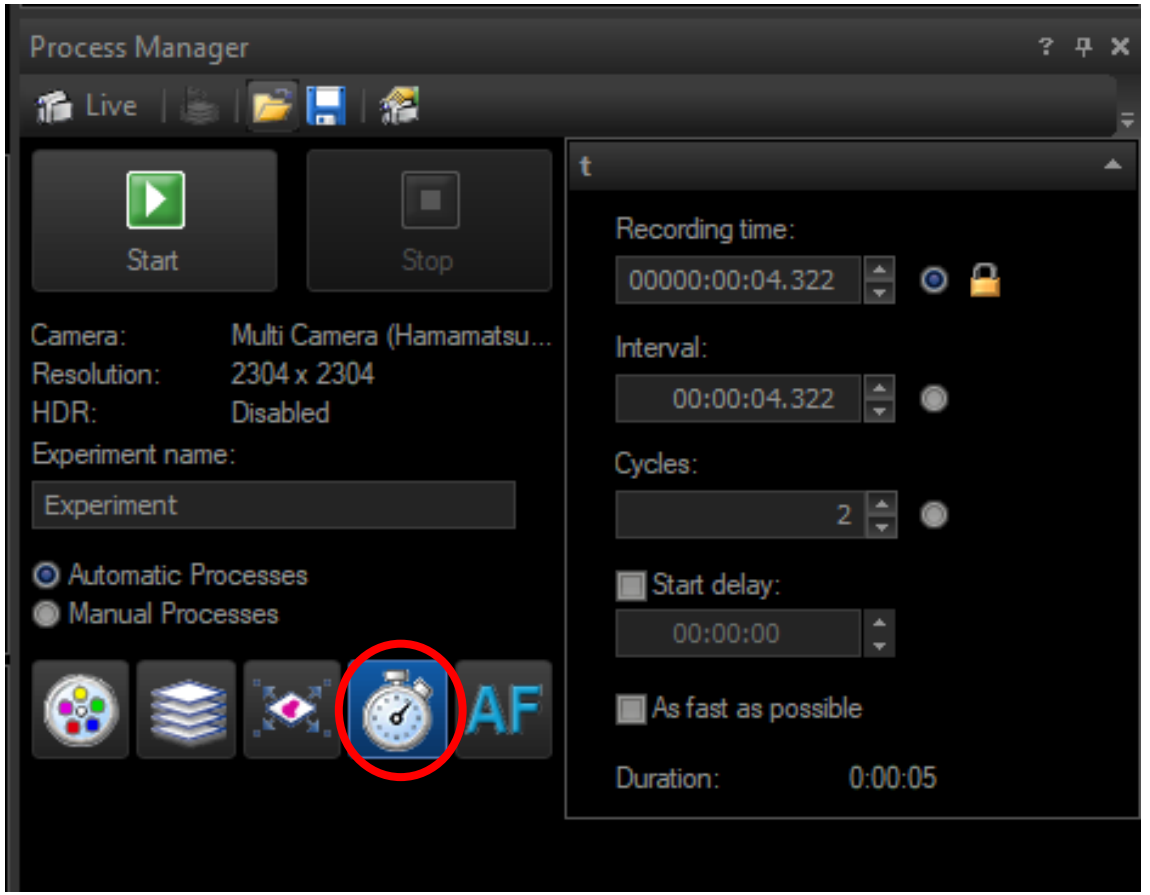
- Next, click Start to capture images.





5. Time-lapse imaging:

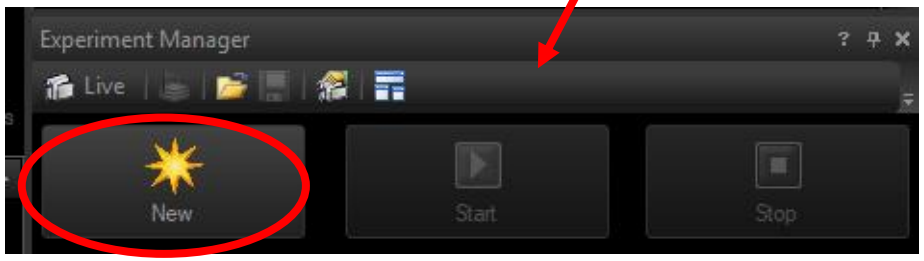
- Click on  to switch to the time-lapse imaging window.
- Recording time: the total duration time you want to set.
- Interval: The time interval between each image.
- Cycles: How many images you will capture within the total duration time.
- If you check “As fast as possible”, the time to capture one image will be applied to the Interval.

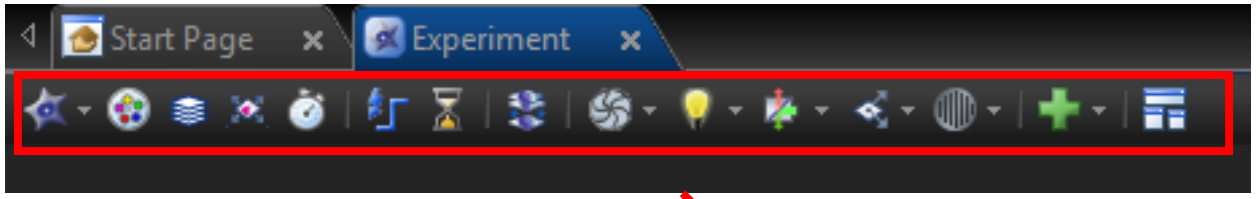


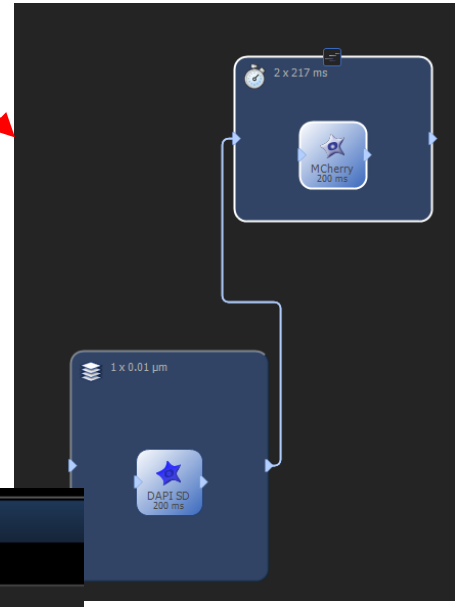
All the functions in the Process Manager can be combined as needed. When you click Start, all selected functions will be engaged. Do not forget to uncheck the ones you do not need.

Experiment Manager

The Experiment Manager is a very useful and user friendly tool to customize your experiment.



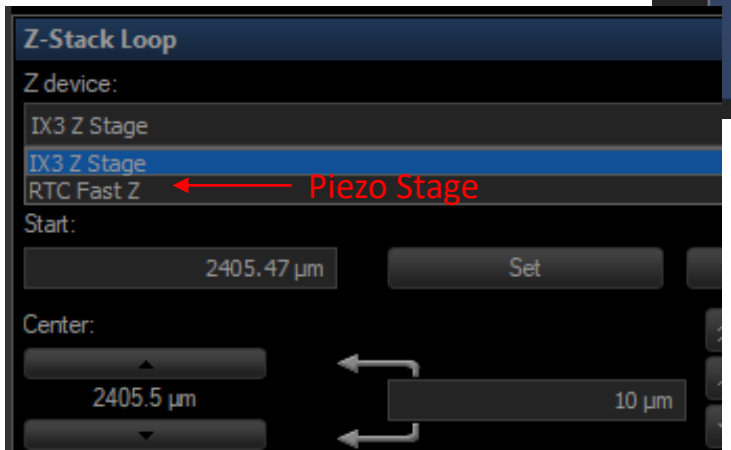




- Click on New to open a new Experiment Manager window.

- In the new Experiment Manager window, you can add different elements to set up customized experiments. For instance, combine single camera and dual camera settings into one experiment; Add Wait in a time loop, or exclude a specific laser light from a time loop; Use bright field light to bleach your sample, then resume imaging. The Experiment Manager is more advanced than the Process Manager. You can choose between the Experiment Manager and the Process Manager to set up your experiment.

- You can only use the Piezo Stage in Experiment Manager.



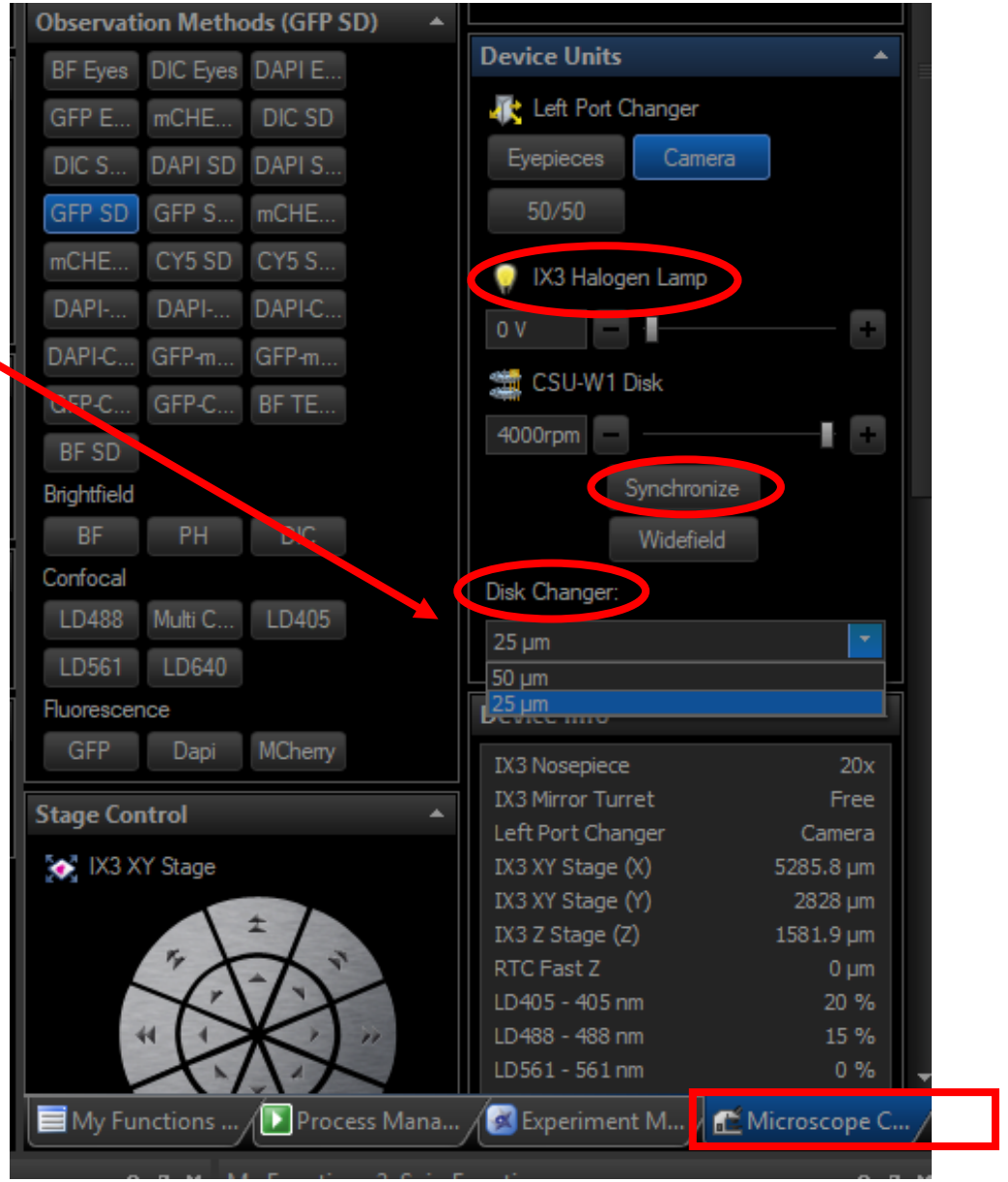
Microscope Control

Three commonly used functions in the Microscope Control window.

1. Disk Changer
Our spinning disk unit is loaded with two disks of different pinhole sizes. A 25µm pinhole disk and a 50µm pinhole disk. The default disk is the 50µm disk. The 50µm disk is suitable for all objectives. If you prefer to have sharper images for 10x and 20x objectives, you can switch to the 25µm disk. The 25µm disk is not suitable for 40x, 60x, and 100x objectives.

2. Synchronize
If you see pinhole track patterns in your image that does not disappear after adjusting camera exposure time, you can click Synchronize to synchronize the spinning disk rotating speed and the camera exposure time to eliminate the pinhole tracks.

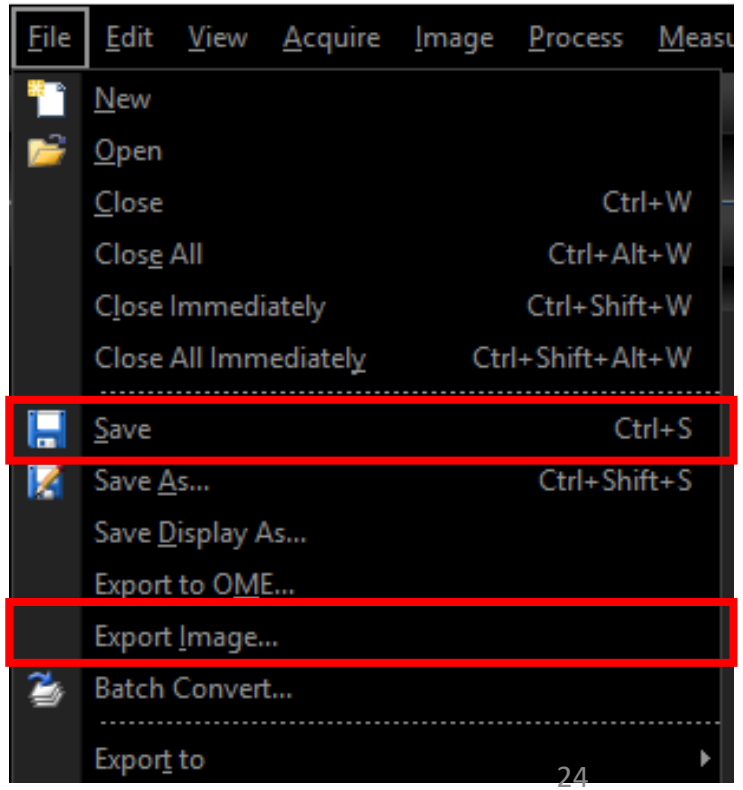
3. Halogen lamp (bright field imaging)
You can control the halogen lamp through the microscope touch pad, or through the halogen lamp controller in the Microscope Control window.

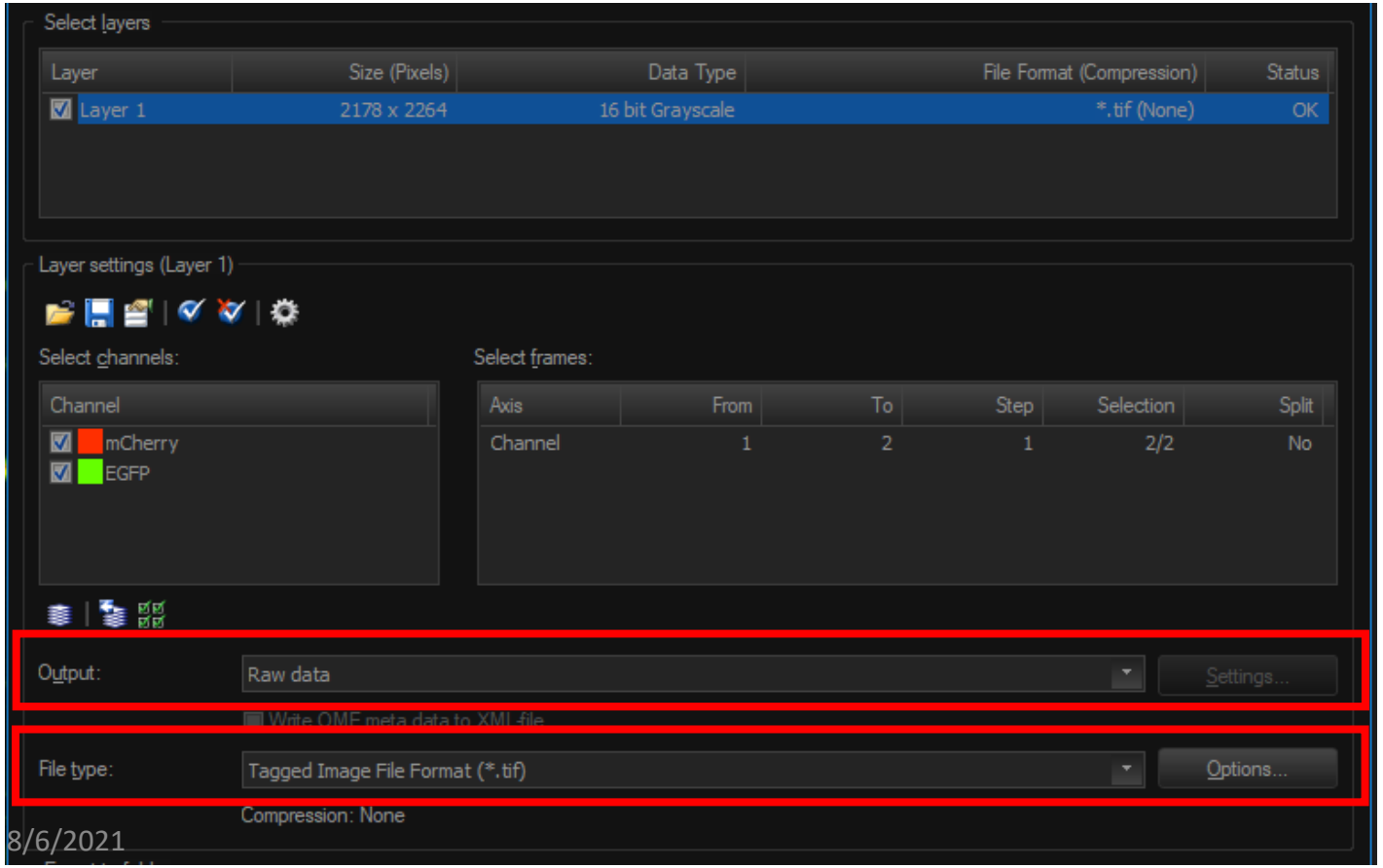


3. Save and Export

Save an image

- After acquisition click File→Save to save the image as .vsi file.
- Then click File→ Export Image to export the saved .vsi file to TIFF files.
- Export into a merged multichannel image and export into individual-channel images are two separate steps. In the Export window, click on the dropdown menu for Output, and choose 24bit RGB for individual channels, and merged for merged channels. Also make sure the File type is .tif. Set output destination to your folder in the E drive.
- **Save images to E drive, do not save any images on the desktop or in C drive**.





4. Live cell imaging

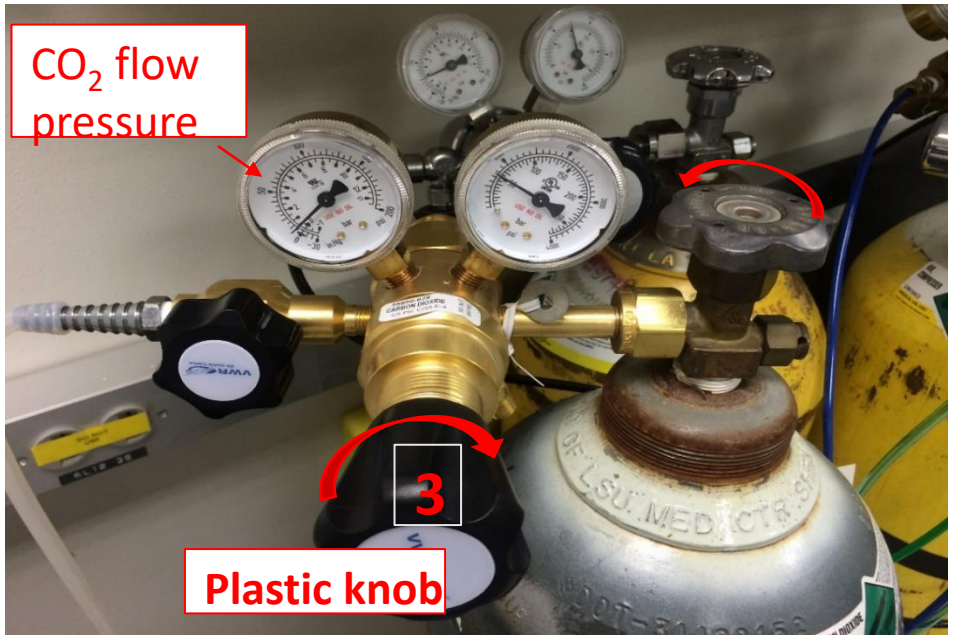
- 1. For live cell imaging, when booking the calendar, **reserve at least 1h extra time for the objective to cool down**. For example: If you need to use the microscope from 8am-3pm, reserve the microscope from 8am to 4:00pm, and stop the experiment at 3:00pm. This will allow the objective to cool down to avoid focus drift for the next user.
- 2. For live cell imaging, when booking the calendar, **specify "Live Cell".**
- 3**. For live cell imaging, it is best practice to turn on the temperature controller 30min-45min before you start your scan.** This will avoid focus drift during live cell imaging.
- 4. For fixed sample imaging users, if you doubt that the objective/microscope has not cooled down and it may affect your imaging, please inform the core staff.
- 5. If cell media spills out, **wipe clean the stage and microscope, then clean with ethanol**.
- 6. If virus infection is involved in live cell imaging, please inform the core staff to evaluate whether it can be brought in to the microscope room or not. Biosafety rules should be followed in the microscope room.

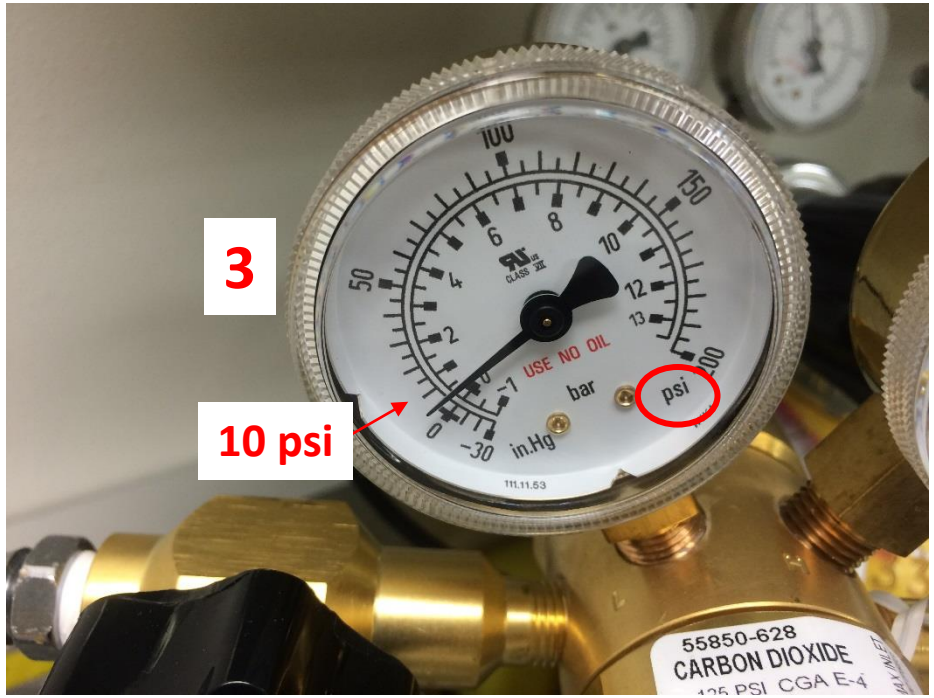
Turn on the live cell imaging system

1. Press the yellow button to turn on the Tokai Hit live cell incubator motor. **Do not change any other settings on the motor**.

2. Turn the metal wheel counter clockwise to open the CO_2 tank (Open and Close directions are marked on the wheel).

3. Turn the plastic knob clockwise to increase the CO_2 flow pressure to 10-13 psi. Make sure The CO_2 tank pressure is **below 15** psi. It will take 15 min for the chamber to heat up and for the CO_2 to increase to 5%. When CO2 is filling up the incubator, the CO_2 flow pressure may drop, readjust it to around 10-13 psi. **Attention: Pressure higher than 15 psi is over the limit of CO_2 tubing and will cause damage.**





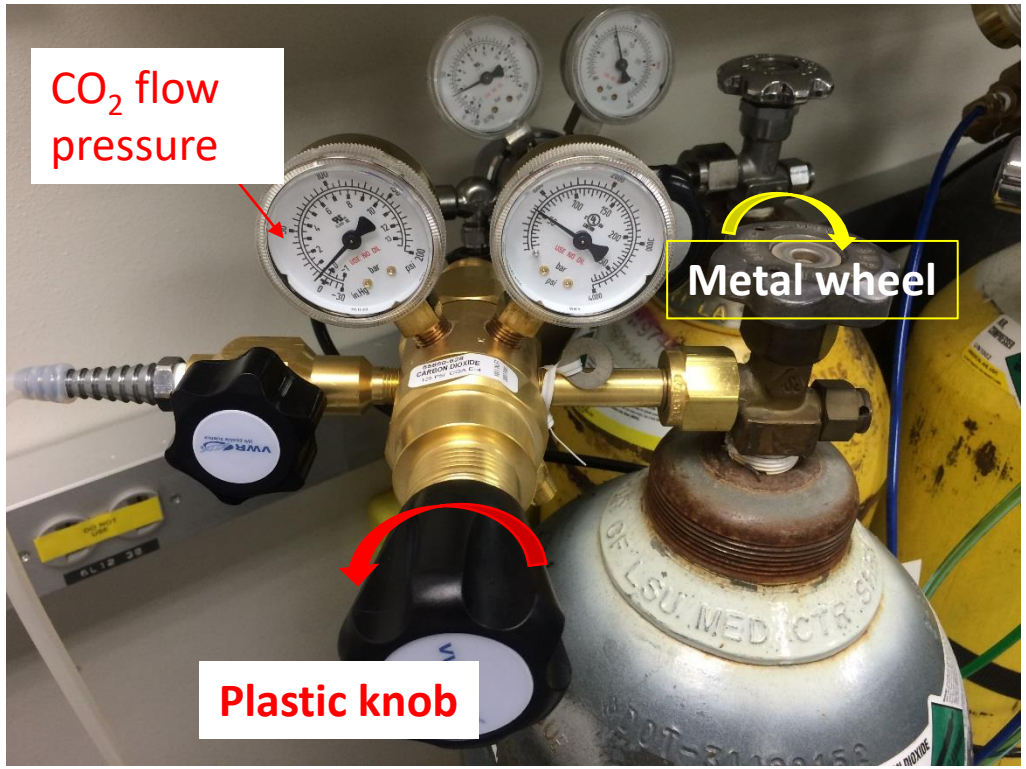
Shut down the live cell imaging system

1. After live cell imaging, turn the plastic knob counter clockwise to decrease CO_2 flow pressure (The CO_2 pressure will not drop immediately). **Then, make sure to close the CO_2 tank by turning the metal wheel clockwise**.

   A $50 penalty will be charged if the CO_2 tank is not closed after live cell imaging.

1. Aspirate distilled water from the live cell stage holder and dry it with paper tower.

2. To sign out the computer and shut down the microscope, follow the steps for regular shut down.



5. Shut down the system

1. Close the software through “File->Exit”.

2. Check the calendar (shortcut on the desktop) to see whether anyone else will use the scope after you. If there are users using with in 2 hours, log off the computer and leave the system on.

3. Clean the oil objectives you used, microscope stage, and the table. Lower the objectives to the bottom, and switch it back to 4x.

4. **Turnoff the touch pad first, then shut down the system, following the steps 4->6->5->3->2->1**.

5. Sign the ending time on the login sheet.