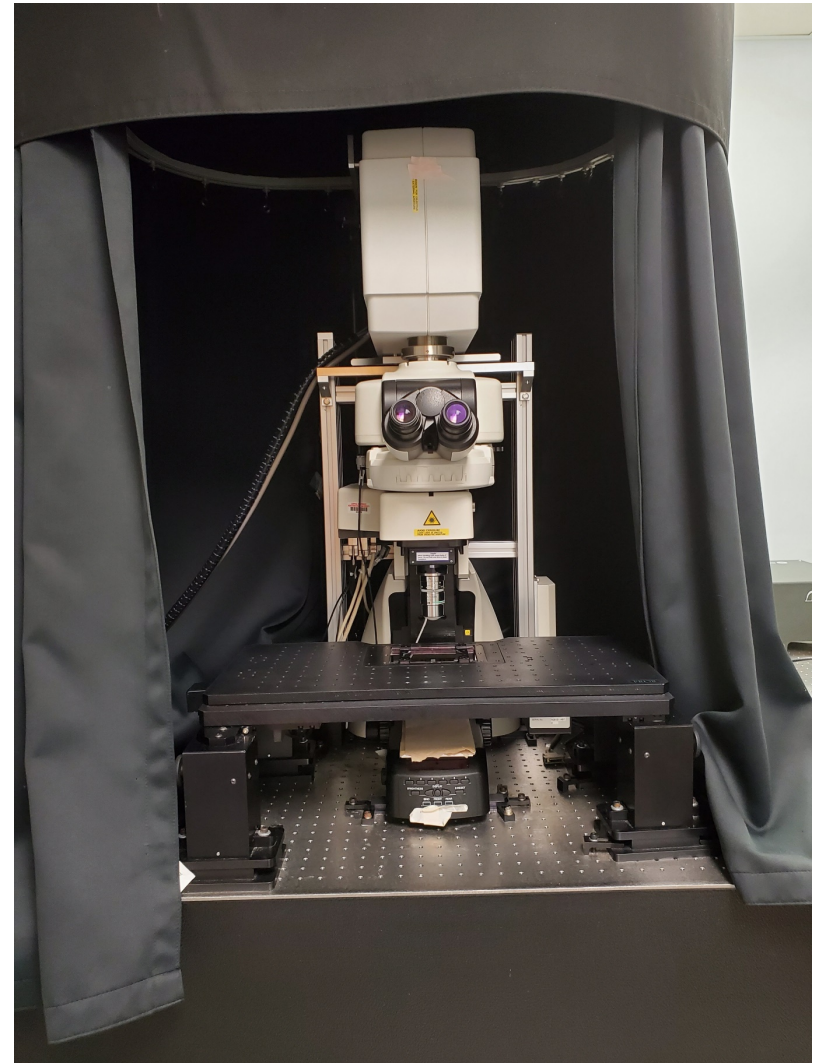


# Notice!

- **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



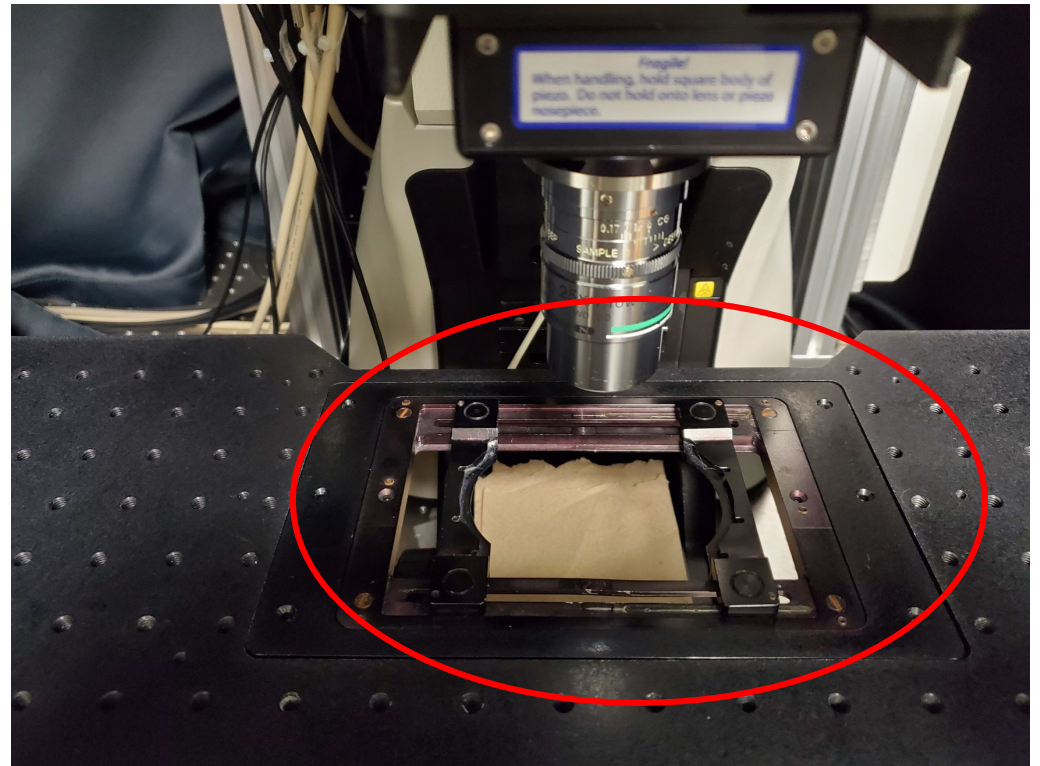
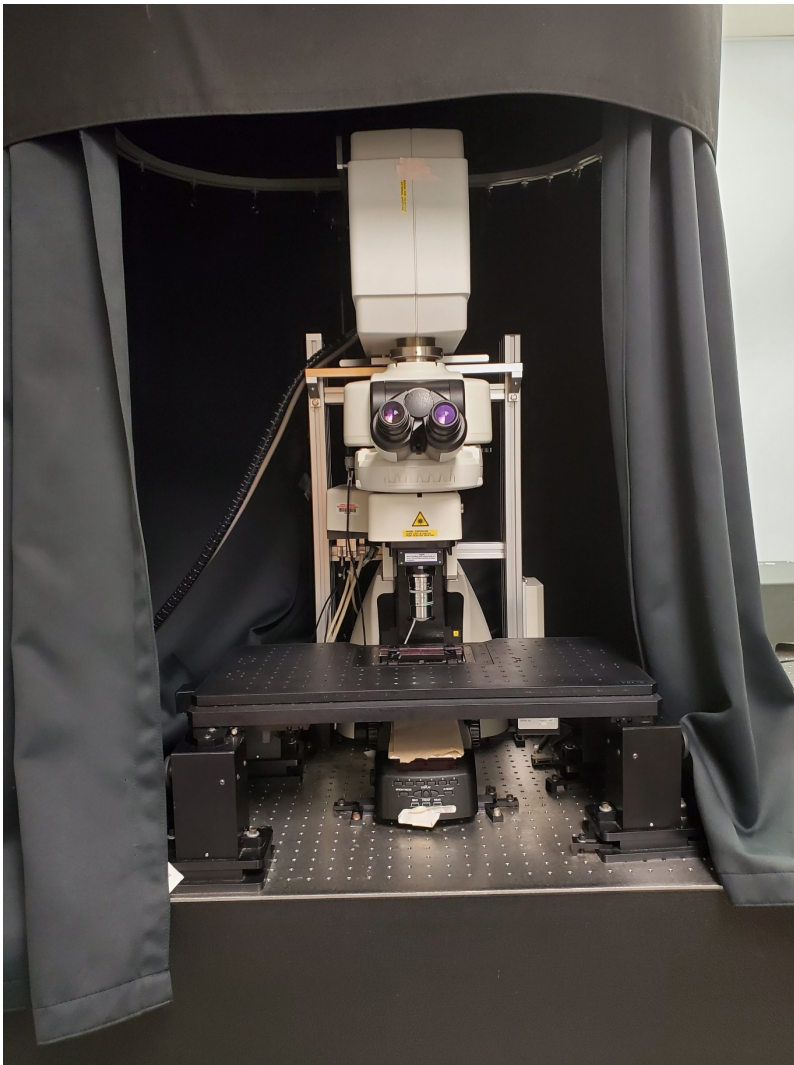
**DISINFECT  
LAB EQUIPMENT  
BEFORE AND AFTER  
EACH USE.**



**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

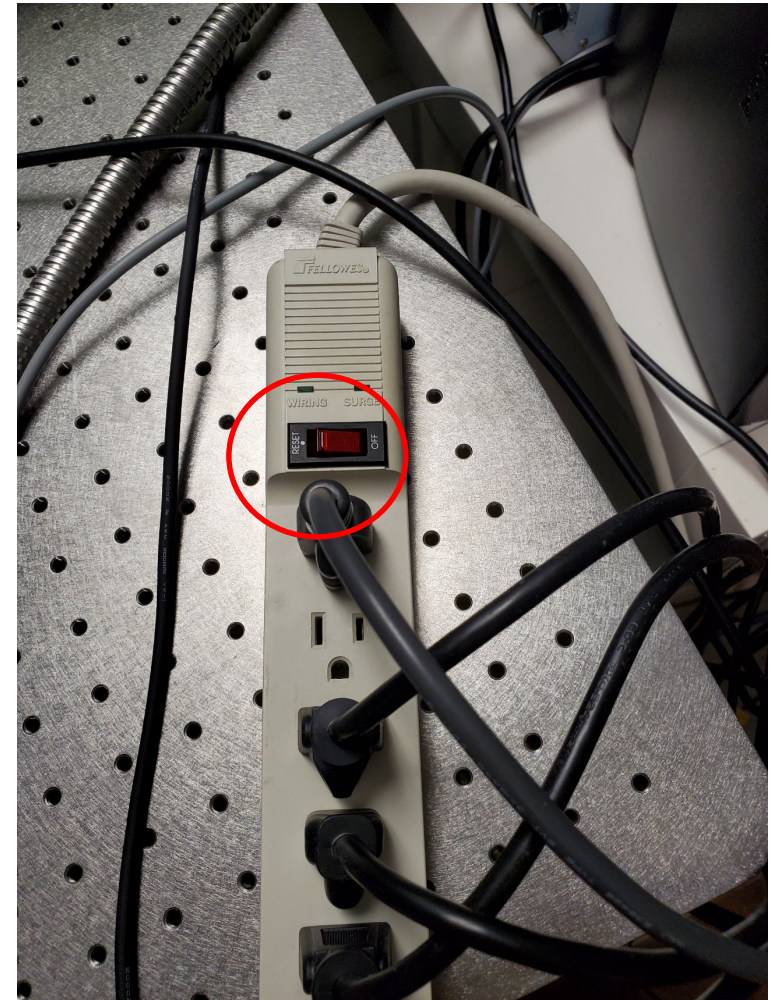


Laser Status

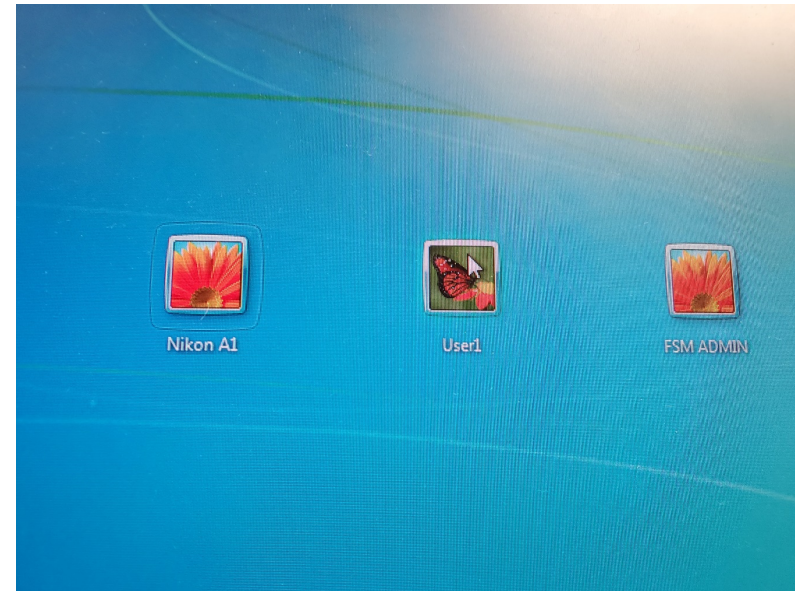
Wavelength can be adjusted by software

Status must be "OK" to use

# Power on the Microscope and control unit



# MP computer and Login info

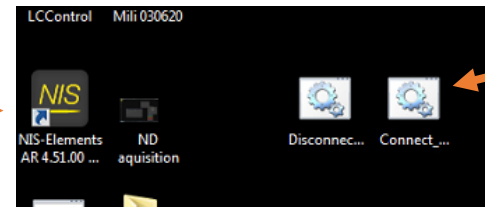


Password found on tape on monitor



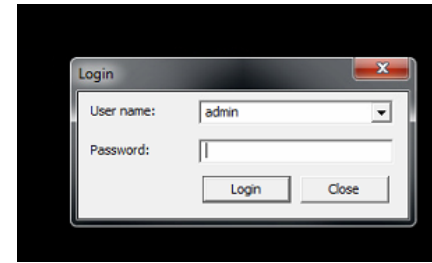
# Logging into computer and software

Open the NIS Elements software:



Connect and disconnect from FSMResFiles server

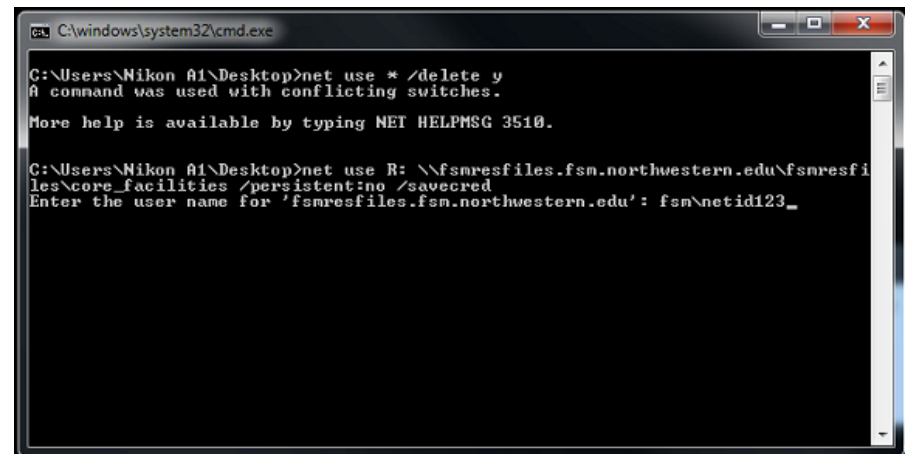
You will be prompted for your NIS Elements username (NetID) and password:  
(Created during the training)



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

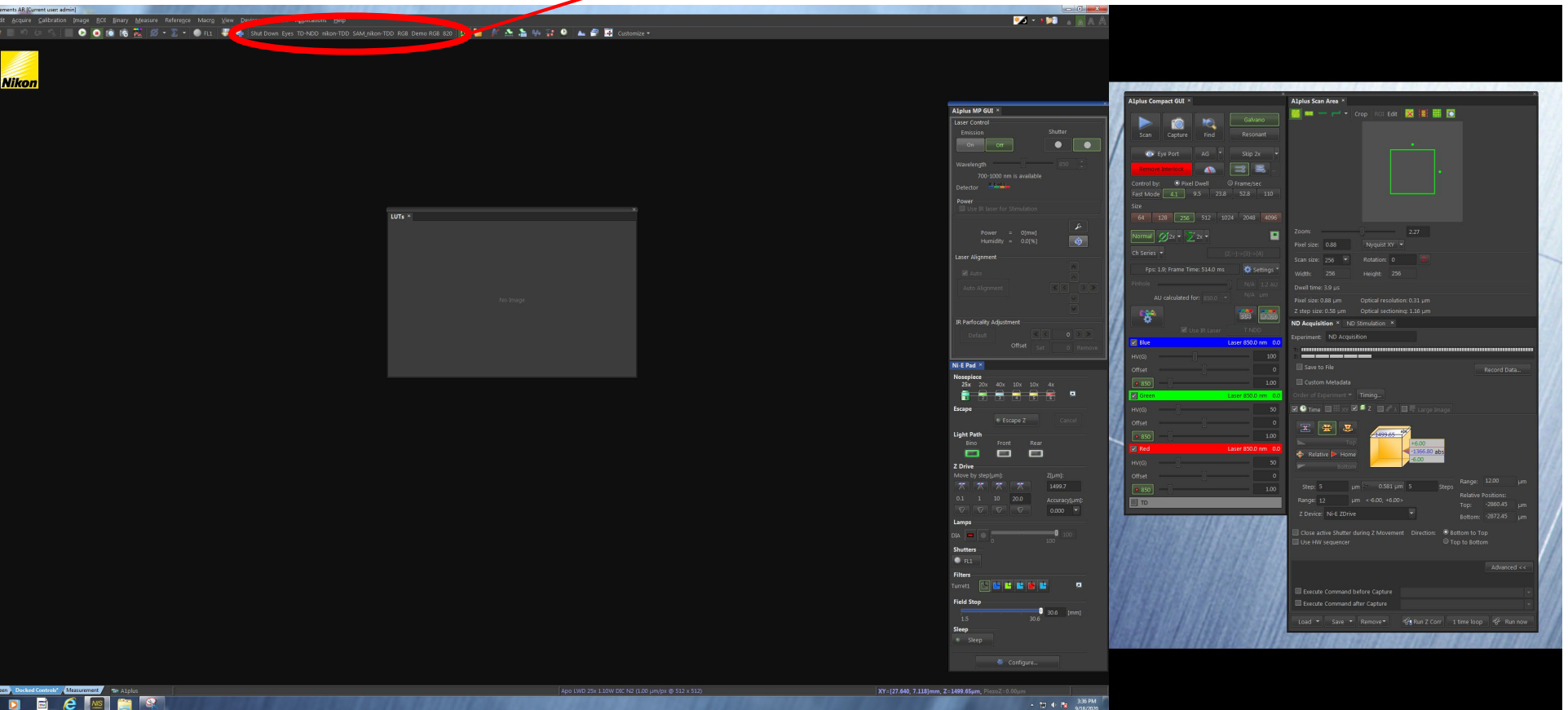
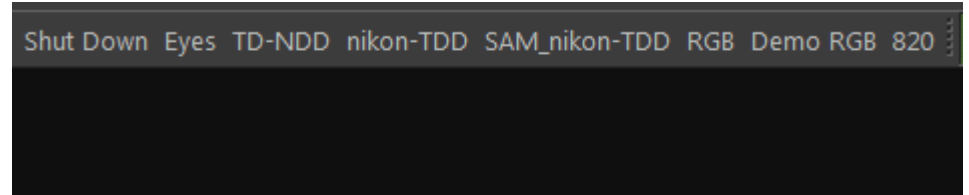
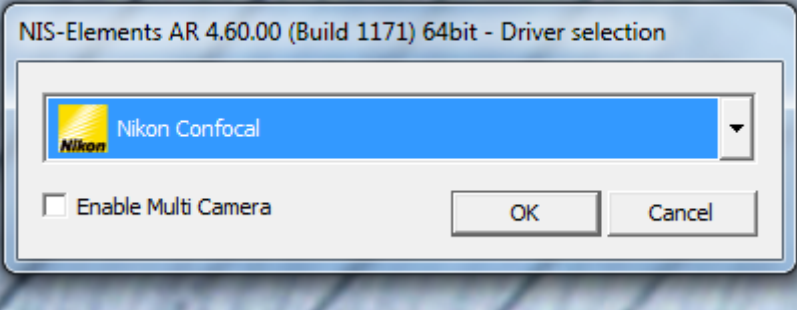
fsm\  
(your netid)

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:

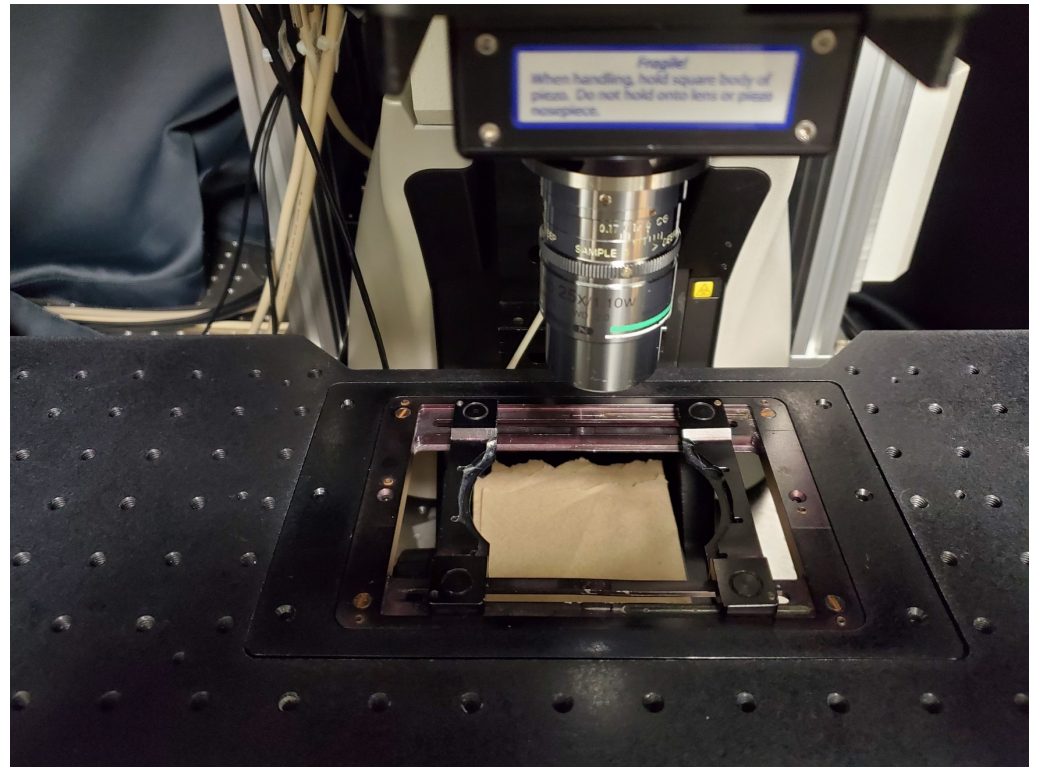
## Step 1:

Lift the Z-deck to proper position to objective and fix the position

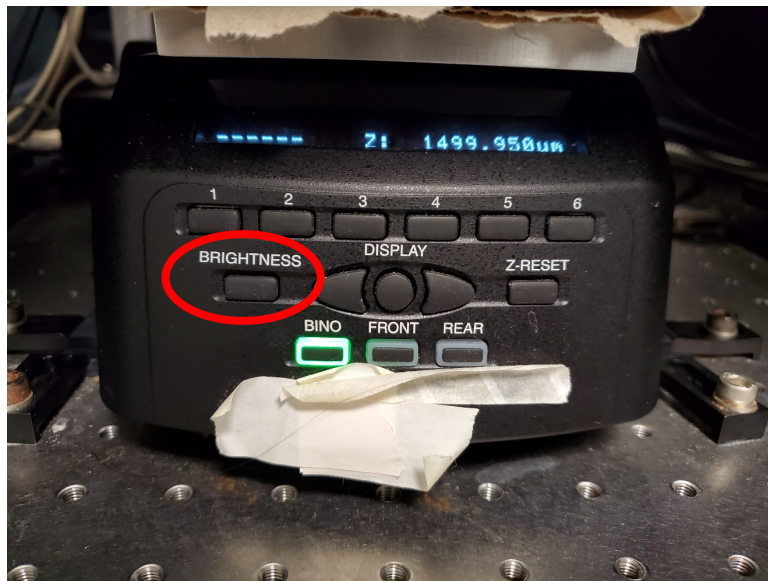


## Step 2:

- Make sure a paper towel is underneath.
- confirm water between the sample and objective
- Lower the objective towards the sample



# Prepare the imaging

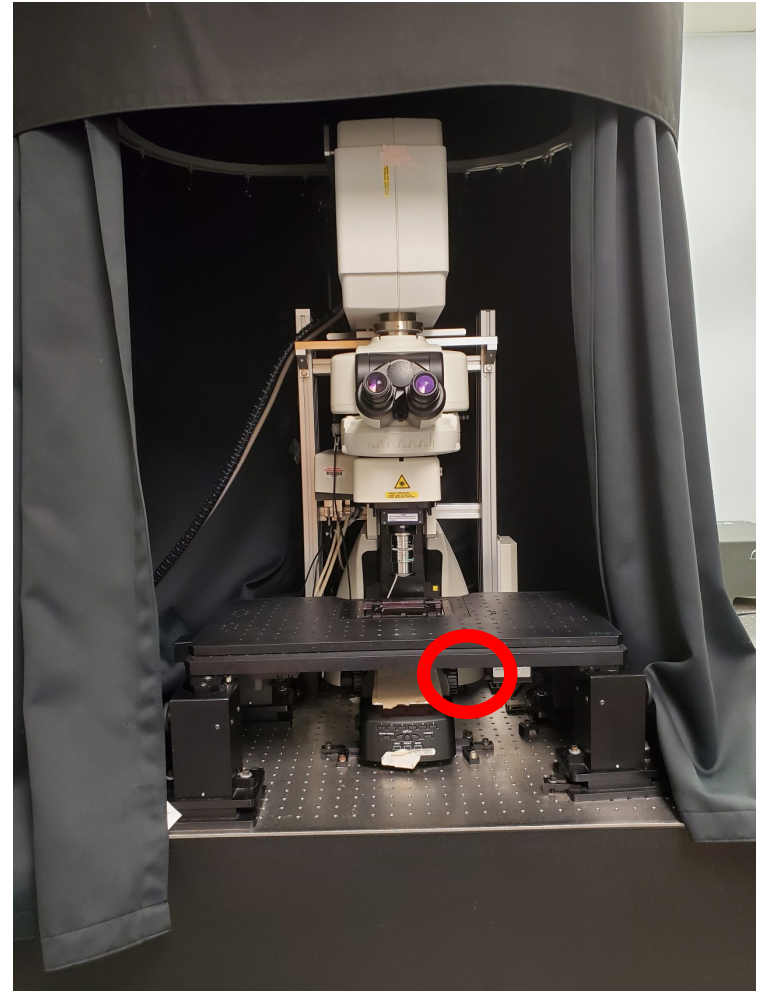


Press "Brightness" twice to turn off all the display and LED

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

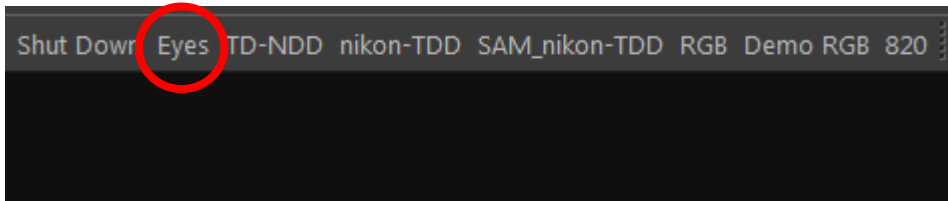


X-Y



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



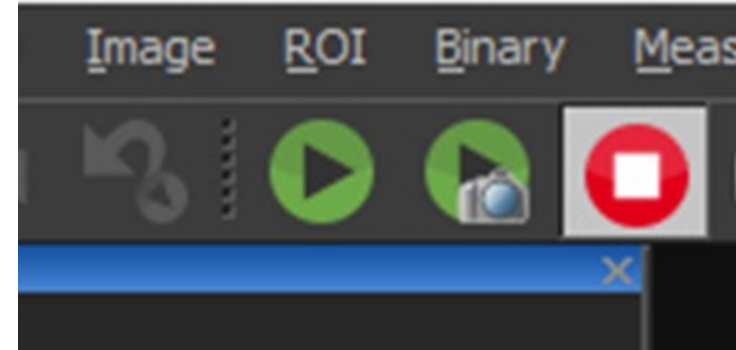
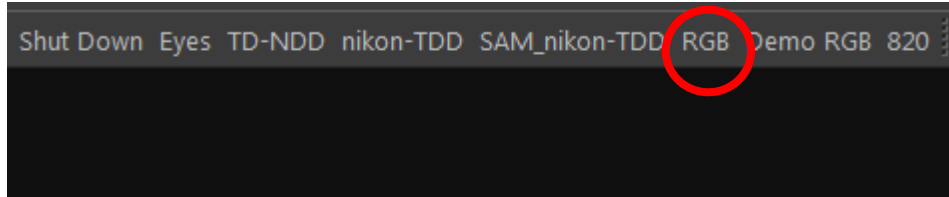
On



Close the curtain and room light before imaging

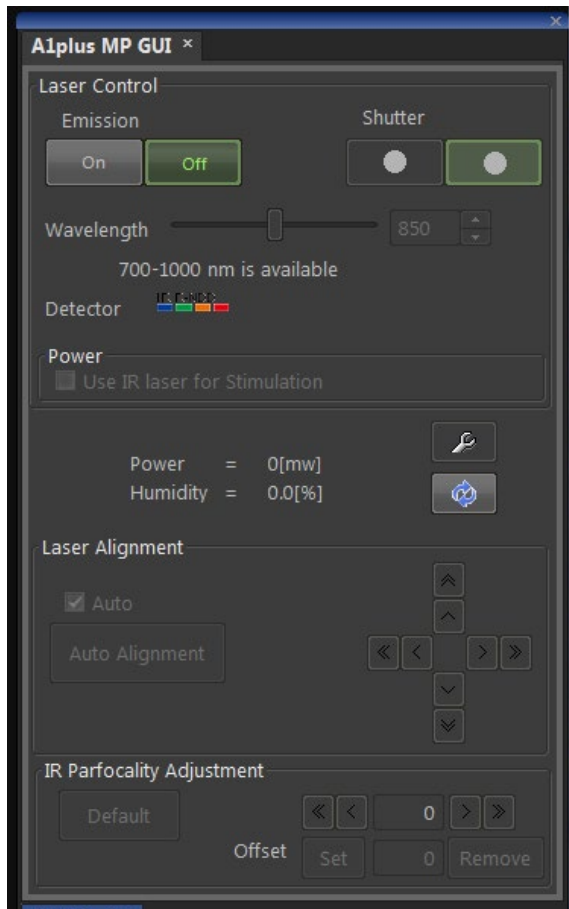


# Switch to Imaging mode: “RGB” or so



“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

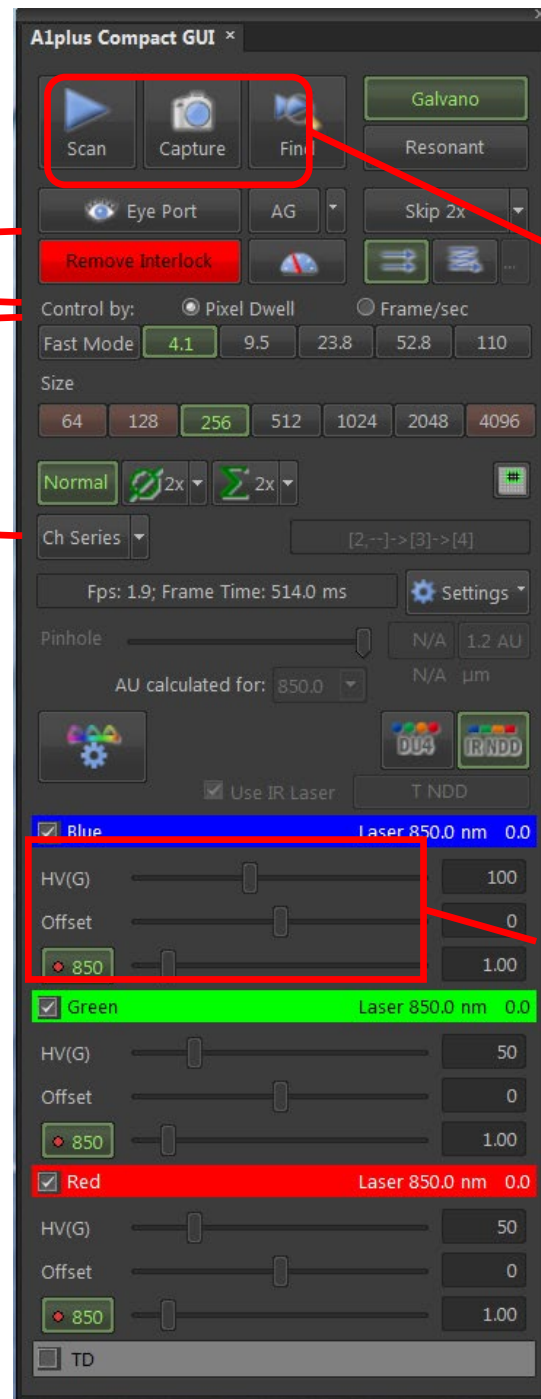
**Remove interlock:** Press the red button to allow lasers to fire. Only allowed when microscope is not in Eyes position.

**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

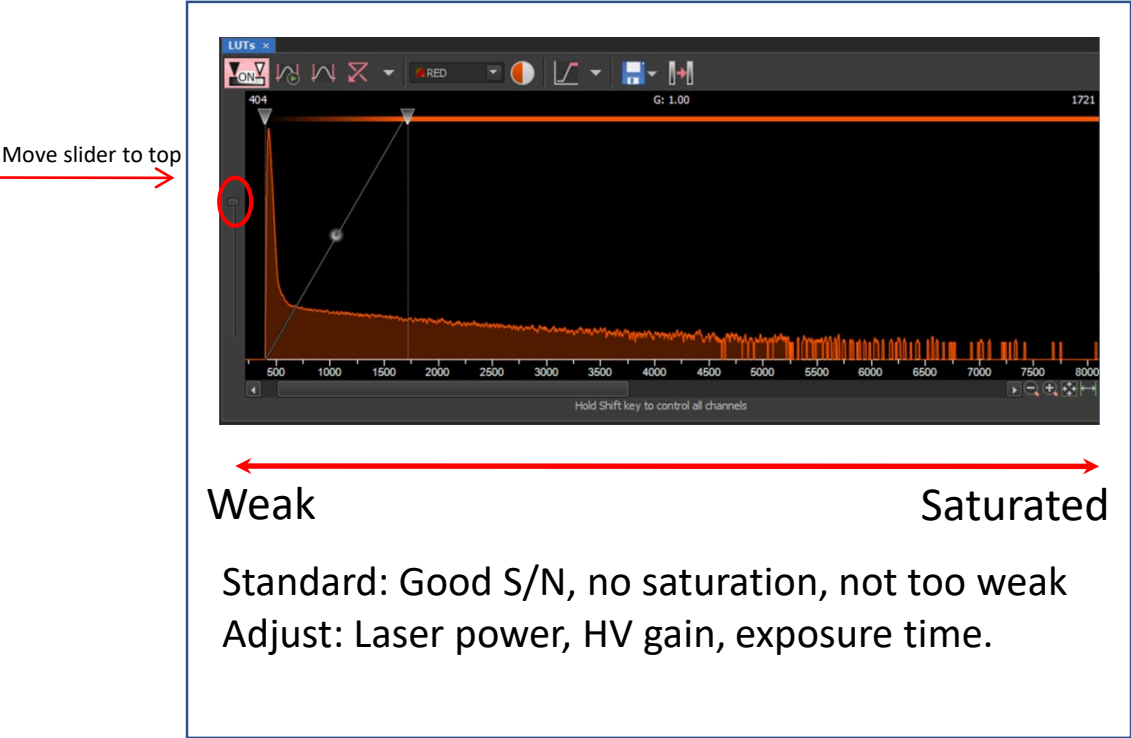
**Capture:** Single frame capture in a separate image window, will not overwrite

**Find:** fastest imaging, single color only, low res scan in Live window

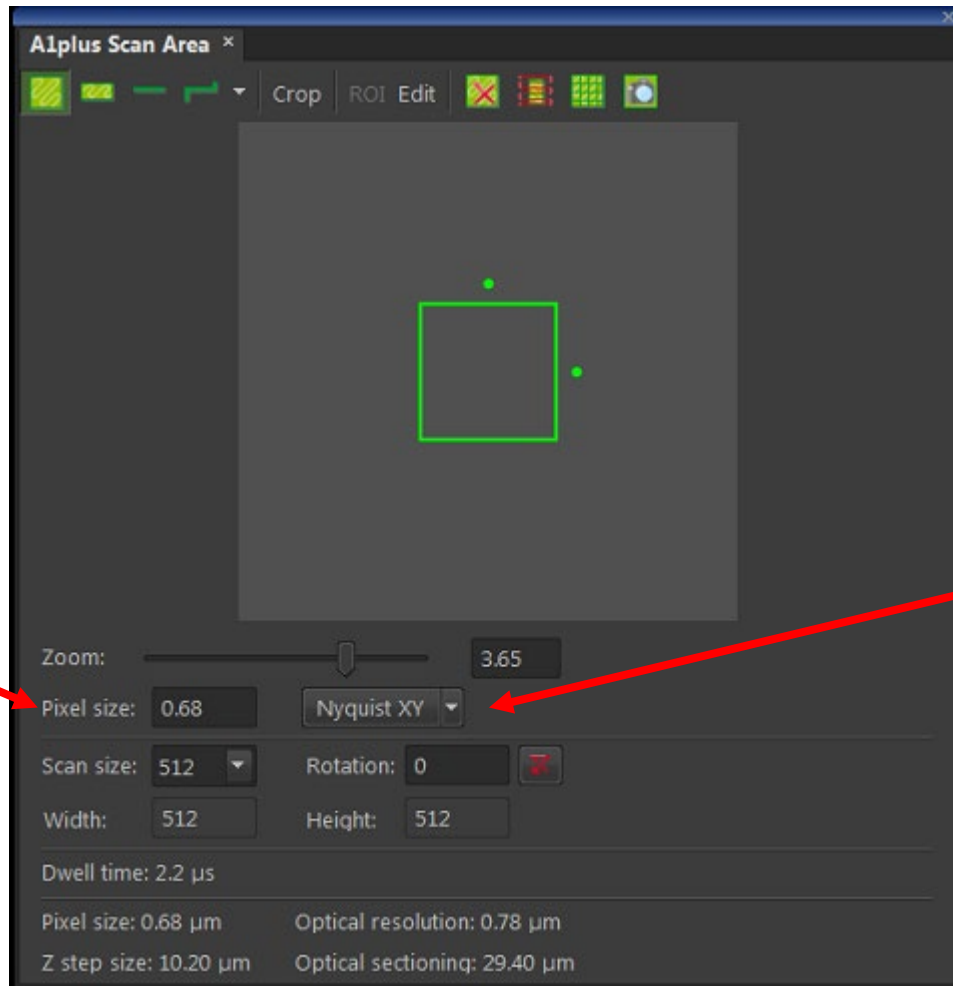
Collection settings for each channel:

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

# software GUI



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

Experiment: ND Acquisition

λ: [Progress Bar]

Save to File

Path: R:\Core\_Facilities\Cores\CAM\CAM\_Labshare\QC\bi2 wf\2020 [Browse...]

Filename: 4 color slide new filter turret june2021.nd2 [Record Data...]

Custom Metadata

Order of Experiment [Timing...]

Camera exposure signal is connected to Ti2 IO.

Time  XY  Z   $\lambda$   Large Image

Setup

Opt. Conf.	Name	Comp. Color	Focus Offset
<input checked="" type="checkbox"/> DAPI	DAPI	[Blue Bar]	
<input checked="" type="checkbox"/> GFP	test gfp	[Green Bar]	0
<input checked="" type="checkbox"/> RFP	RED	[Orange Bar]	0
<input checked="" type="checkbox"/> FrRed	test rfp1	[Red Bar]	0

Close Active Shutter during Filter Change  Use PFS  Use Trig. Acq.

Use Ratio [Define Ratio...]

[Load] [Save] [Remove] [Advanced >>] [1 time loop] [Run now]

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

The screenshot shows the main software interface. At the top, there is a status bar with a green message: "Camera exposure signal is connected to Ti2 IO." Below this, the "Order of Experiment" dropdown is set to "XY", which is highlighted with a red box. Other acquisition modes like "Time", "Z", and "Large Image" are also visible. The "Points" table is empty. At the bottom right, the "Custom..." button is circled in red, with a red arrow pointing to the "Custom Multipoint Definition" dialog box shown below.

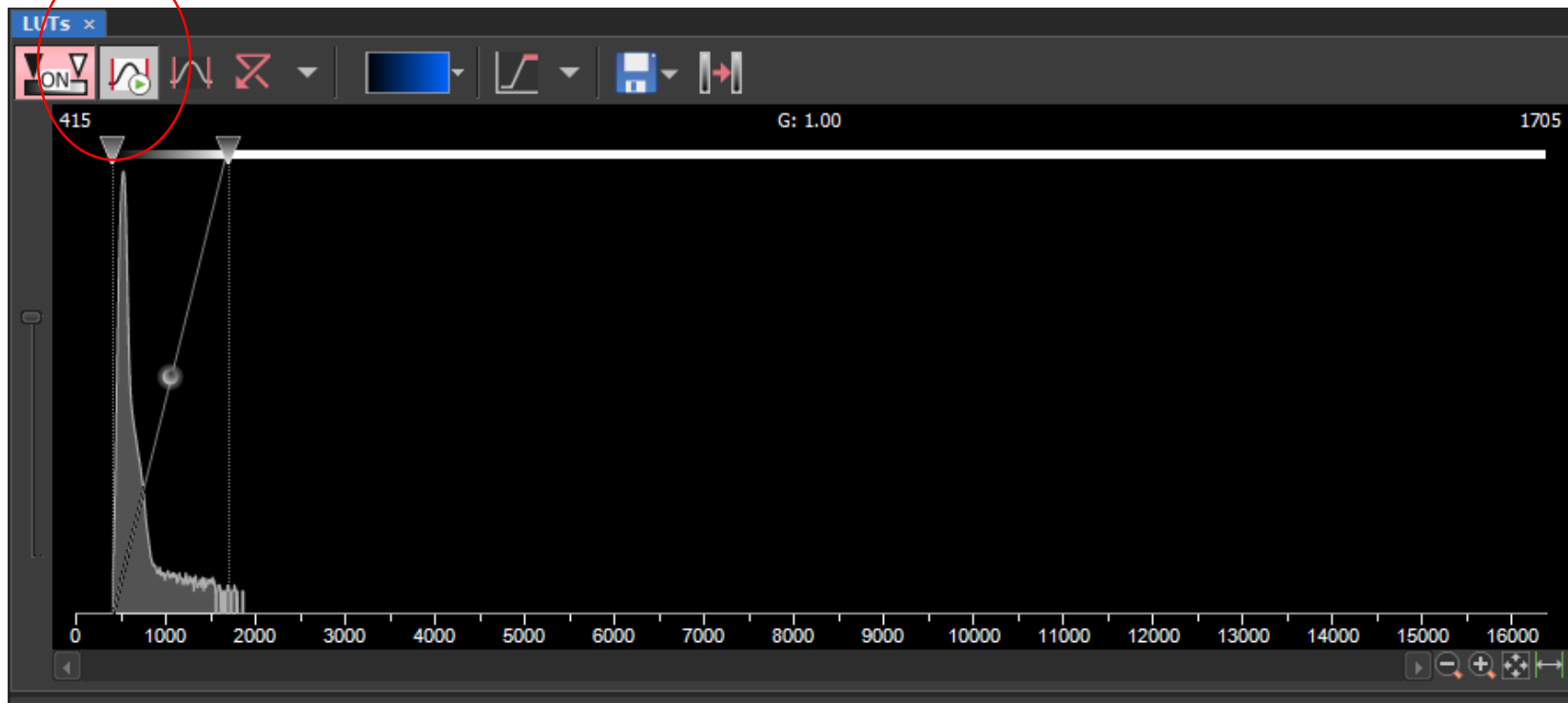
Point Name	X [mm]	Y [mm]

The "Custom Multipoint Definition" dialog box is shown. It has three tabs: "Well Plate", "Large Image", and "Random". The "Large Image" tab is selected. The "Scan Area" section has two radio buttons: "fields" (selected) and "mm". The "fields" option is set to 2 x 2. The "mm" option is set to 10.000 x 10.000. The "Camera" is set to "DS-Q12" and the "Objective" is set to "4 - S Plan Fluor ELWD 40x Ph2". The "Overlap" is set to 15%. There are "Finish" and "Cancel" buttons at the bottom.

1. Scan area
2. Objective!

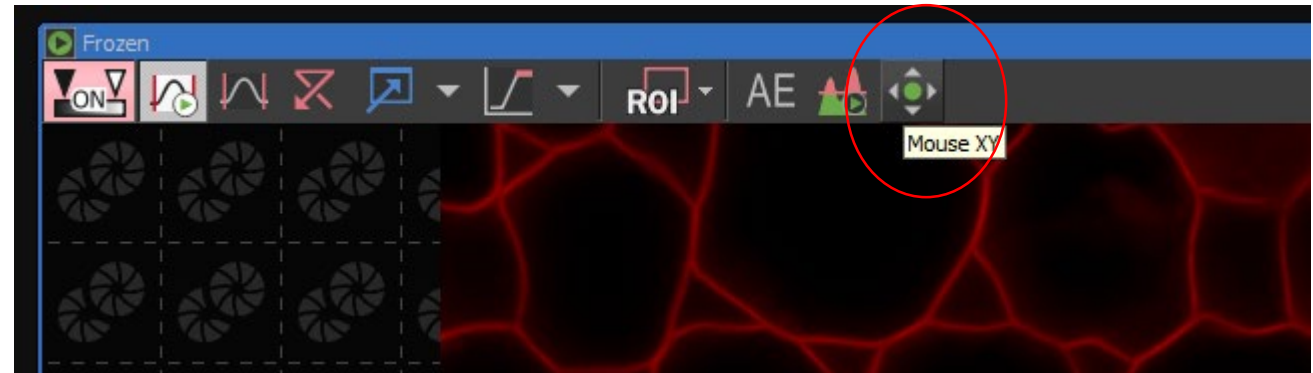
# Tips:

Living autoscale for preview



# Tips:

Use mouse to move sample in XY



Mouse right click the image for specific position or others

