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 A1/R Confocal 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**

Powering on the A1 microscope

- Login to NUcore from your phone and begin your reservation

- Turn on the X-cite LED lamp

- Turn on microscope power from the power strip

- Turn on the A1 controller

- Turn on the lasers: A1B and A1C use one key on the A1 controller. A1 Spectral has four different keys on the lasers

- Clean ALL objective lenses with sparkle and lens paper. Sign the log book

- 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

- If you are the laser user of the day you must completely turn off the instrument and the lasers!

- ALL users must clean ALL objectives with sparkle and lens paper when they finish using the microscope!

Powering on the A1 microscope: Ti-E

Turn on microscope power from the power strip toggle switch.

Each A1 has a power strip in a slightly different location:

- A1R A (Spectral): Left leg of instrument table near A1 controller
- A1R B (GaAsP): On top of instrument table to the left of microscope
- A1 C: Right leg of instrument table near the computer
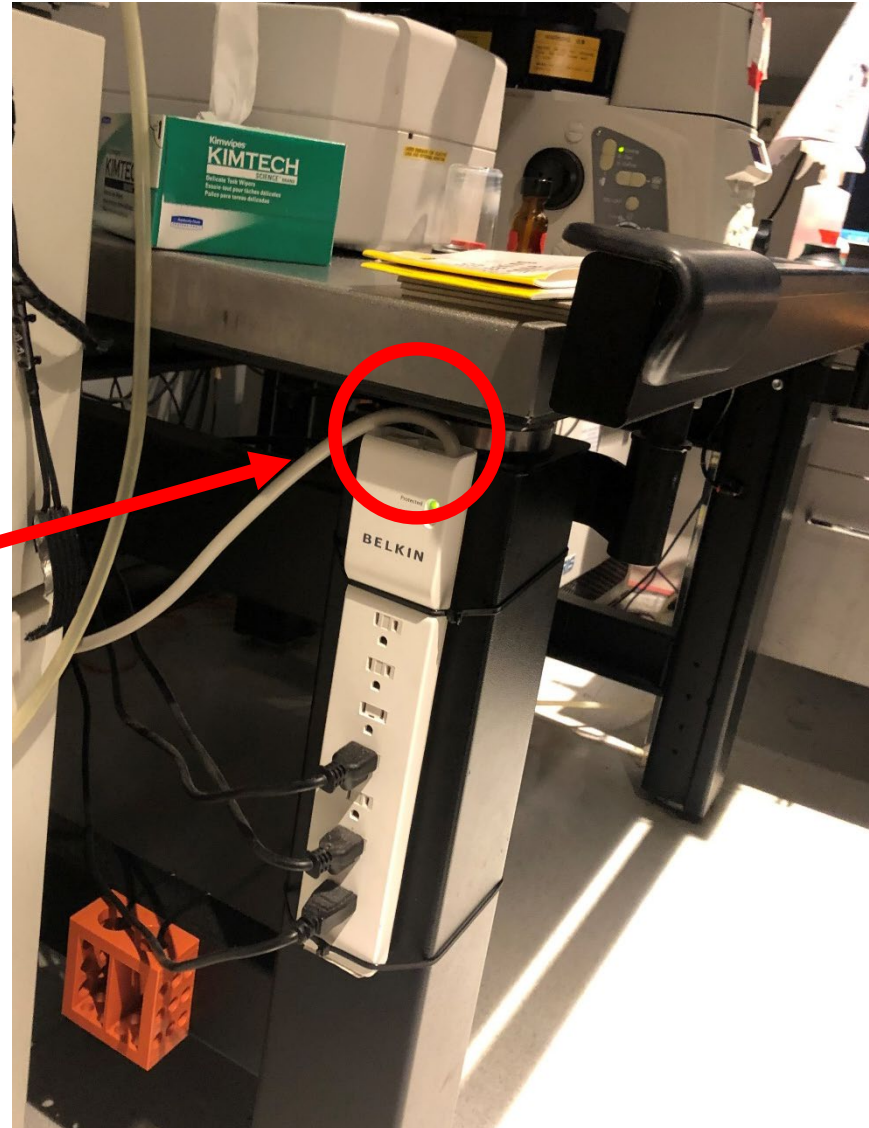
Powering on the A1 microscope: A1 controller

Power on the A1 controller from a recessed button on the left side of the A1 control tower.
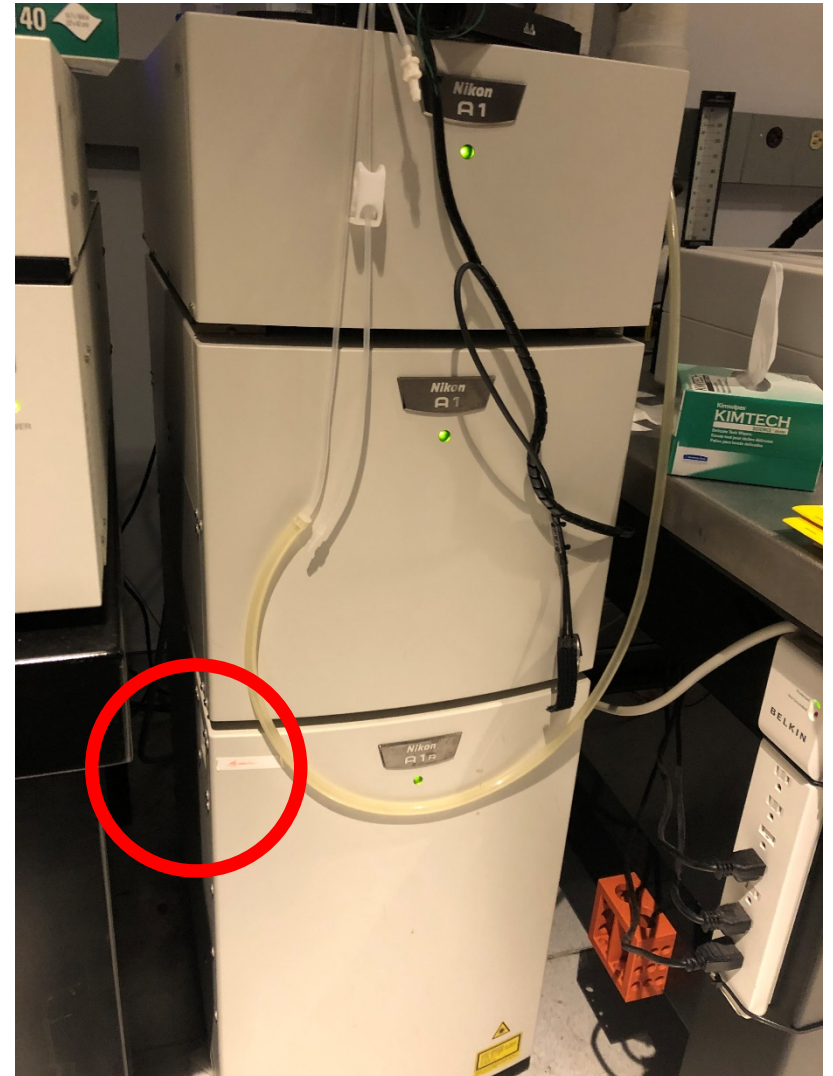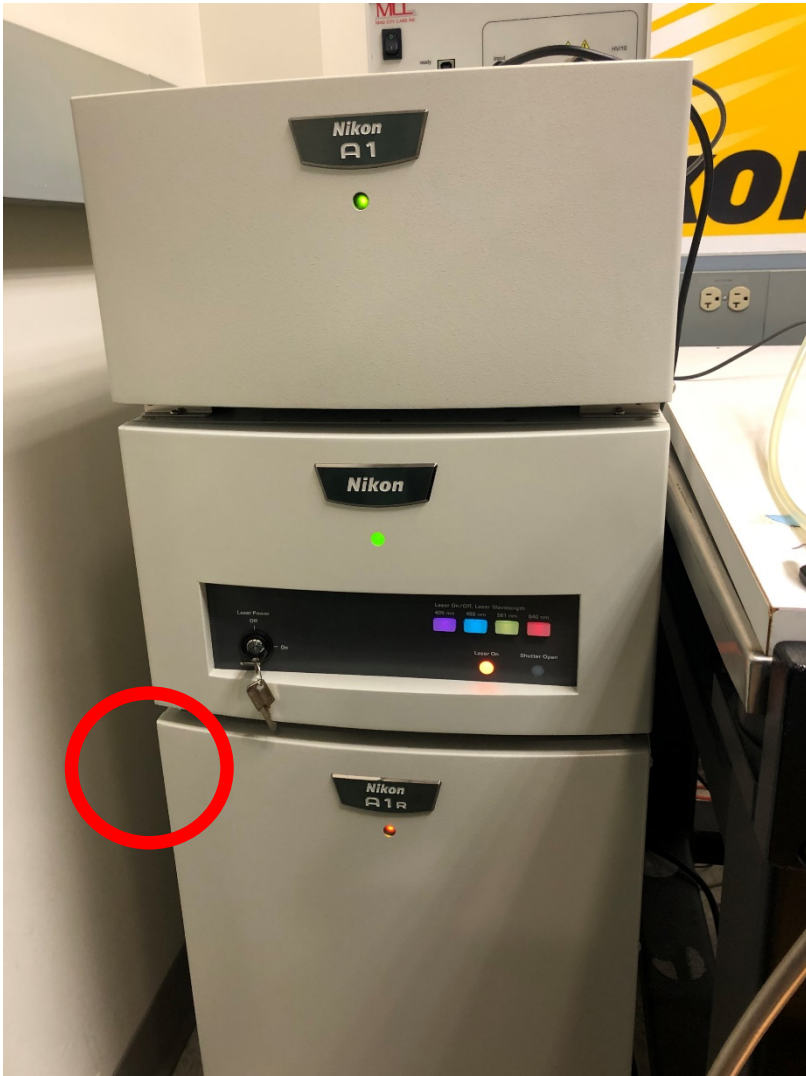
Powering on the A1 microscope: Lasers (A1R B and A1 C)

Power on the four system lasers (405, 488, 561, 640 nm) using the power key on the front of the A1 controller. Turn the key to the right to power on, and to the left to power off.

Four colored lights will come on to indicate the lasers are ready.

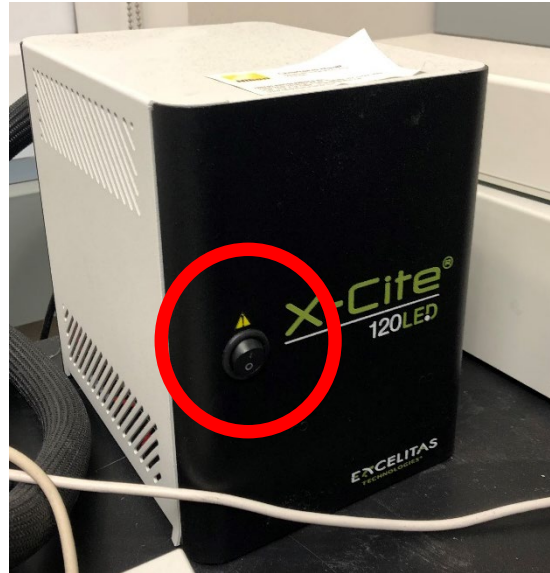# Powering on the A1 microscope: Lasers (A1R Spectral)

Power on the four system lasers with four separate keys. Turn each key to the right to power on, and to the left to power off.

The Melles Griot laser (457/488/514) is an Ar$^+$ gas laser and has a fan attached. It is normal for the fan to run for a few minutes after the laser is turned off.

Powering on the A1 microscope: LEDs

Power on the Xcite epifluorescence LED from the front power supply

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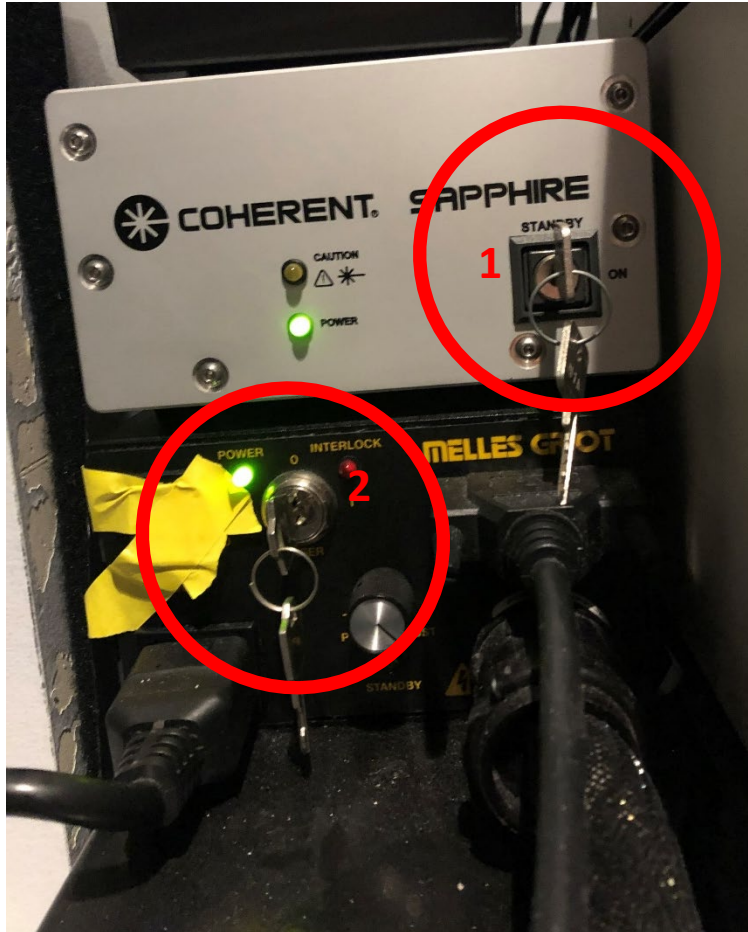Turn the knob to change the LED intensity 1 – 100%

Off

On

Powering on the A1 microscope: Lasers (A1R Spectral) cot'd

Turn each key to the right (to ON) to power on, and to the left (to STANDBY) to power off.

488nm and 561nm laser controllers:

405nm and 640nm laser controllers:

Layout of the Ti-E microscope

Left operation panel

Focus speed selector:
Coarse (fast)
Fine (medium)
Extra-fine (slow)

Focus knob

Objective turret
selector button

Diascopic/transmitted
brightfield light
halogen lamp on/off

Transmitted light
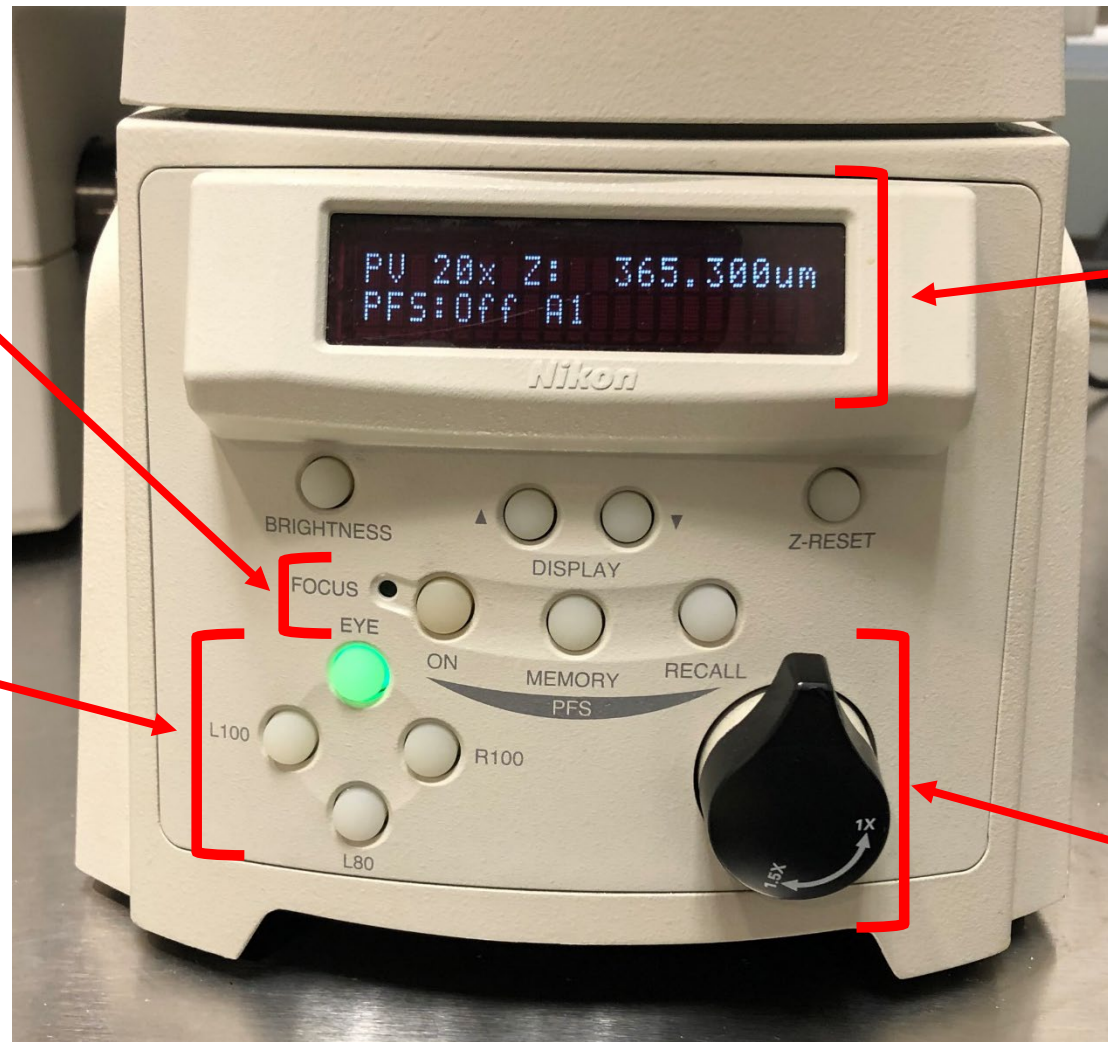intensity dial

Layout of the Ti-E microscope

Front operation panel

Perfect Focus System (PFS) controls: ON to set PFS, it will automatically lock when in proper range

Microscope configuration controls:
EYE: view sample though eyepieces with LED
L100: use confocal scanner to collect image in software

Front display panel:
- Objective
- Z position (in microns)
- PFS status
- Filter position:
(A1 for confocal imaging, DAPI, FITC, TRITC, D/F/T for triple filter)

Zoom knob should ALWAYS be set to 1x facing upward

Layout of the Ti-E microscope

Right operation panel

Focus speed selector:
Coarse (fast)
Fine (medium)
Extra-fine (slow)

Focus knob

Escape button drops the current objective to a preset height. **Refocus** button drives the objective back to the original location. BE EXTREMELY CAREFUL if you use these commands not to crash the objective into your slide! Remove the slide and make sure the stage is clear before pressing Refocus!
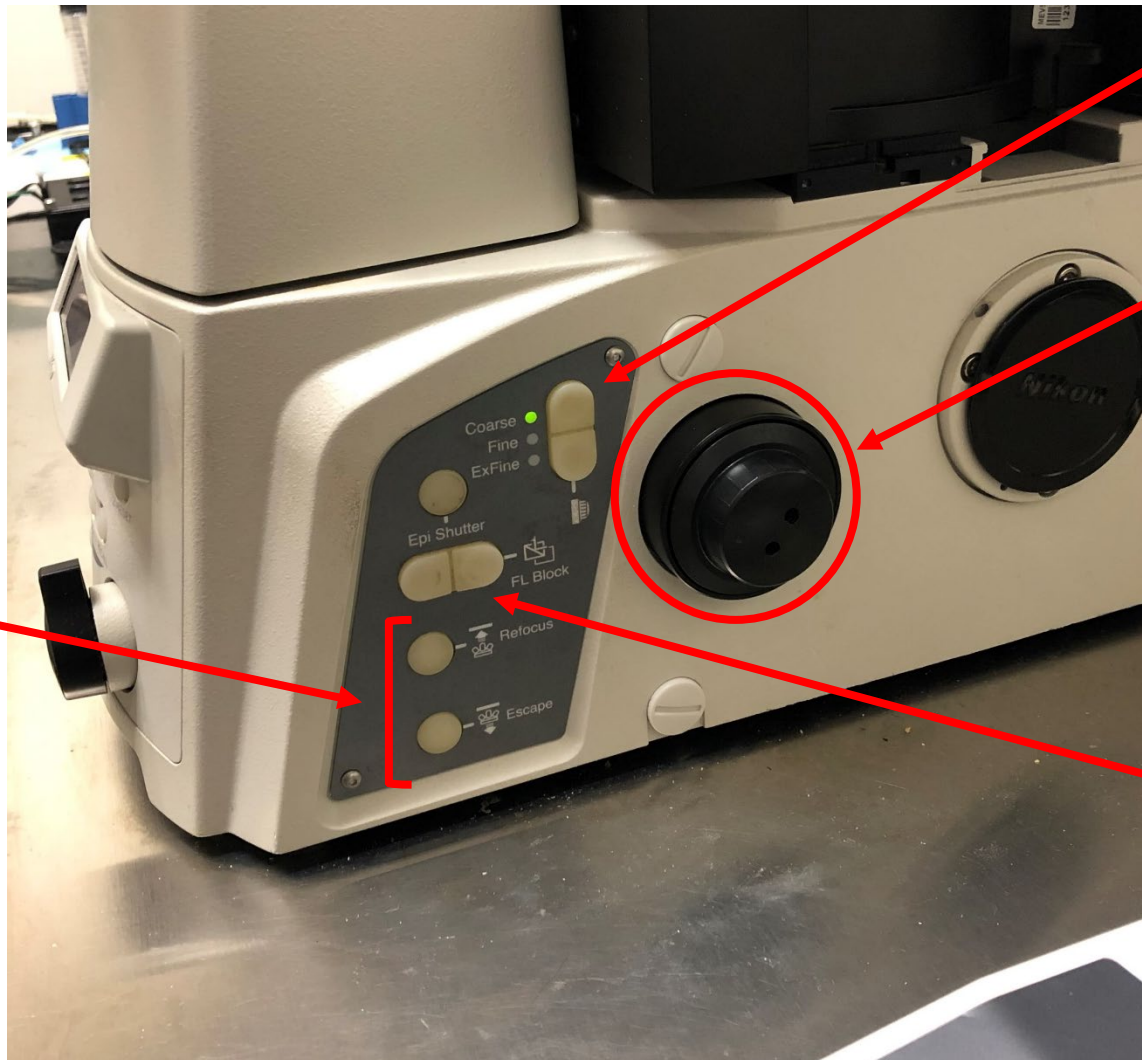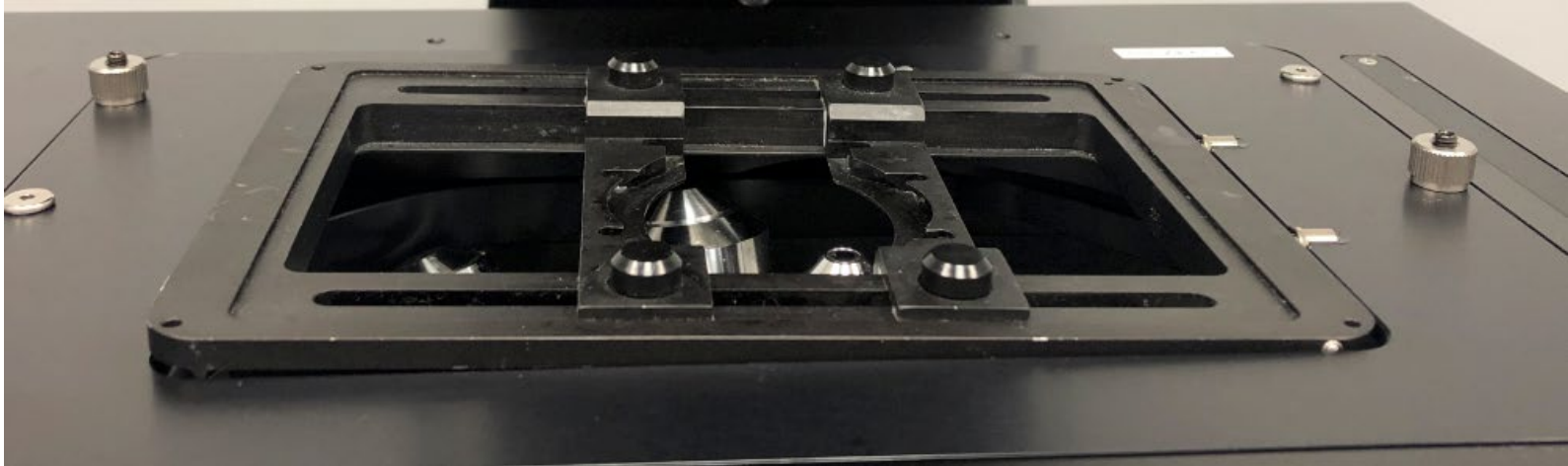
FL block button: rotate the filter turret to select a viewing filter to view the sample with LED illumination
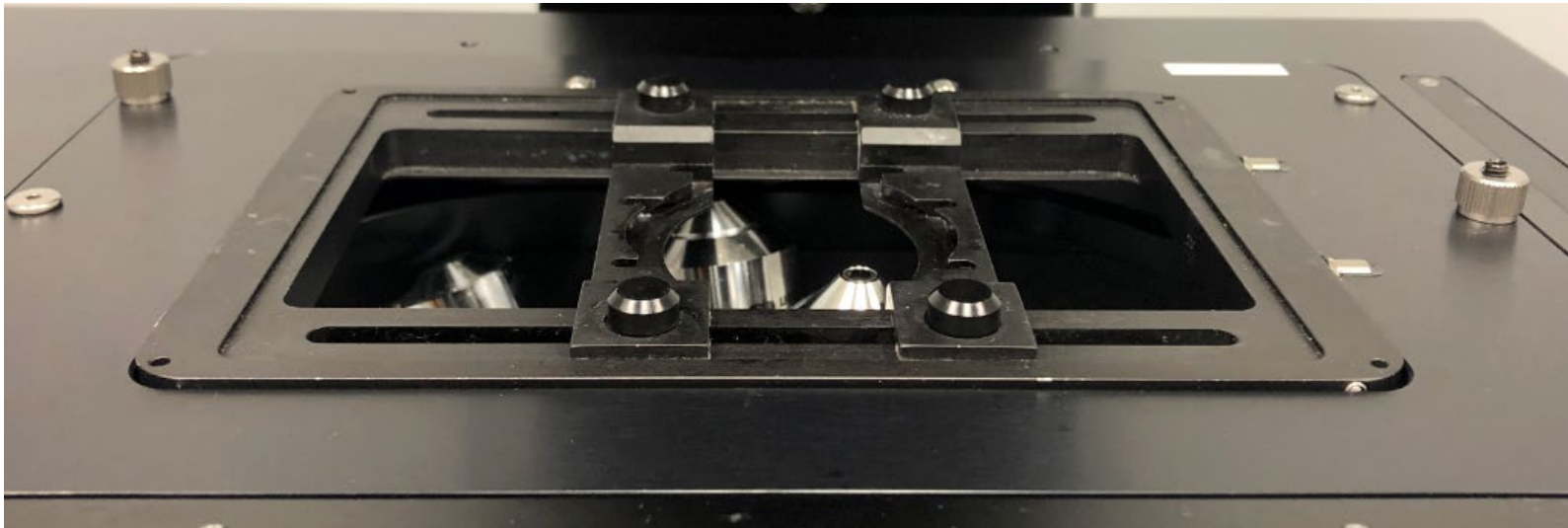
Layout of the Ti-2 microscope

Stage sample holder

If the objectives are raised too high, they may press on the sample holder and cause them to pop the stage insert out:
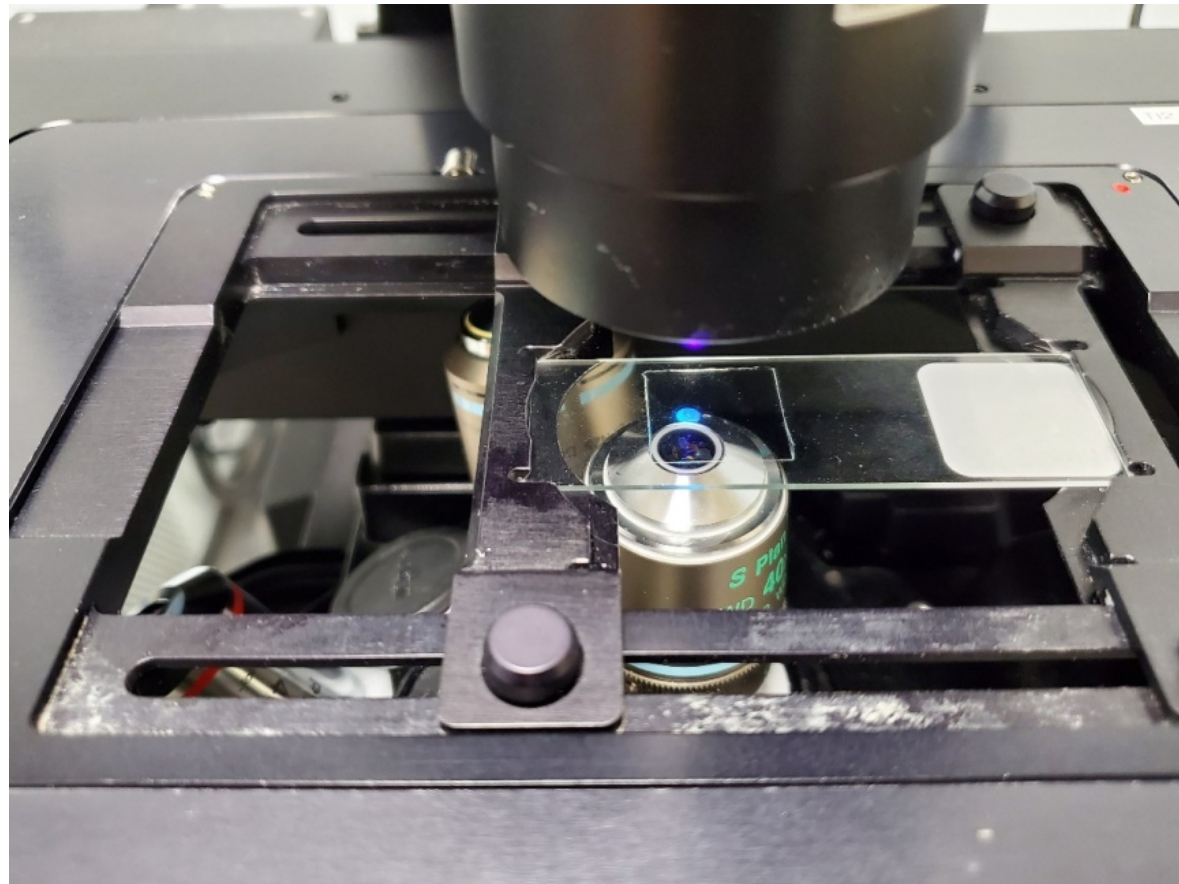

When the stage insert is properly set it will look like this:

Layout of the Ti-2 microscope

Stage sample holder

Layout of the Ti-E microscope
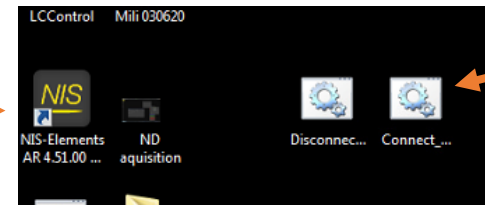
Joystick controller

Focus speed selector:
Coarse (fast)
Fine (medium)
Extra-fine (slow)

Focus knob

Twist Joystick to change speed
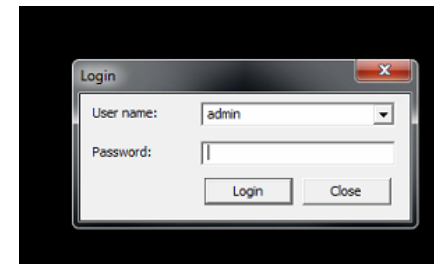Coarse (fast)
Fine (medium)
Extra-fine (slow)

Logging into computer and software

Open the NIS Elements software:

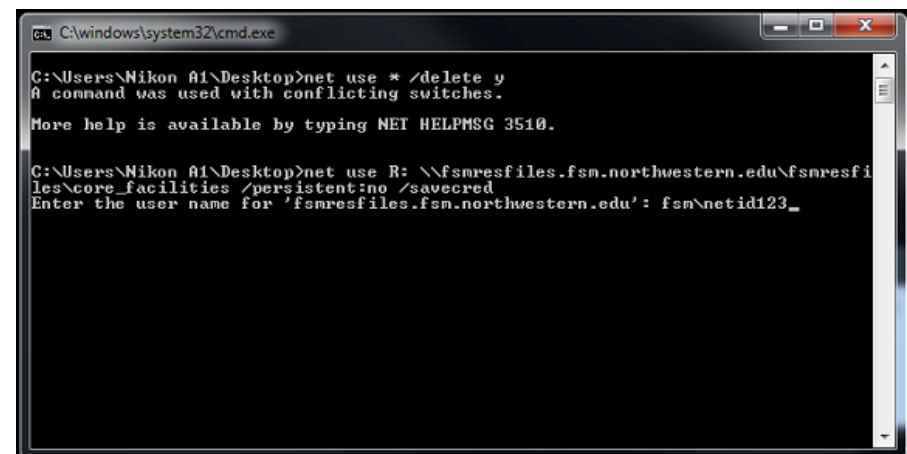Connect and disconnect from FSMResFiles server

You will be prompted for your NIS Elements username (NetID) and password:

Run the 'Connect' script on the desktop to connect to the FSMResFiles server. The username is:
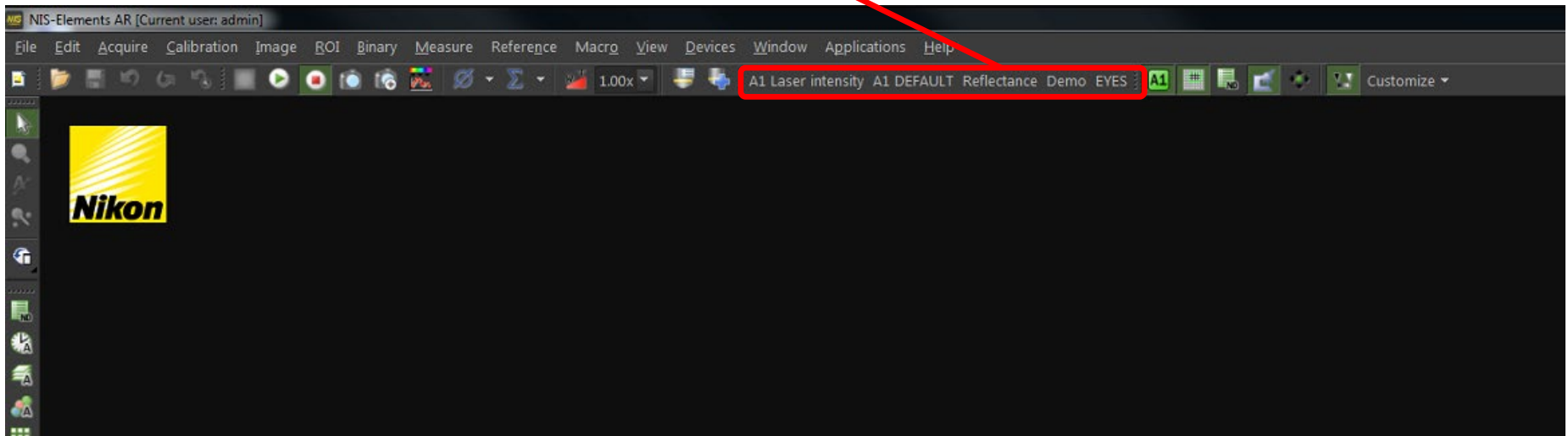
fsm\(your netid)

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

NIS Elements v4.6 software GUI

Monitor 1: Image area and Toolbars

Optical configuration toolbar: OCs can capture image collection software settings as well as physical microscope stand instrument state settings. Right click to save settings for each OC.

Right click A1 Default and duplicate the

NIS Elements v4.6 software GUI

Monitor 2: GUI and image collection parameters

**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.
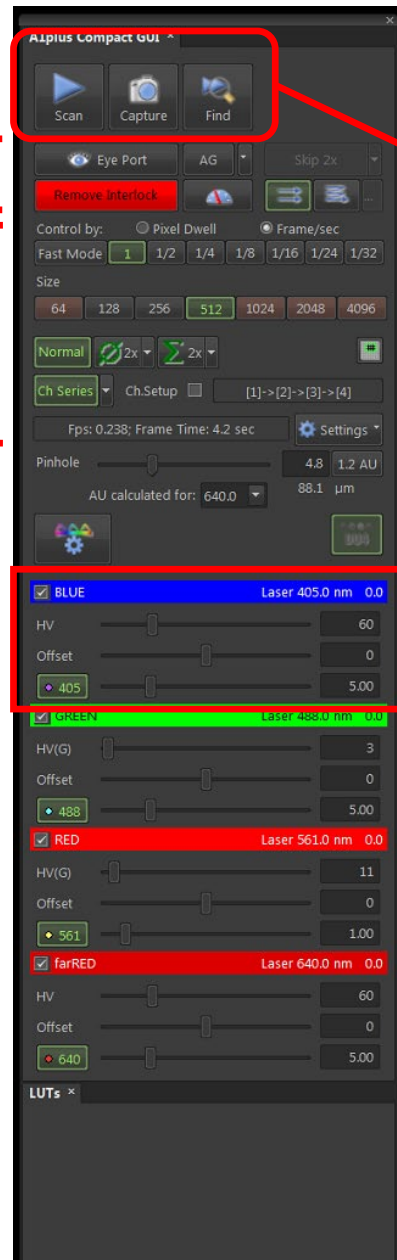
Scan: fast imaging, every collected frame overwrites themselves in Live window

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:
- **HV**: Gain settings for PMT detector. Usually a value of 80-120.
- **HV(G)**: Gain settings for high-sensitivity GaAsP detector. Usually a value of 10-50.
- **Offset**: zero level of the PMT detector. Usually a value of 0.
- **Laser**: laser power (in %) for each laser line. Usually a value from 1-10%

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

NIS Elements v4.6 software GUI

Pinhole setup for each objective
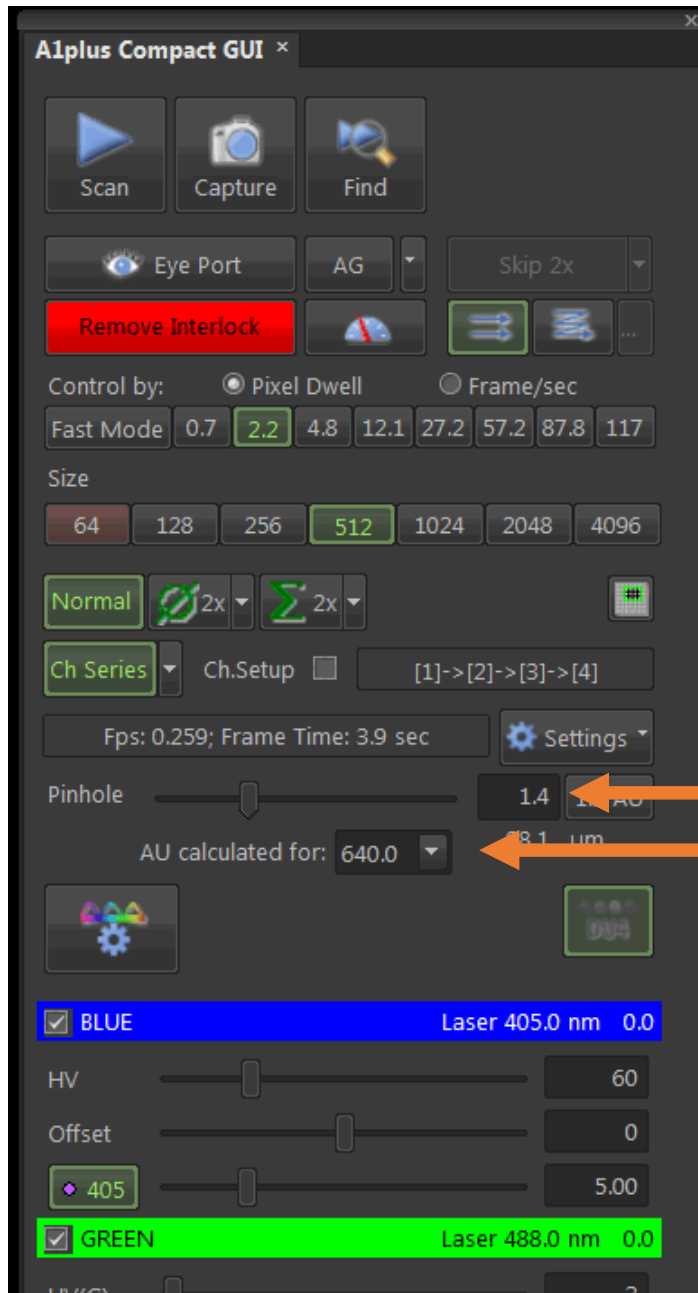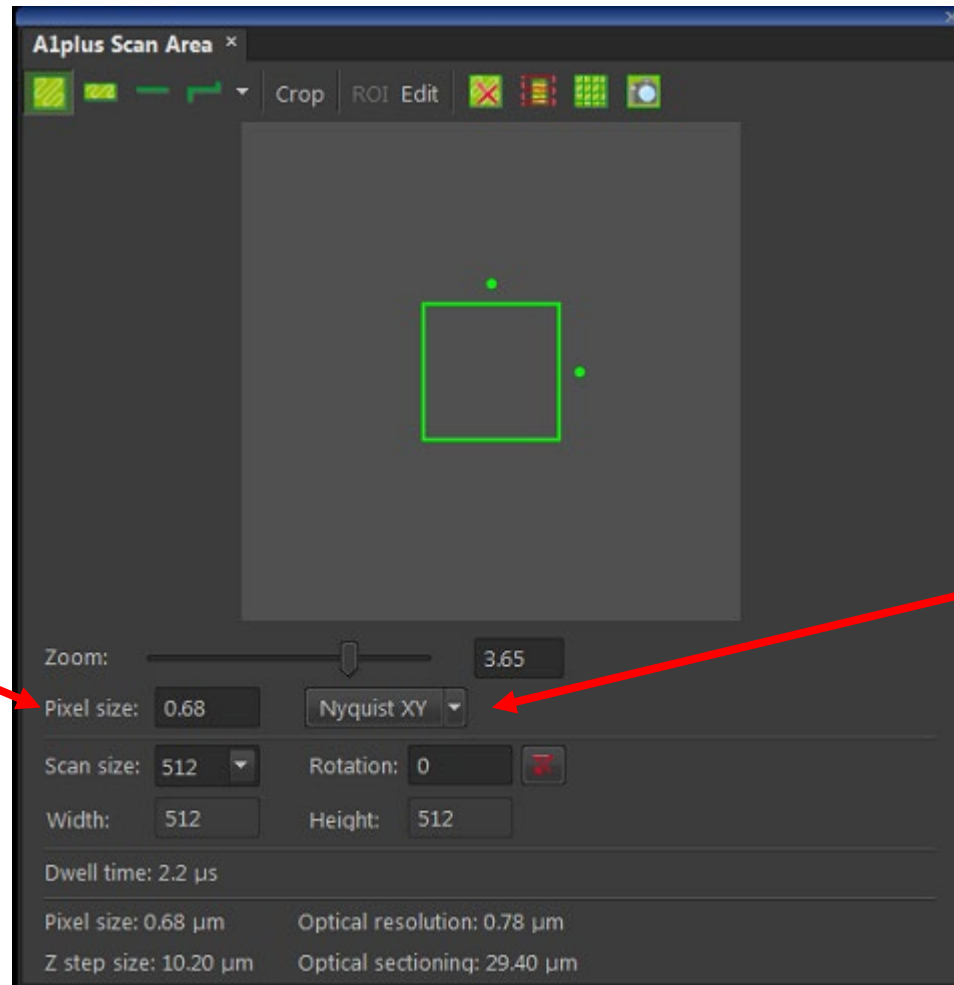

NEED TO DO THIS ON EVERY OBJECTIVE!

2. Change this to 1.0

1. Change this to 488

3. Save the optical configuration settings

NIS Elements v4.6 software GUI

Monitor 2: GUI and image collection parameters

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.

NIS Elements v4.6 software GUI

Move slider to top
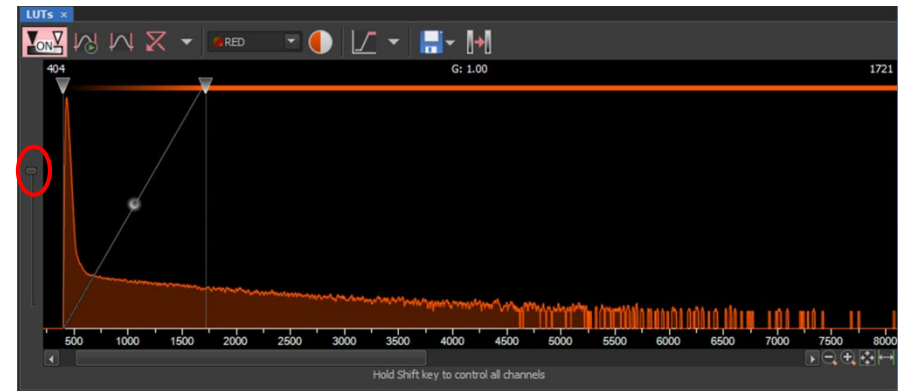
Weak   Saturated

Standard: Good S/N, no saturation, not too weak
Adjust: Laser power, HV gain, exposure time.