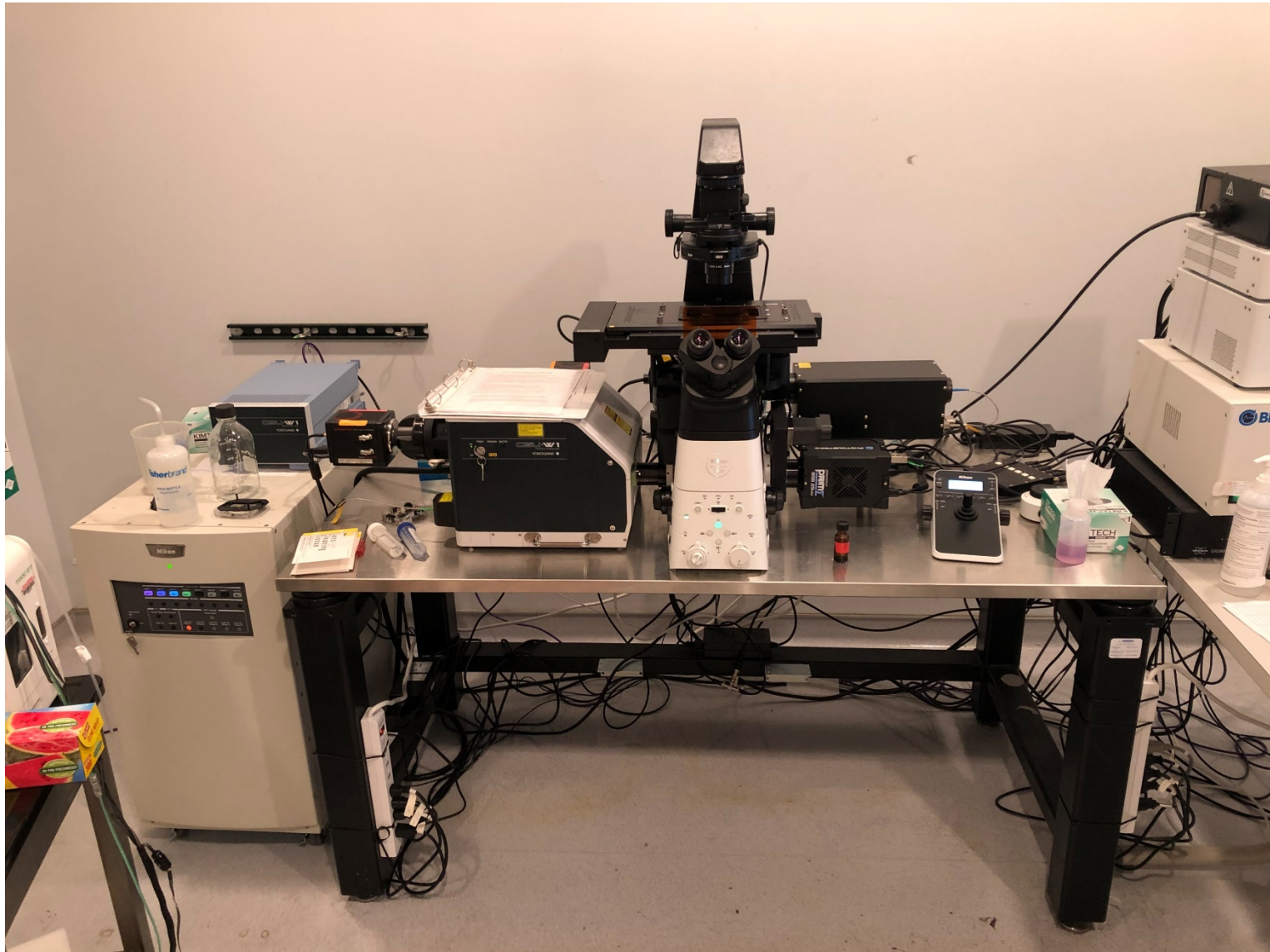


Notice!

- **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 the FSMResFiles 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) or Dina (312-503-7139).

Nikon W1 Spinning Disk Microscope



Before you begin

(Important)

- Clean the microscope and working area according to the instructions next to the computer monitor
- Start and end your reservation in NUcore from your phone



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



**CLEAN
SURFACES**

Powering on the W1 microscope

- **FIRST MAKE SURE THE COMPUTER IS POWERED OFF. IF IT IS ON, THEN TURN OFF THE COMPUTER BEFORE YOU MOVE ON. IF YOU DO NOT DO THIS THE SOFTWARE WILL CRASH.**
- Power on the W1 using two power strips on the legs of the table.
- Wait until the Prime 95B camera is fully initialized before powering on the computer. (Make sure light is no longer blinking- see slide 6).
- Power on the lasers with the single key.
- Turn on the computer power.
- Log into the Windows account – username: .\nikon
- Sign into FSMResFiles server with netid and password
- Sign into NIS Elements with username (NetID) and password

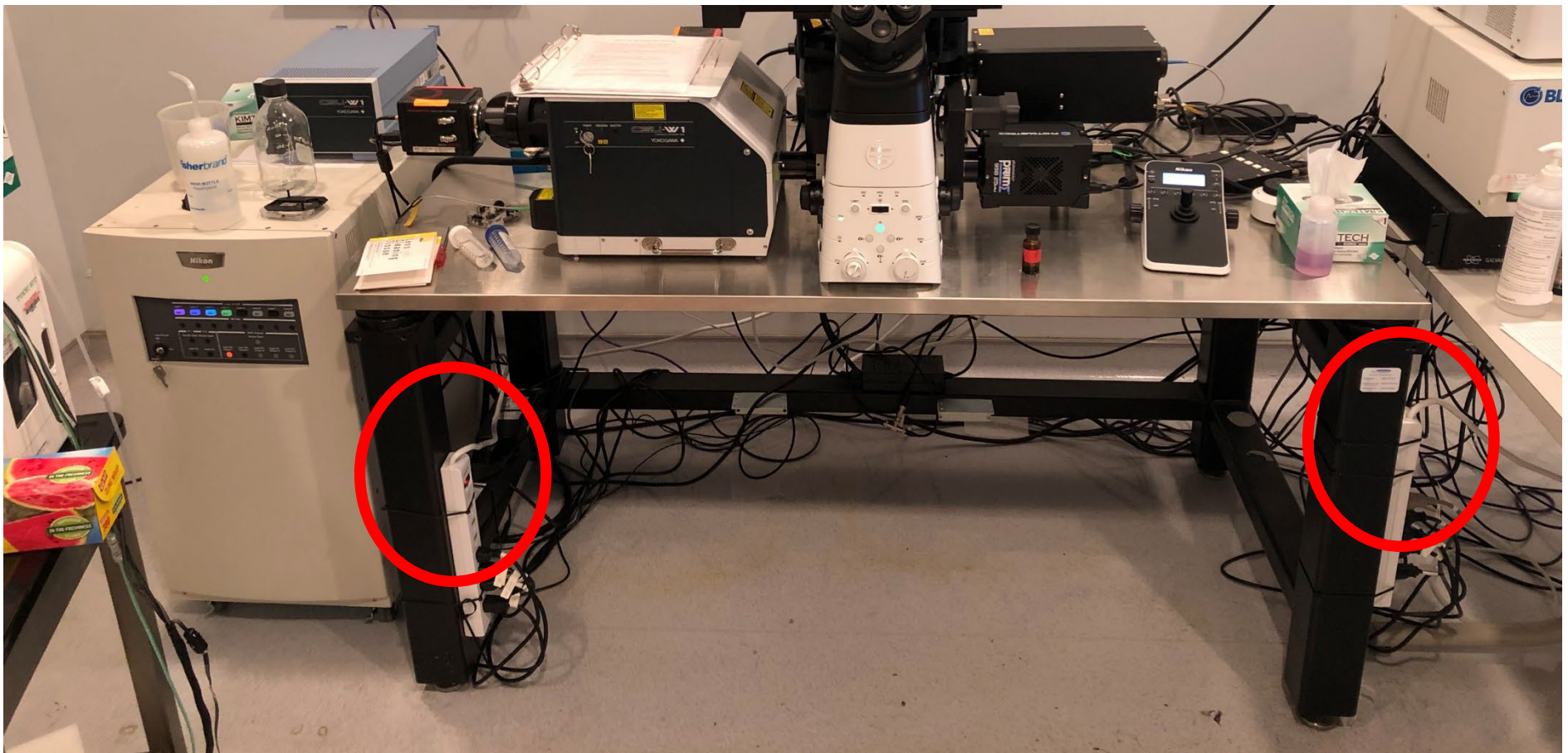
Powering on the W1 microscope: Lasers

Turn on all 6 laser lines (405, 457, 488, 561, 640 nm) using the single key. The colored lights will blink while the lasers are initializing and show solid when the lasers are ready.



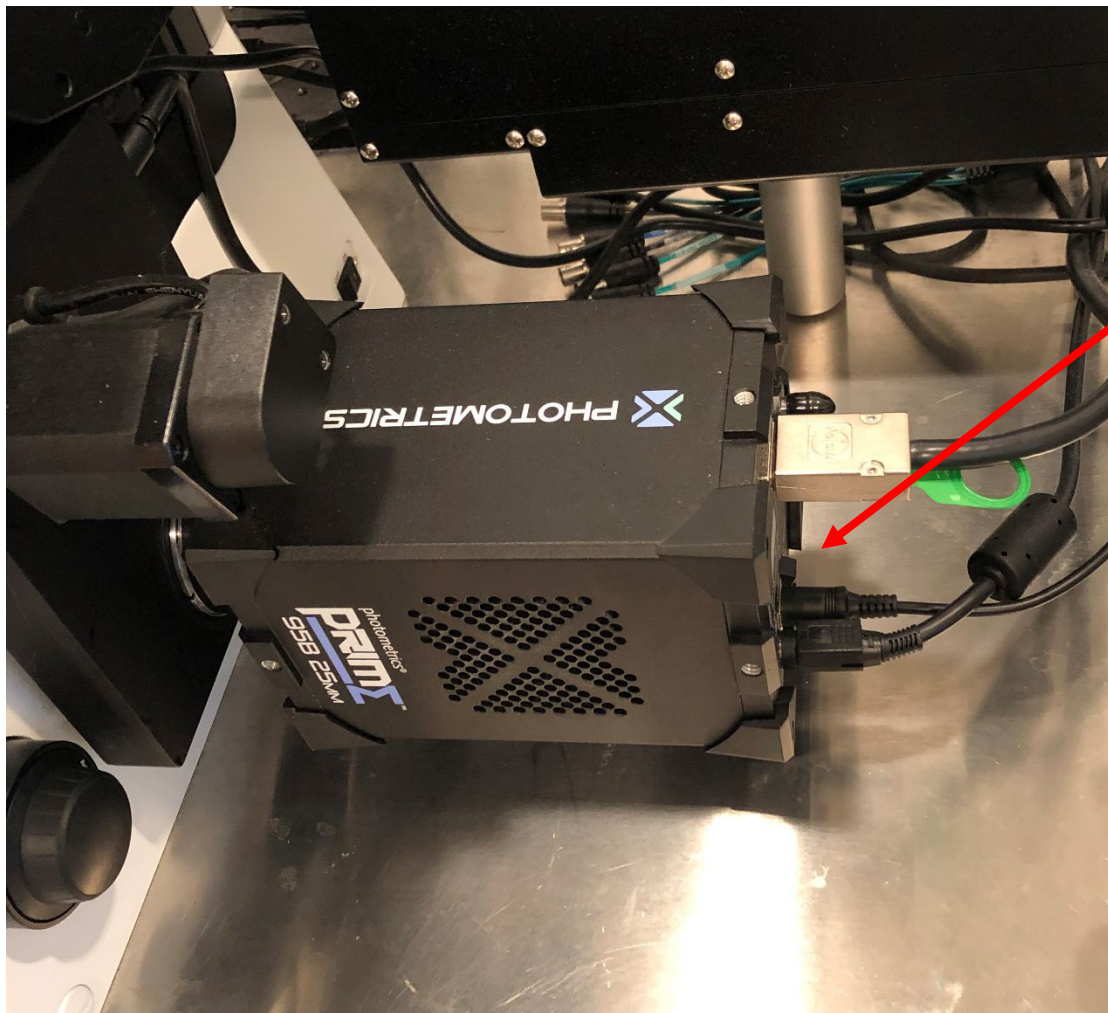
Powering on the W1 microscope

After making sure the computer is turned off, turn on the power to the W1 microscope stand, the dual Flash 4.0 and Prime95B cameras, and the Confocal Scanning Unit (CSU) by pressing the two power strip switches circled in red below.

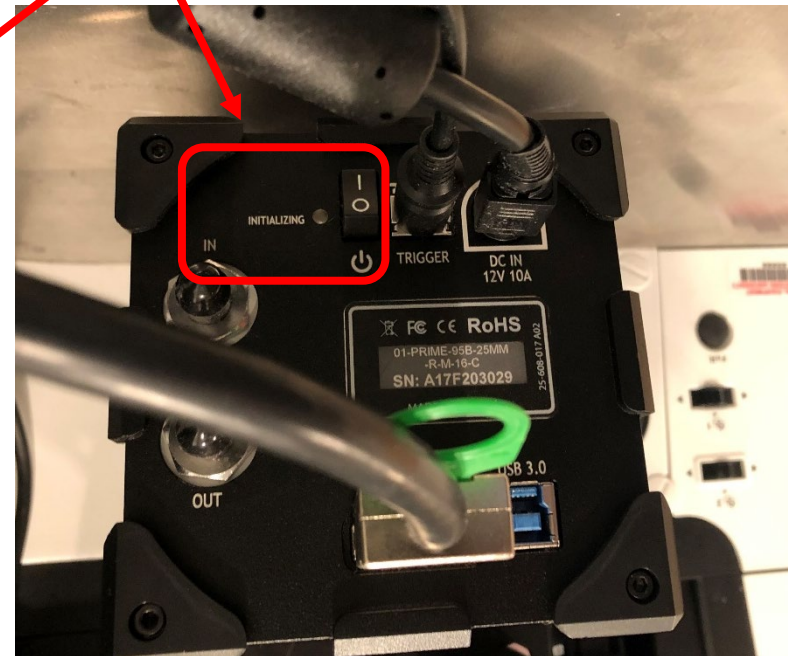


Powering on the W1 microscope, cot'd

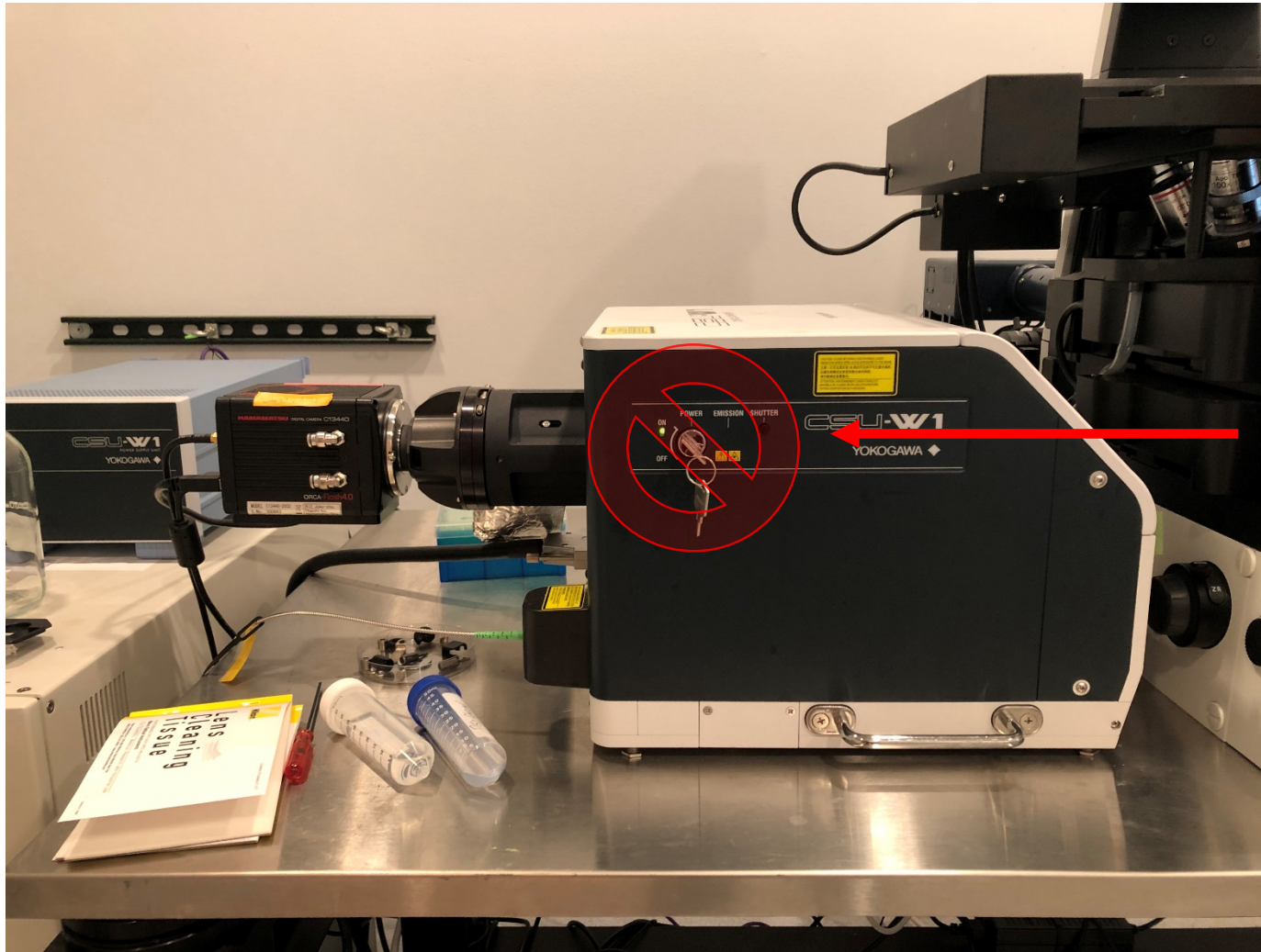
When turning the system on, make sure the Prime 95B camera is fully initialized (which takes about 60 seconds) before powering on the computer.



The initializing light will blink while the camera is initializing. When it stops blinking the computer can be turned on.



Powering on the W1 microscope: CSU



**DO NOT turn off
the power key
to the CSU!**

Layout of the Ti-2 microscope

Front operation panel

Microscope configuration panel:

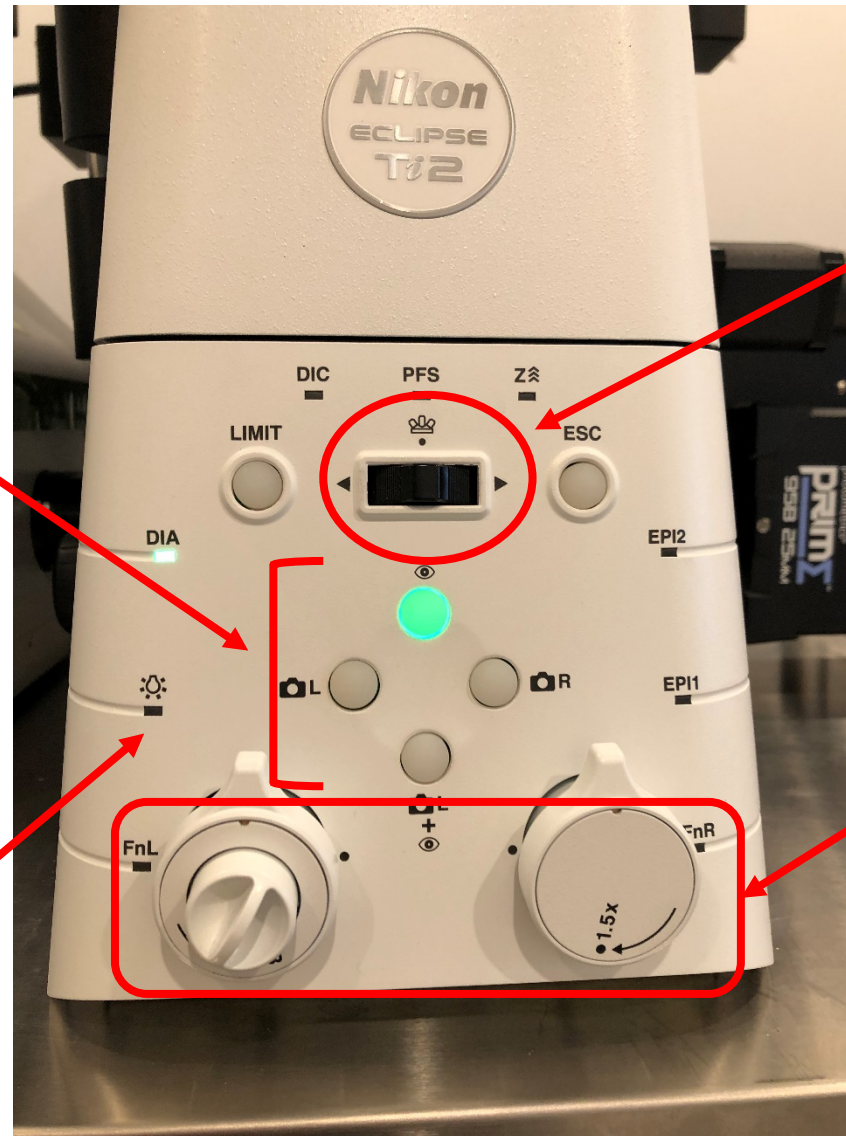
EYES: Eyepiece viewing

L: Confocal scanning with CSU and Flash 4.0 cameras

R: Widefield epifluorescent / TIRF imaging with Prime 95B camera

Microscope position will be automatically selected when you choose an optical configuration in the software – normally you will not change the position by yourself.

Brightfield lamp on/off indicator. The button to the left of this indicator turns on/off the lamp.



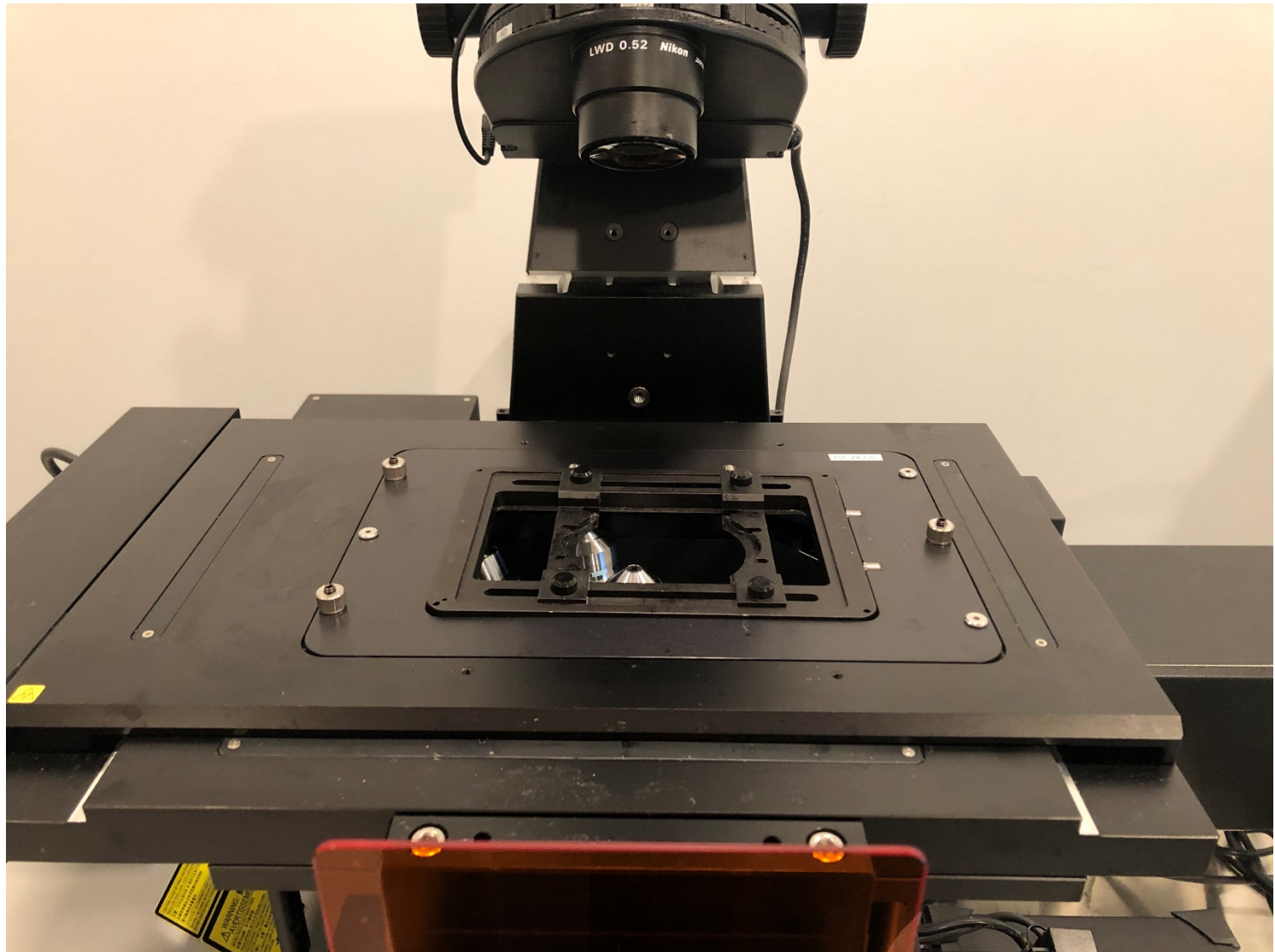
Rotate objective turret left/right

Left dial should always face up

Right dial is 1.5x zoom lens: You may sometimes use the zoom lens for extra magnification (especially when using low mag objectives)

Layout of the Ti-2 microscope

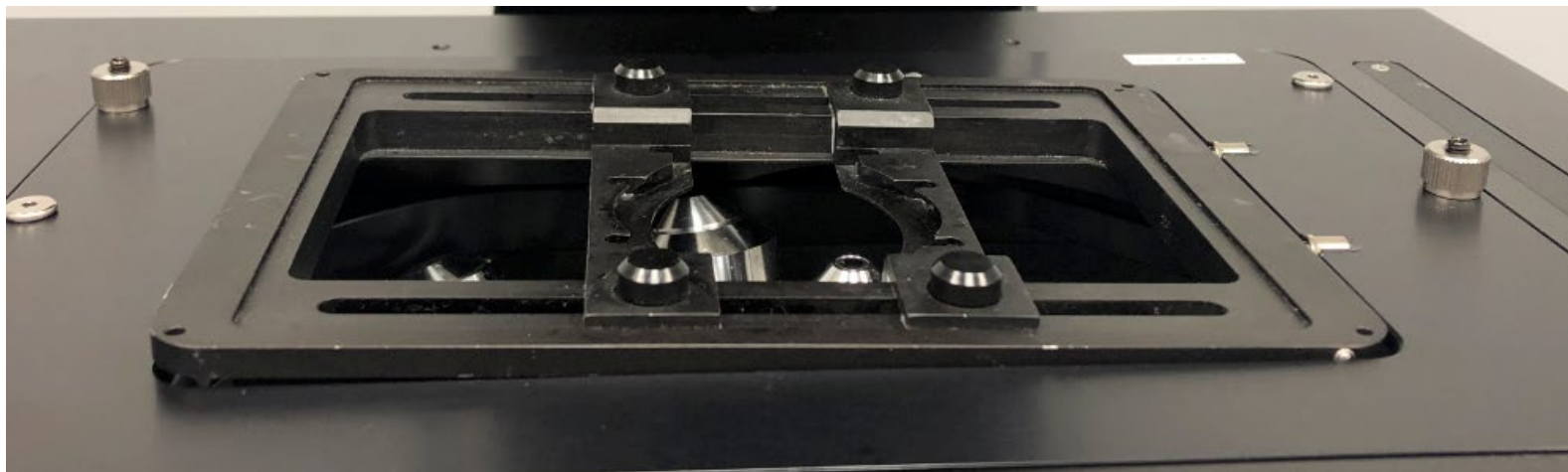
Stage sample holder



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:

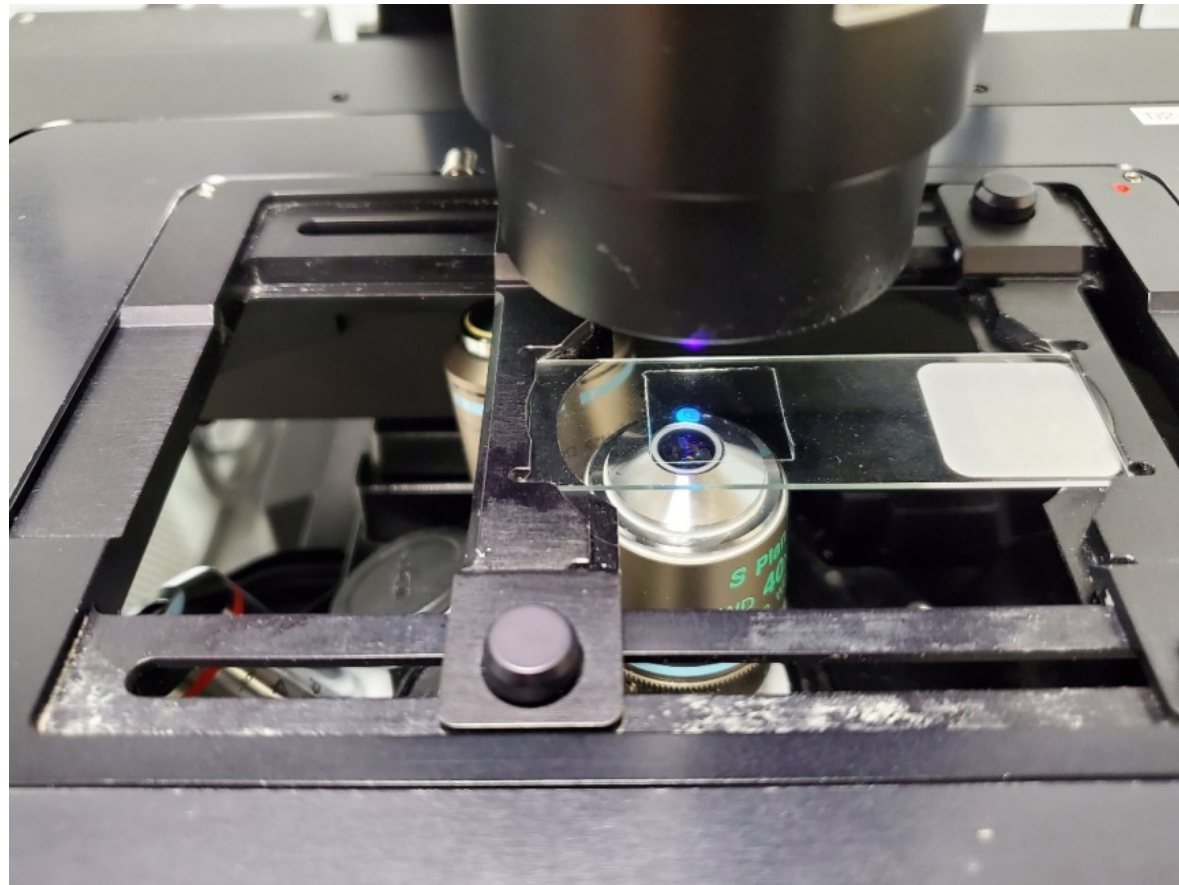


When the stage insert is properly set it will look like this:



Layout of the Ti-2 microscope

Stage sample holder



Layout of the Ti-2 microscope

Left operation panel

Focus knob.
Same controls as on joystick.



Turns brightfield light on/off

Brightfield intensity dial.

FnL moves all the
components so you can see
transmitted light through
the eye pieces

Layout of the Ti-2 microscope

Joystick operation panel

Display screen:

Objective

PFS Status (On/Off)

XY Speed (Fast/Med/Slow)

Z Focus Speed (Fast/Med/Slow)

Z Position (microns)

Focus knob

Joystick for XY
movement of the
sample stage



Programmable function
buttons:

1: DAPI-Eyes

2: GFP-Eyes

3: RFP-Eyes

4: Light off

5: FarRed-Eyes

6: n/a

Activation buttons for PFS

Z Focus speed selector
button:

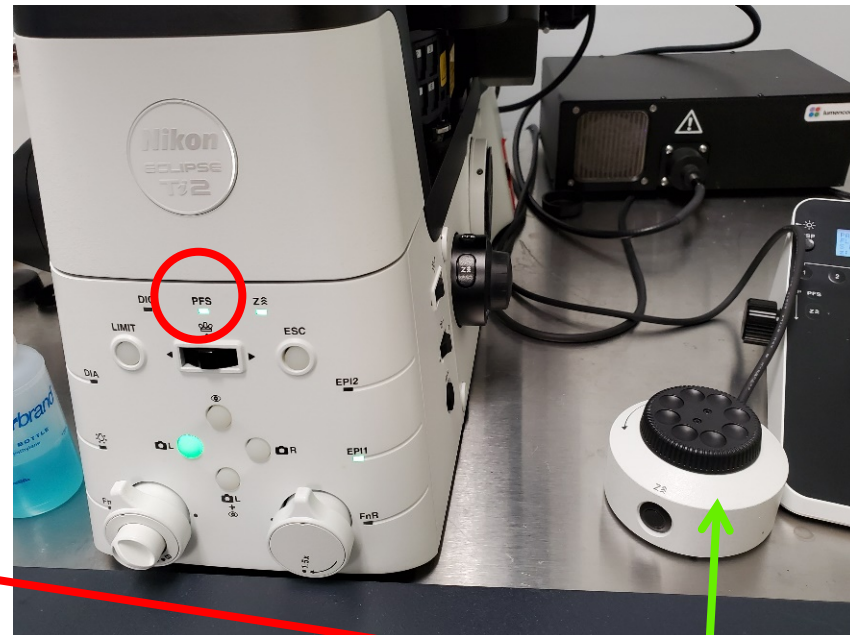
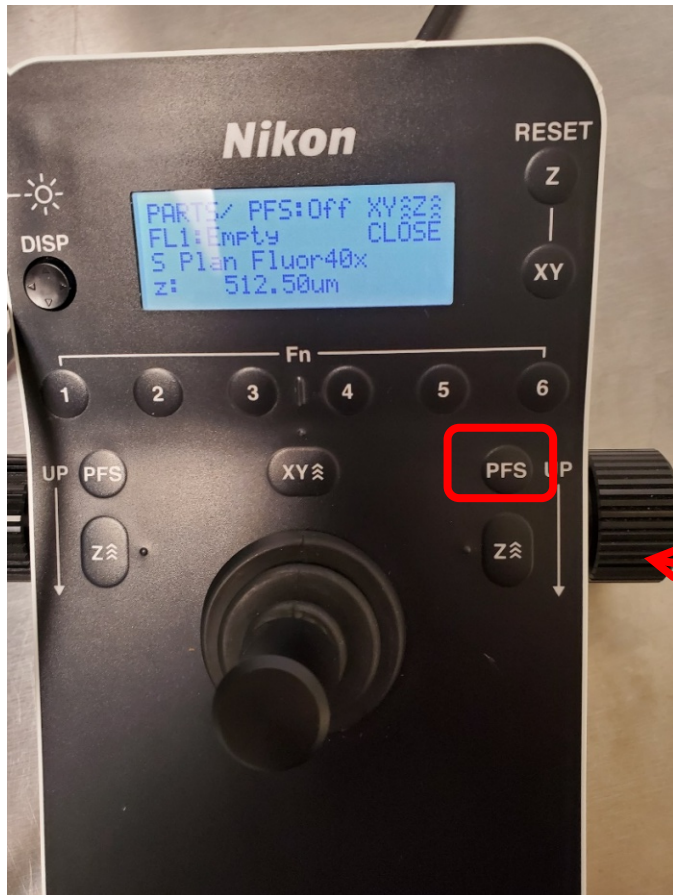
^^^ Fast

^^ Medium

^ Slow

Perfect Focus System (PFS)

PFS: automatically maintains the focus, especially over a long period of time or over a large area



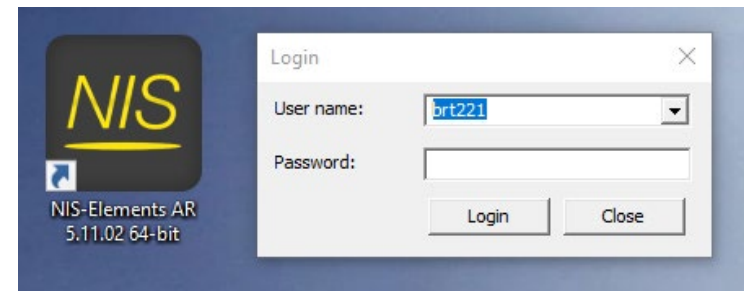
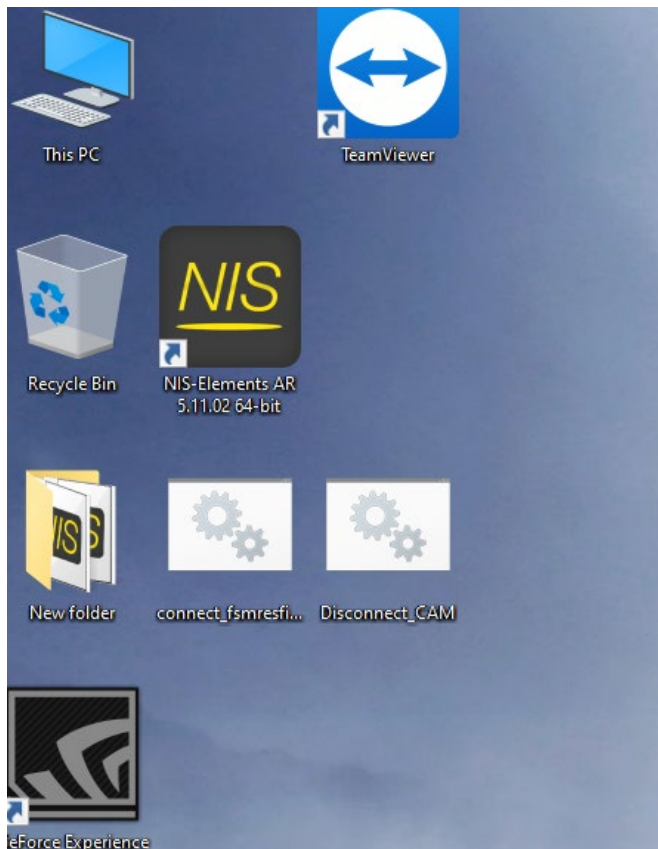
Blinking PFS LED: Not locked, use the z focus knob to focus the stage
Solid PFS LED: Locked, use the PFS focus knob to focus the stage

Log into NIS Elements software and FSMResFiles server

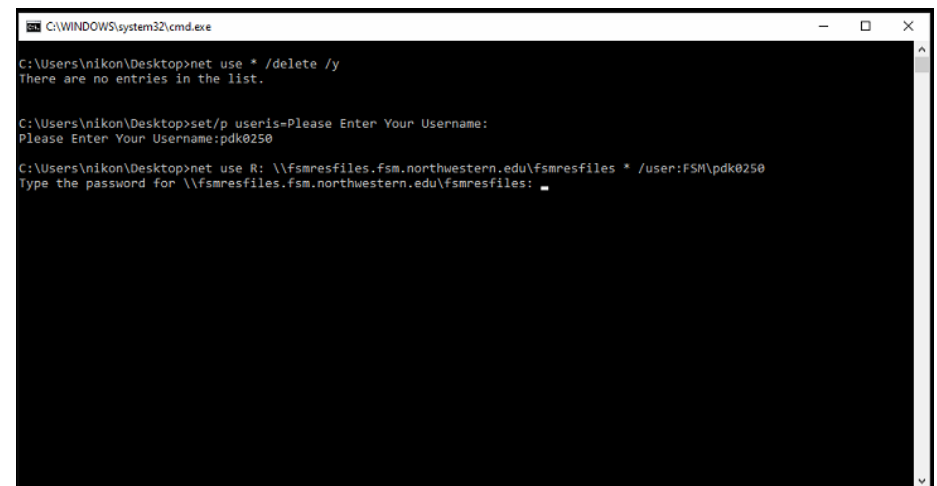
Log into NIS Elements:

Username is your NetID

Password will be set up during training



Use the 'connect' script to log into FSMResFiles:
The username is your NetID, the password is your NetID password. Disconnect before you leave.



NIS Elements v5.11

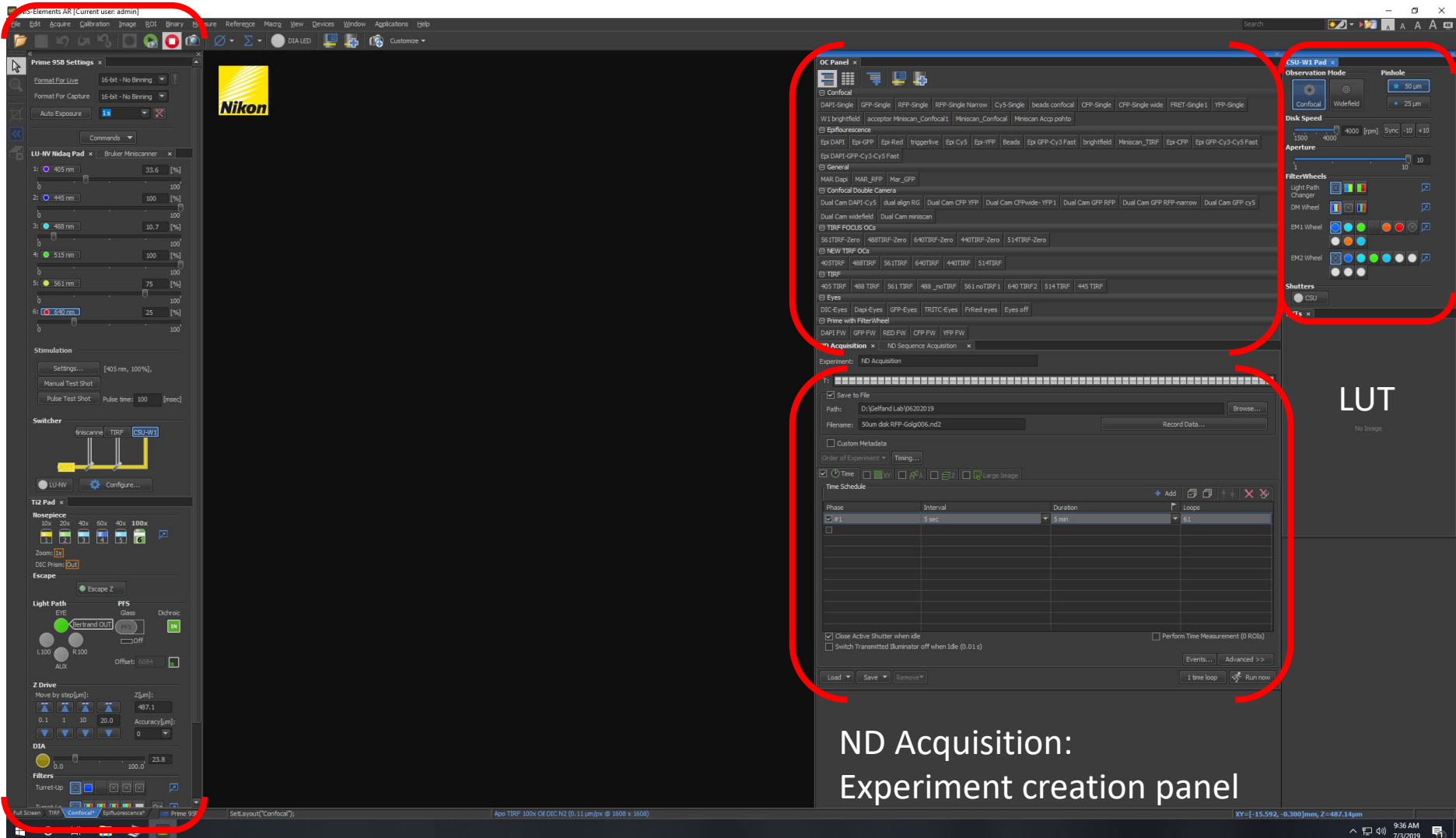
Software layout

Instrument
state

Image area

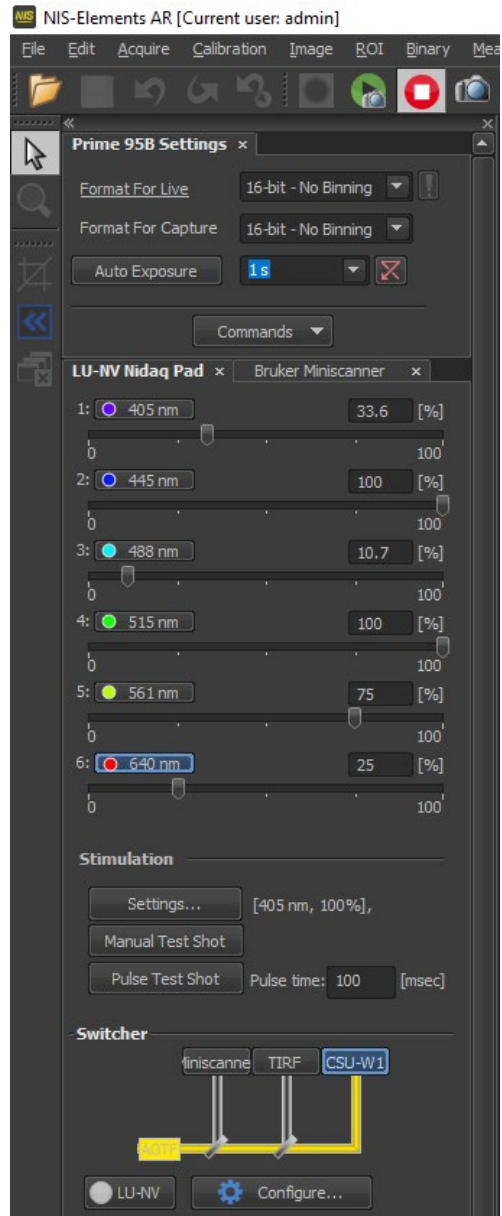
Optical configuration
panel

Confocal
controls



NIS Elements v5.11

Software layout



NIS Elements v5.11

Software layout

Objective selector:

Click to select obj

Hover for obj information



Confocal Scanning Unit controls are mostly defined in the optical configuration. Normally you will not make any changes in this area.



NIS Elements v5.11

Software layout

Pre-configured OC template setting files:

- Users can not modify them.
- Users can duplicate as many as needed.
- Users should use duplications for imaging.

Eyes Group: eyepiece observation. System will automatically choose EYES position and activate the appropriate LED light when you click a filter button

Single Cam Confocal Group: Hamamatsu Flash 4.0 camera acquisition

DAPI – GFP – RFP – RFP Narrow band – Cy5 – CFP – CFP Wideband – YFP

Dual Cam Confoal Group: Dual Hamamatsu Flash 4.0 camera acquisition

Epifluorescence Group: Photometrics Prime 95B camera acquisition

DAPI – GFP – RFP – CFP – YFP

Duplicate these into your own names for imaging

