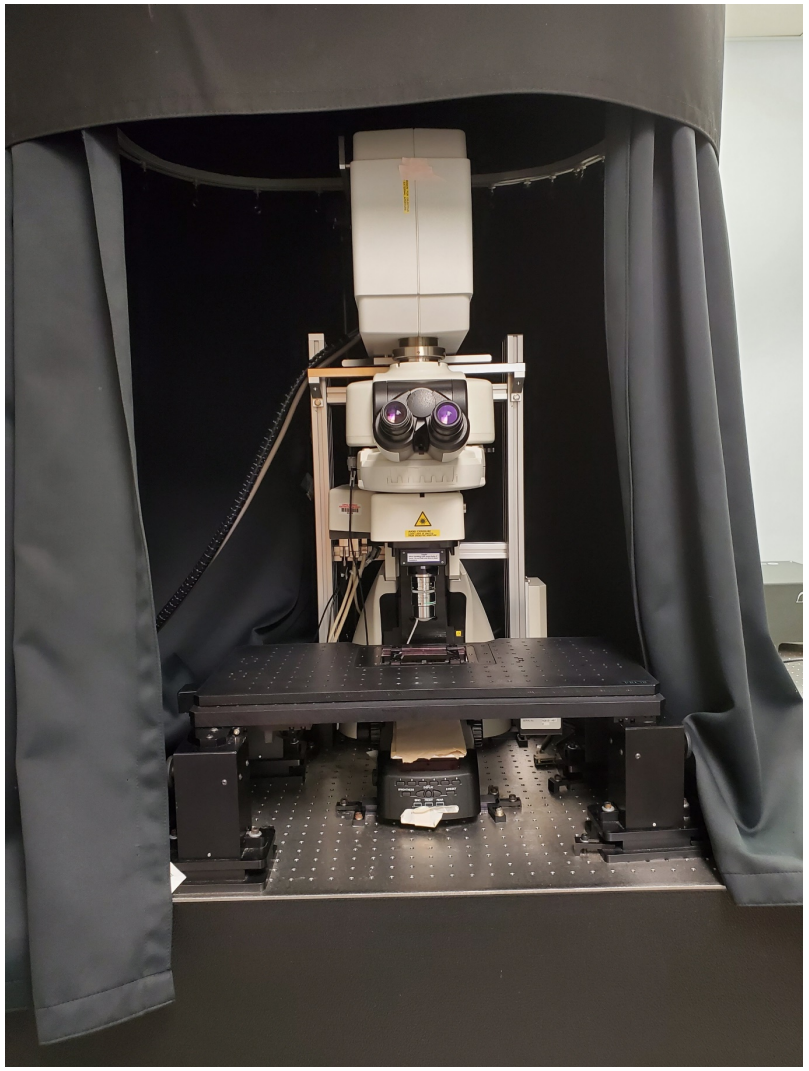
# Notice!

- **CAM Operations and COVID-19**
  - Please read the COVID instructions on our website and watch the video for disinfecting the microscope. https://cam.facilities.northwestern.edu/
  - Please remember to transfer the data to your server after imaging session. Local data will be deleted weekly and without warning.
  - Please remember that ALL users are responsible for cleaning the stage and ALL objectives before and after each use. Failure to properly clean can permanently damage or destroy the objectives.
  - For questions please call Peter (312-503-1823), David (312-503-3184), or Dina (312-503-7139).

# Nikon Multiphoton Microscope

# Before you begin

(Important)

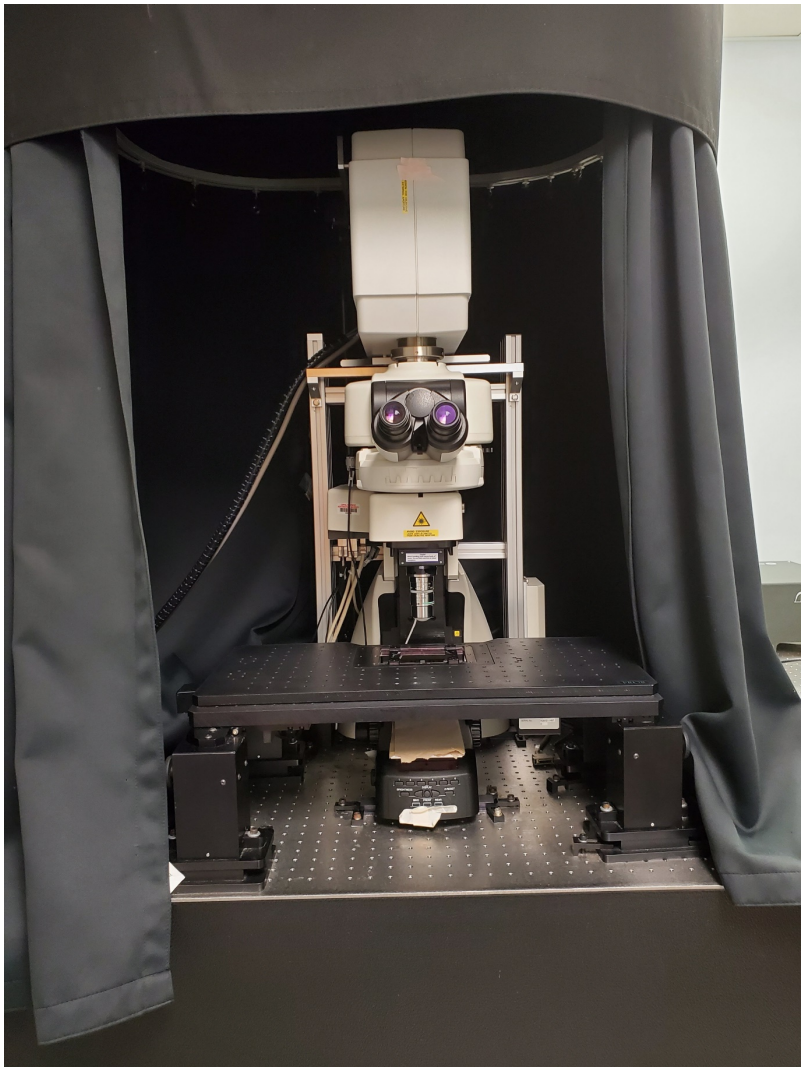
- Clean the microscope and working area according to the instructions on the desk
- Start and end your reservation in NUcore from your phone

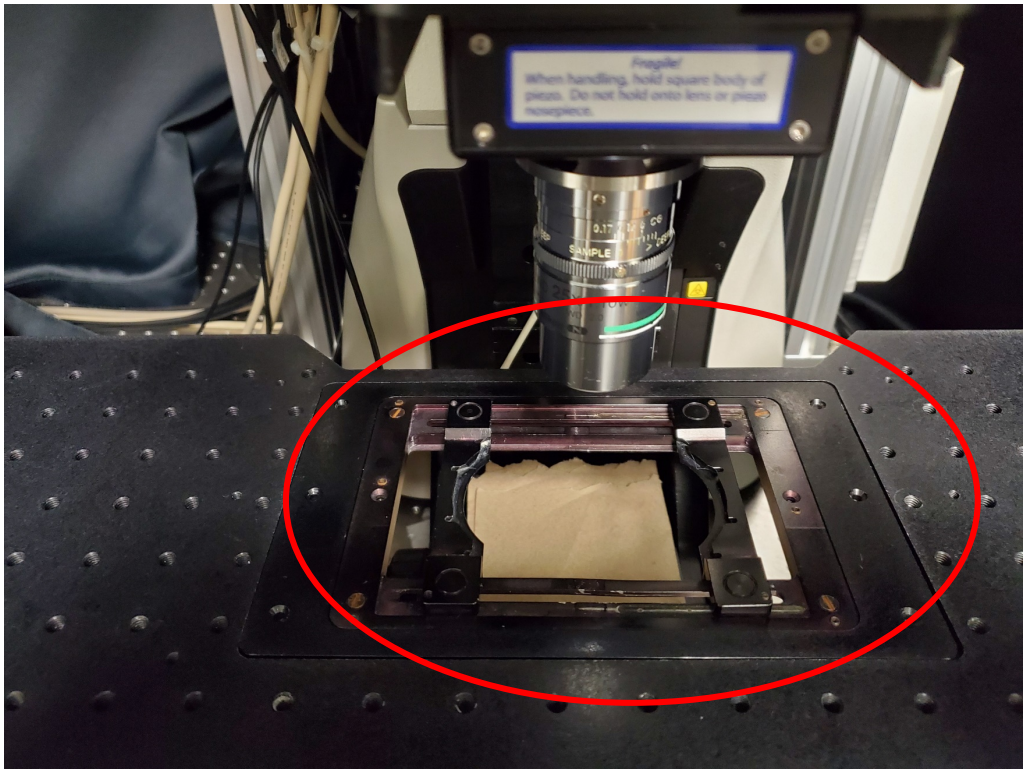
DISINFECT LAB EQUIPMENT BEFORE AND AFTER EACH USE.

CLEAN SURFACES

# Before starting the system:

Check the opening of the adapter and make sure the initialization movement of the objective (very expensive) will not hit the stage





# Powering on the A1 Multiphoton microscope

- Login to NUcore from your phone and begin your reservation
- Start the Multiphoton laser by turn the key clockwise
- Turn on the A1 controller
- Turn on microscope power from the power strip
- Turn on the computer
- Log into the Windows account – username and password are posted on the monitor
- Sign into FSMResFiles server
  - Username: fsm\(your NetID)
  - Password: (your NetID password)
- Log into NIS Elements with your NetID and password (Created during the training)
- If you are the laser user of the day you must completely turn off the instrument and the laser!
- ALL users must clean the objective!

# Multiphoton laser

Laser Key!

Turn it to “on” in the beginning
Turn it to “Standby” after imaging


Laser Status

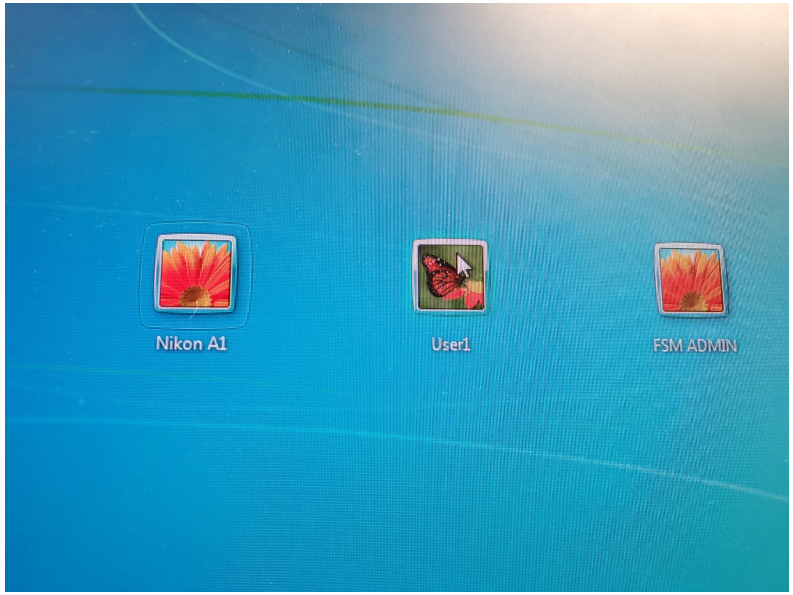
Wavelength can be adjusted by software

Status must be “OK” to use

# Power on the Microscope and control unit



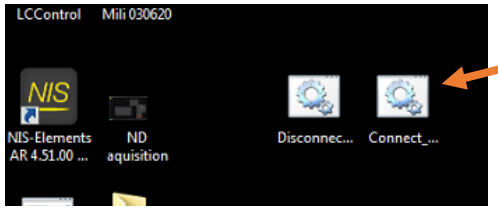
# MP computer and Login info


Password found on tape on monitor

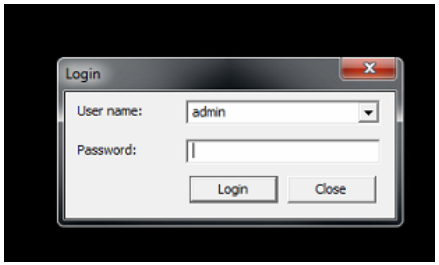
# Logging into computer and software

Open the NIS Elements software:




Connect and disconnect from FSMResFiles server

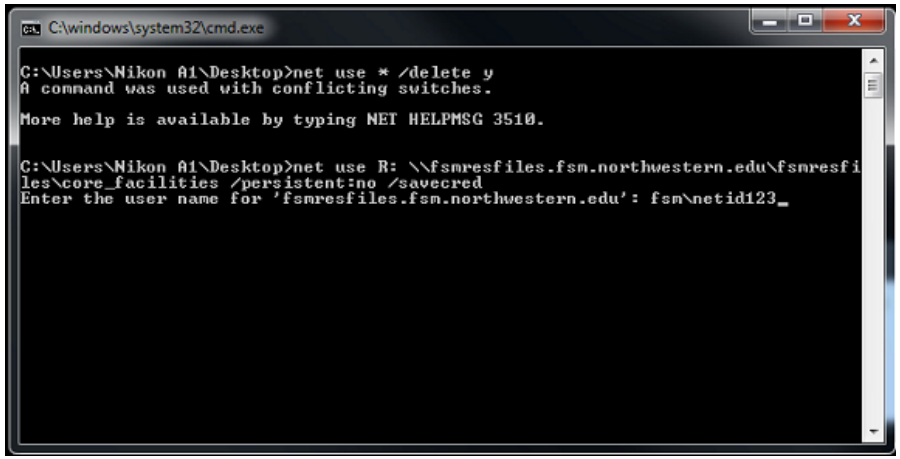
You will be prompted for your NIS Elements username (NetID) and password:
(Created during the training)



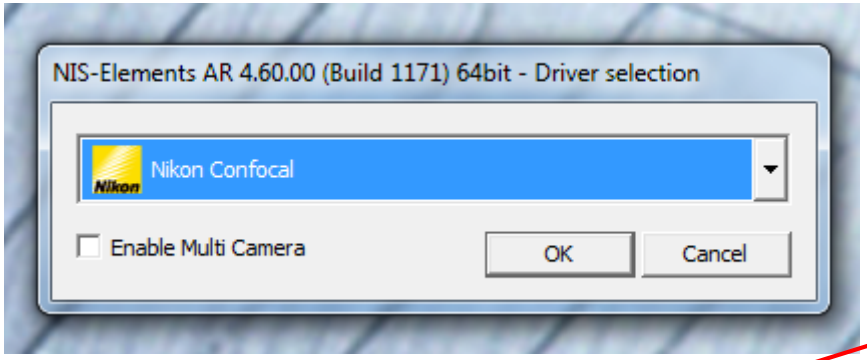

Run the 'Connect' script on the desktop to connect to the FSMResFiles server. The username is:

fsm\(your netid)

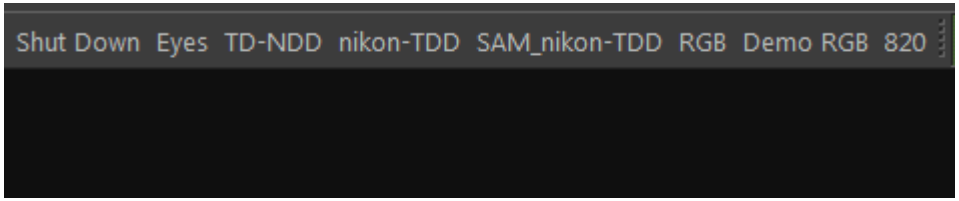
Press Enter. Then enter your password (you will not see any * while you type) and press Enter again.

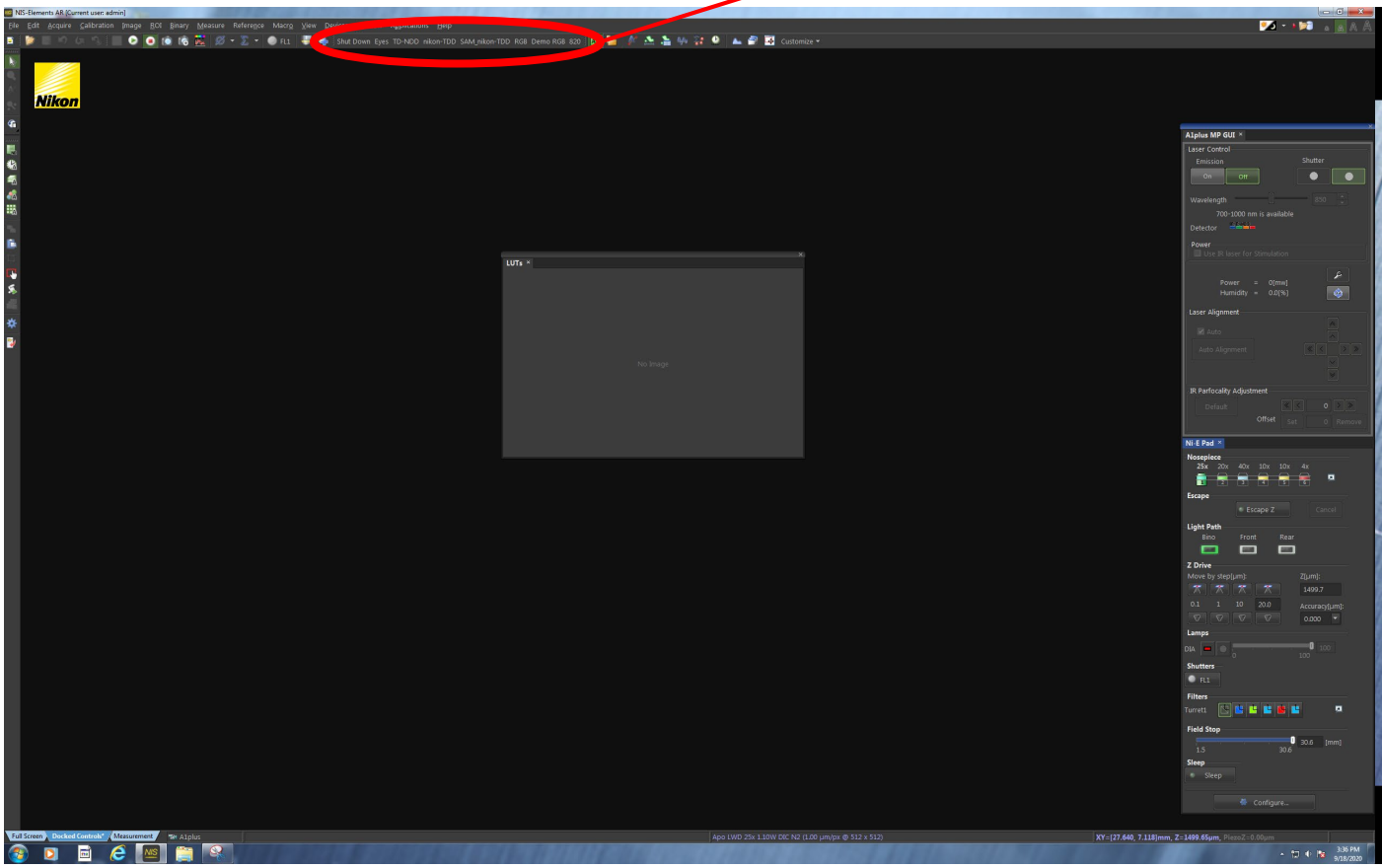


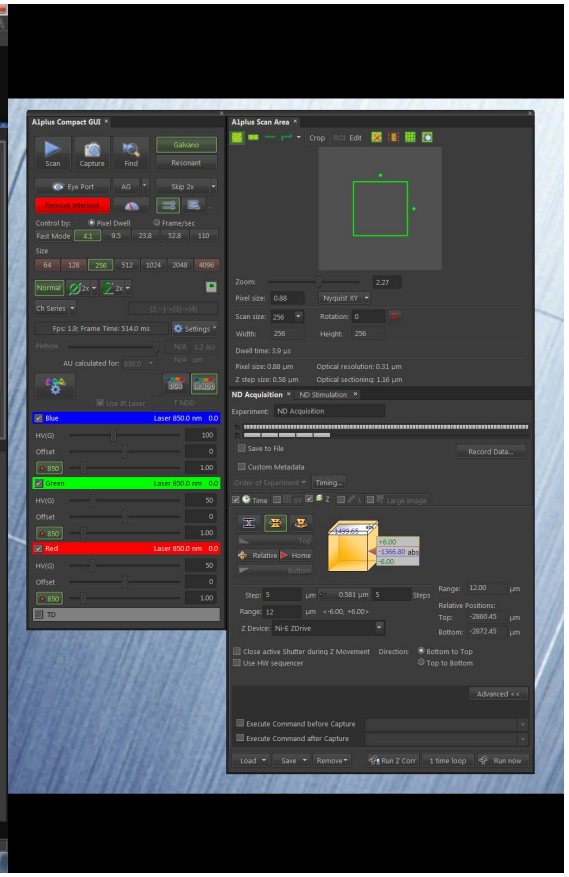
# Software GUI




CAM templates for imaging



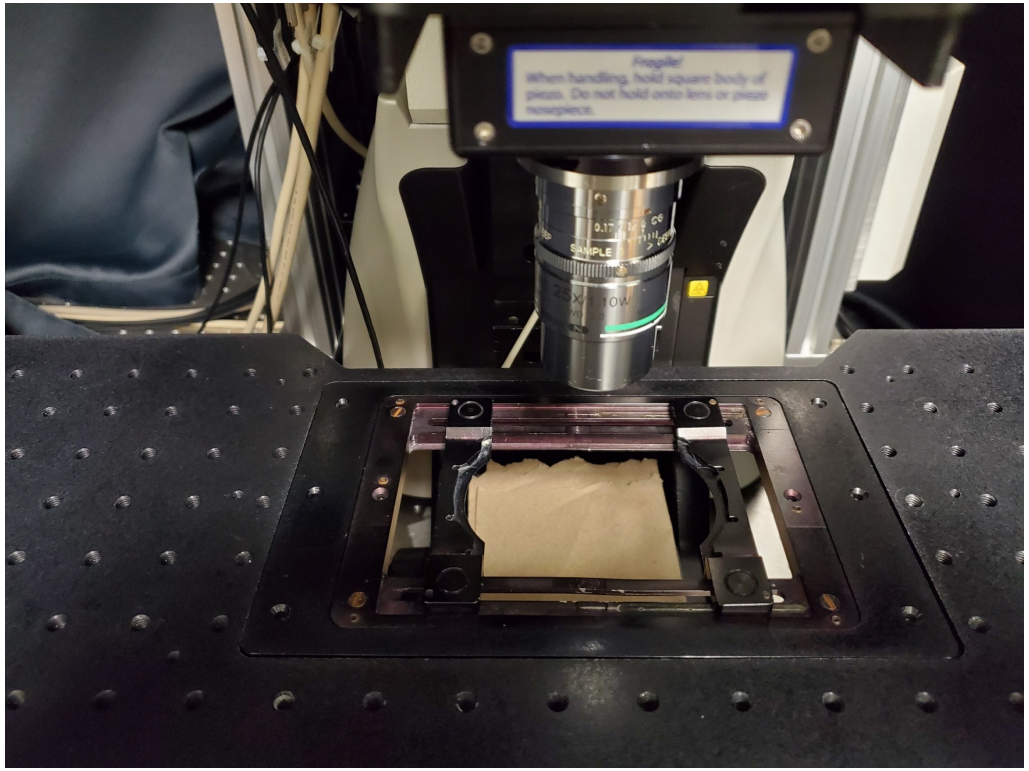





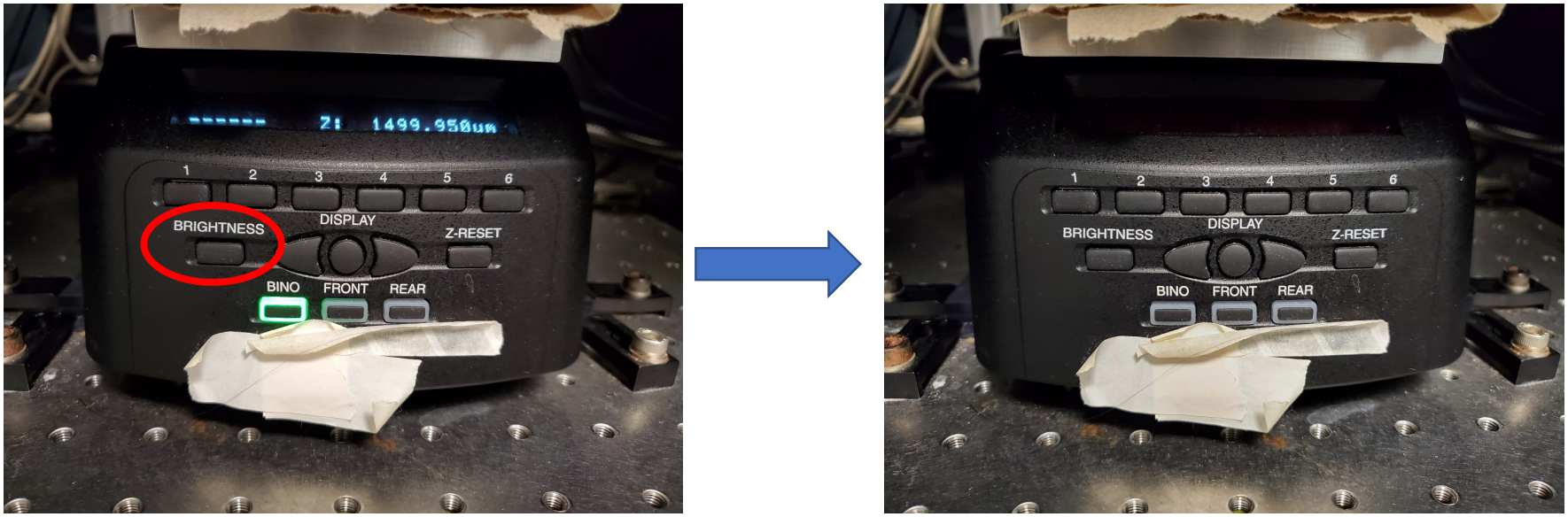
# Loading sample:

Step 1:
Lift the Z-deck to proper position
to objective and fix the position

Step 2:
a. Make sure a paper towel is underneath.
b. confirm water between the sample and objective
c. Lower the objective towards the sample

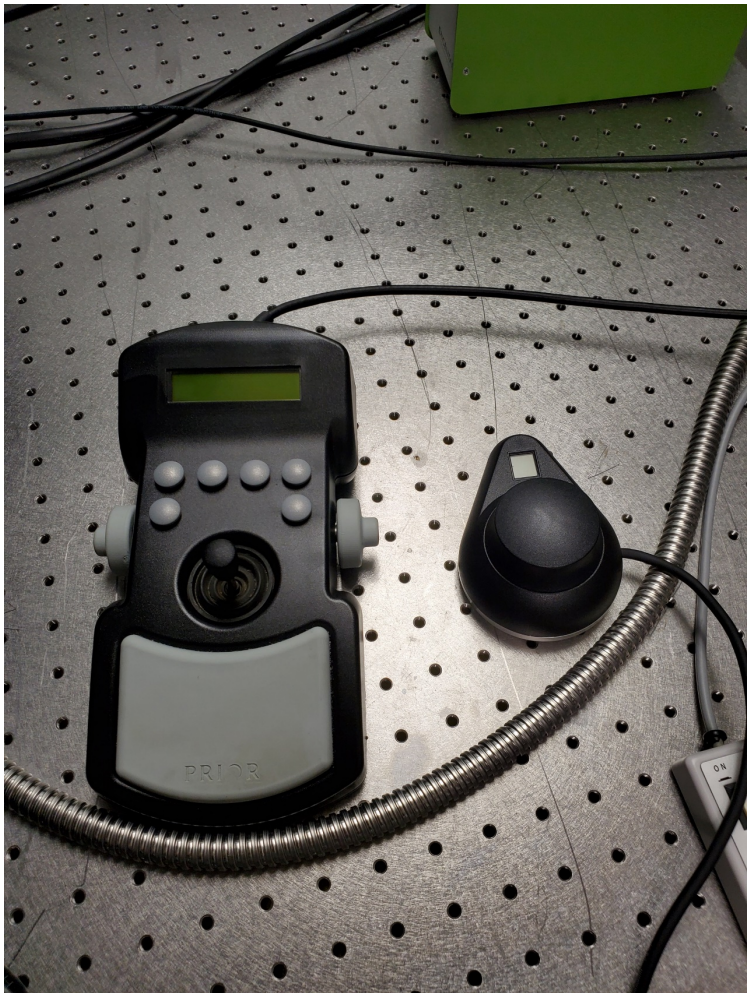


# Prepare the imaging

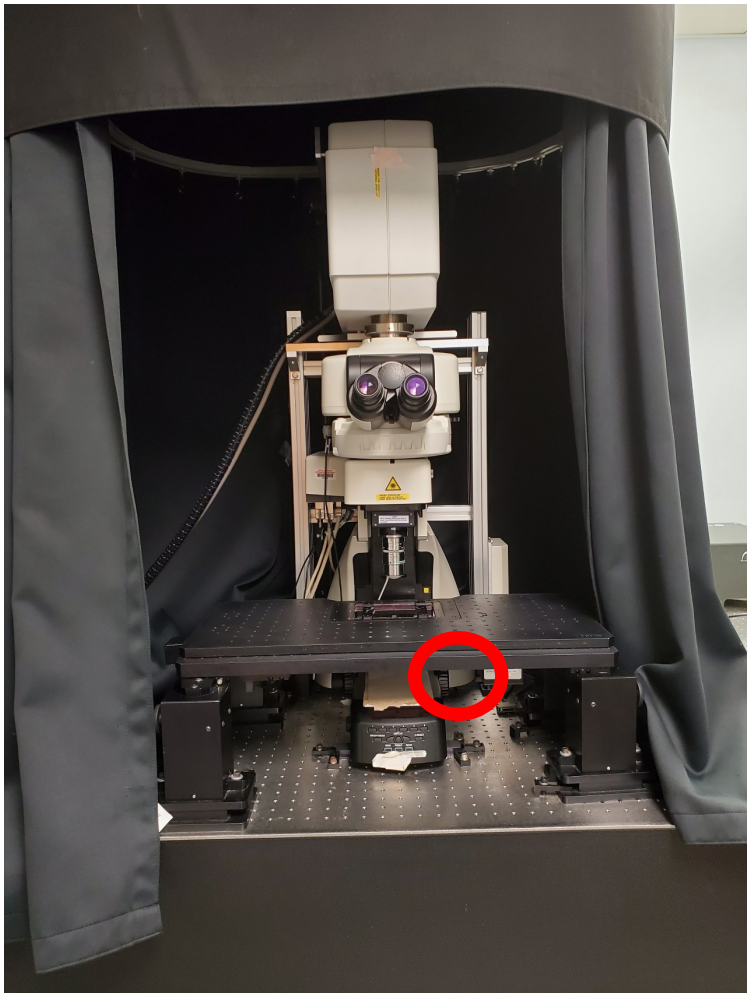



Press “Brightness” twice to turn off all the display and LED

# Move sample by x-y Joystick and z focus knob

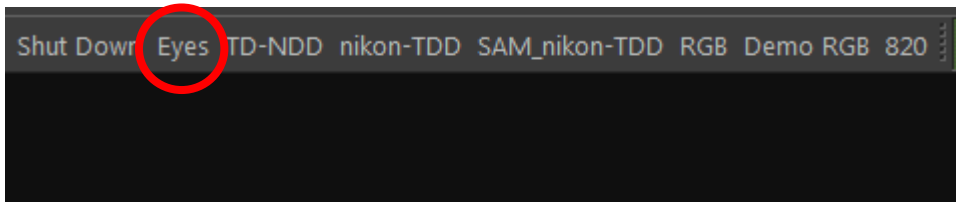



X-Y



Z

# Find the sample by Eye piece in epi-fluorescence




In software, goto “Eyes” mode

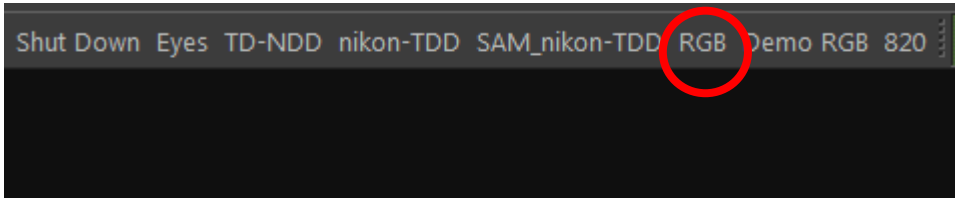
Press the large black knob to turn the LED on and off.
Turn the knob to change the LED intensity 1 – 100%

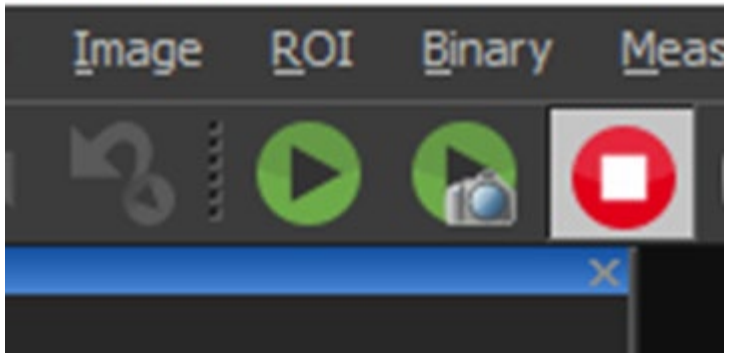
Off


On

# Close the curtain and room light before imaging

# Switch to Imaging mode: “RGB” or so



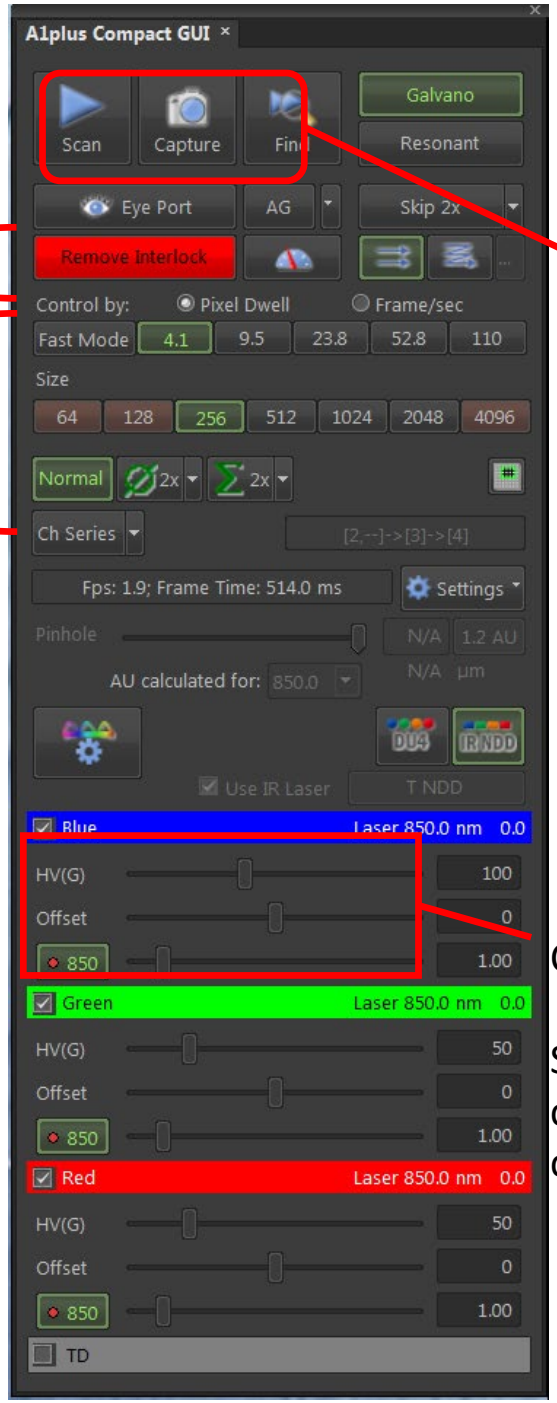

“Live view” and “Stop”


Check Laser mode and open the shutter

If needed, do an “auto Alignment”

**Eyeport**: Do not use. Use the EYES configuration to view the sample
**Remove interlock**: Press the red button to allow lasers to fire. Only allowed when microscope is not in Eyes position.

**Frame/sec settings**: Laser scan speed. Expressed as 1 frame per number of seconds to collect an image. 1/2 is 16x faster than 1/32.
**Size**: Number of pixels that comprise each image
**Normal/Avg/Sum**: The user can collect a frame multiple times to improve SNR
**Ch Series**: Collecting each laser channel (up to 4) in series rather than parallel. Will take up to 4x as long, but will avoid fluorescent crosstalk between channels. Should be activated.



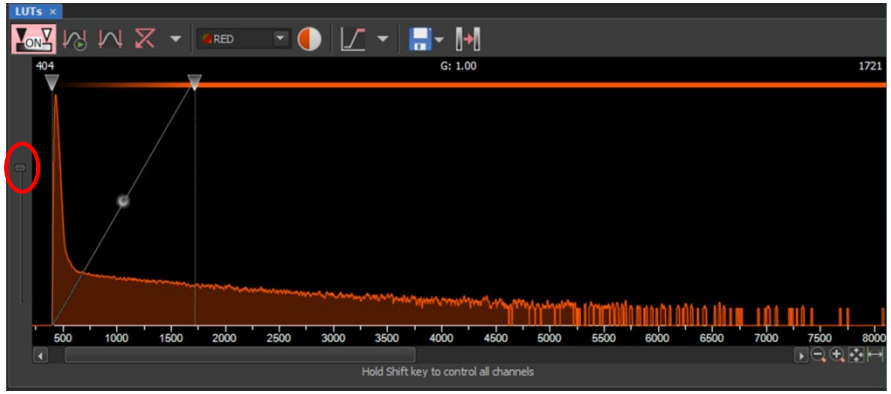

**Scan**: fast imaging, every collected frame overwrites themselves in Live window
**Capture**: Single frame capture in a separate image window, will not overwrite
**Find**: fastest imaging, single color only, low res scan in Live window

Collection settings for each channel:

Set collection parameters for each channel (you can deselect unused channels). Save the optical configuration to preserve the settings

# software GUI

Move slider to top

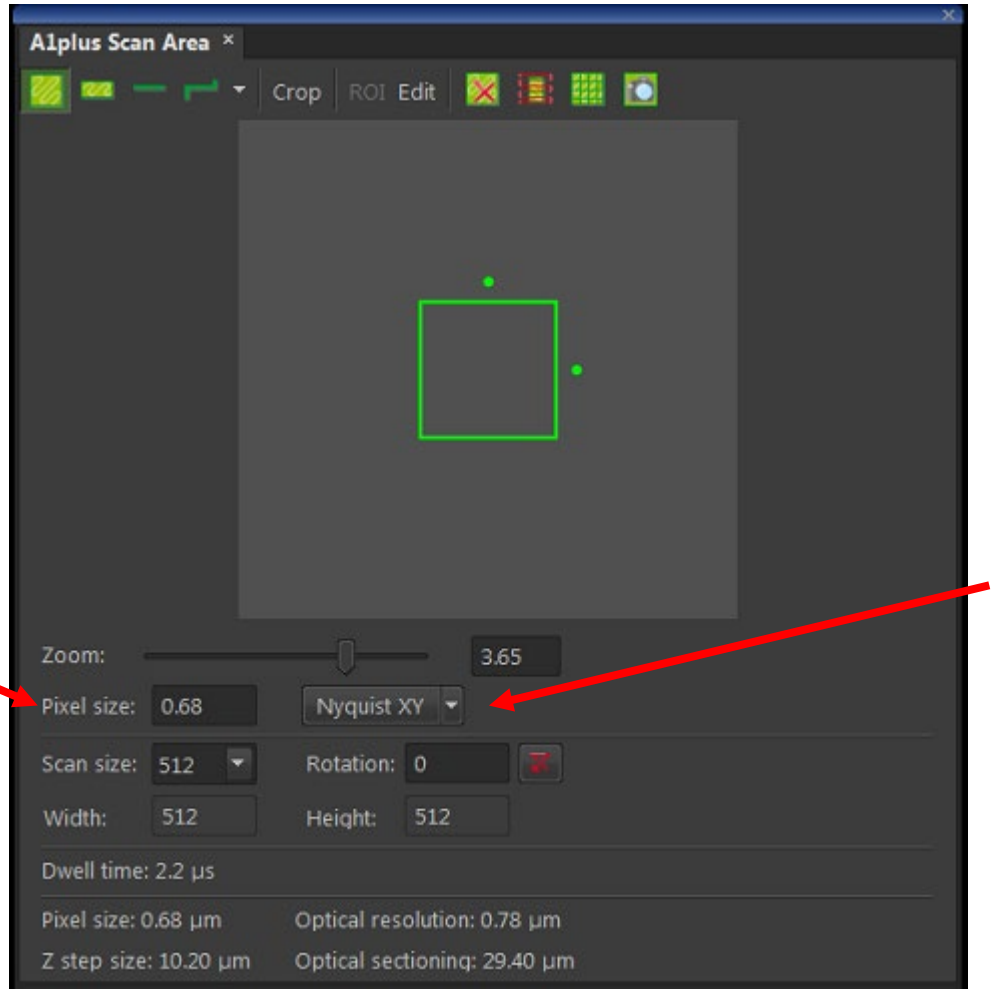



Weak Saturated

Standard: Good S/N, no saturation, not too weak

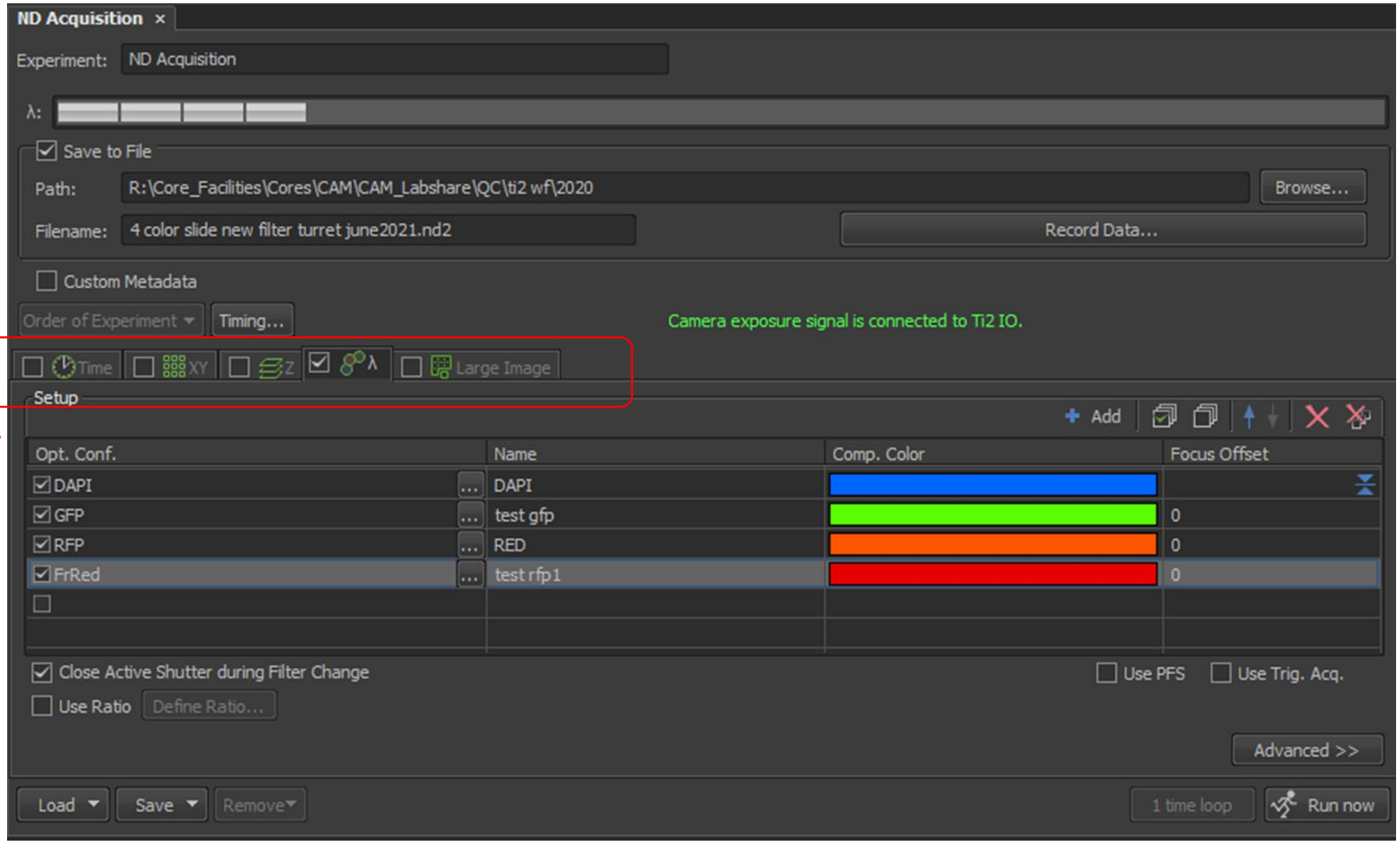
Adjust: Laser power, HV gain, exposure time.

The pixel size is continuously adjustable. The resolution of the image is determined by the Numerical Aperture of the objective and the pixel size in the recorded image.




Nyquist XY:
Sets the pixel size less than half the resolution limit of the objective. This allows the image to record objects that are the size of the resolution limit.

# ND Acquisition




Data acquired based on selection here, can be combined.

Time: Movie or complex time series

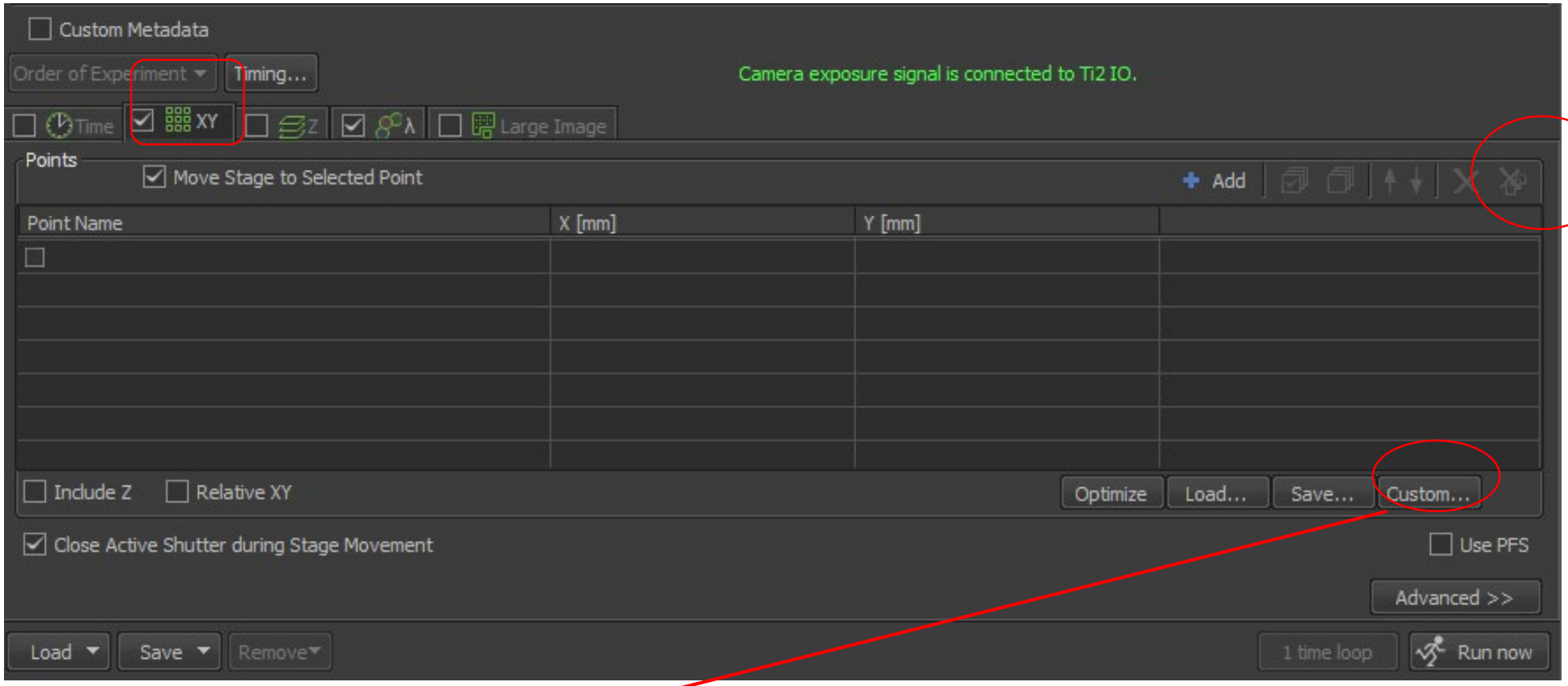
XY: Large image, multi-position images or more

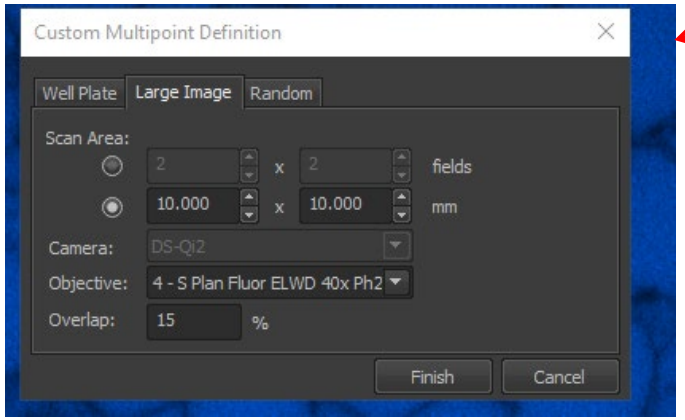
Z: 3D

Lambda: Multi-Channel images

# Using “XY” for large images

Clear first

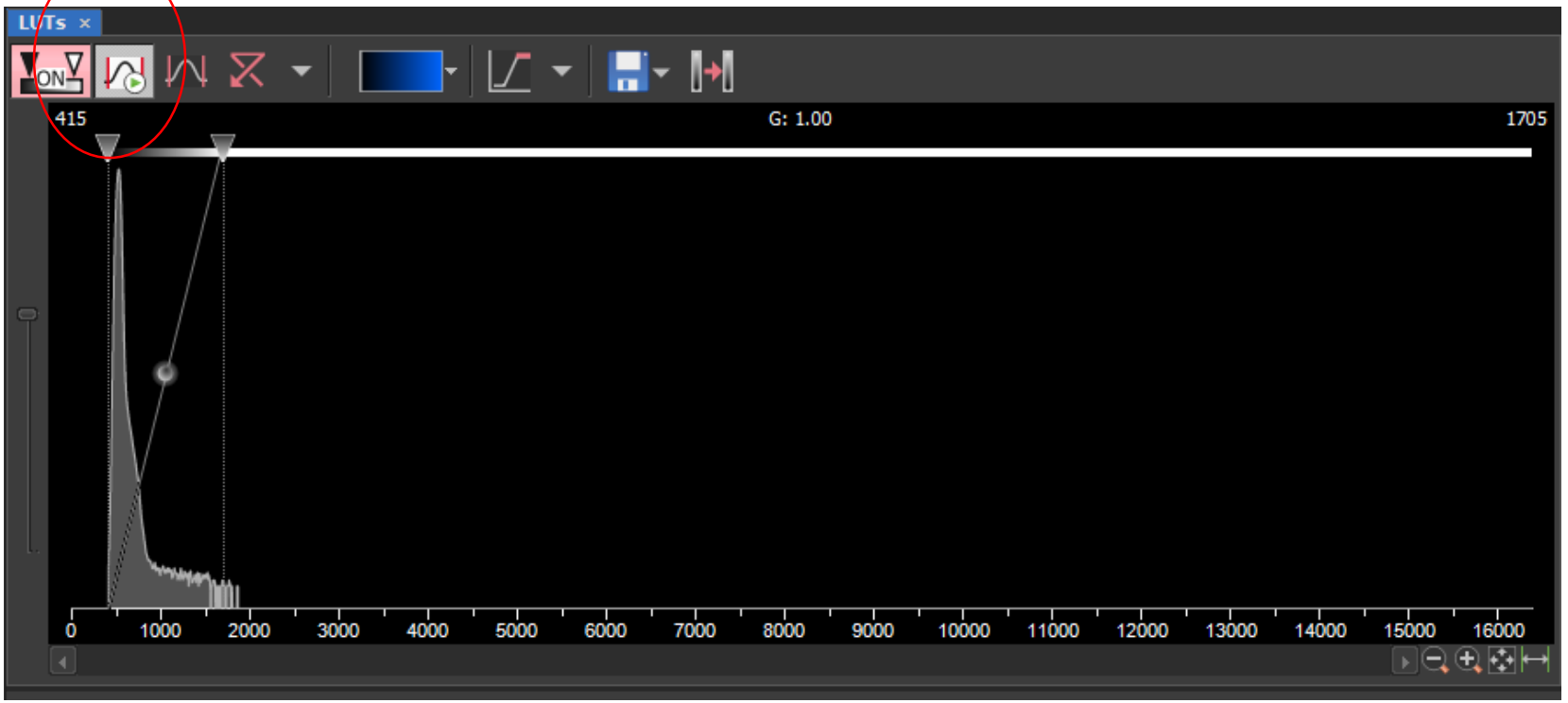



1. Scan area
2. Objective!

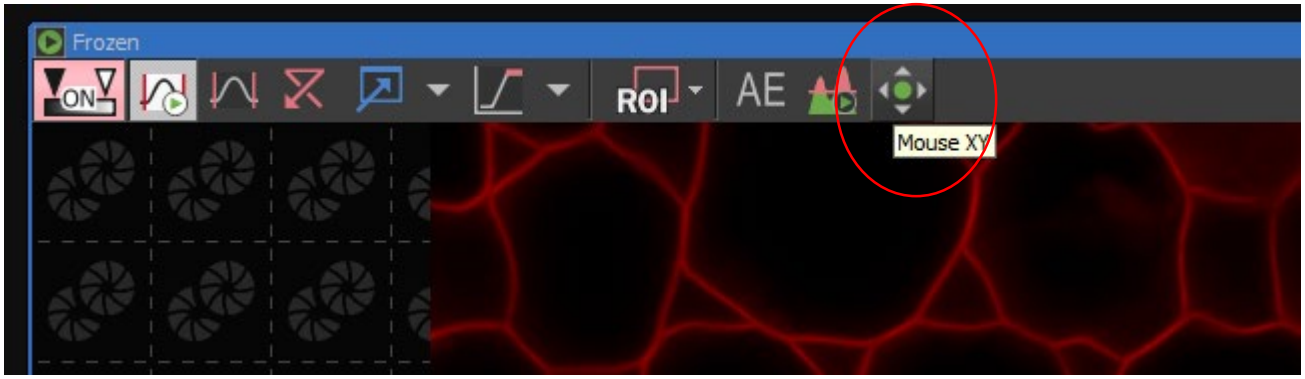
# Tips:

Living autoscale for preview



# Tips:

Use mouse to move sample in XY




Mouse right click the image
for specific position or others