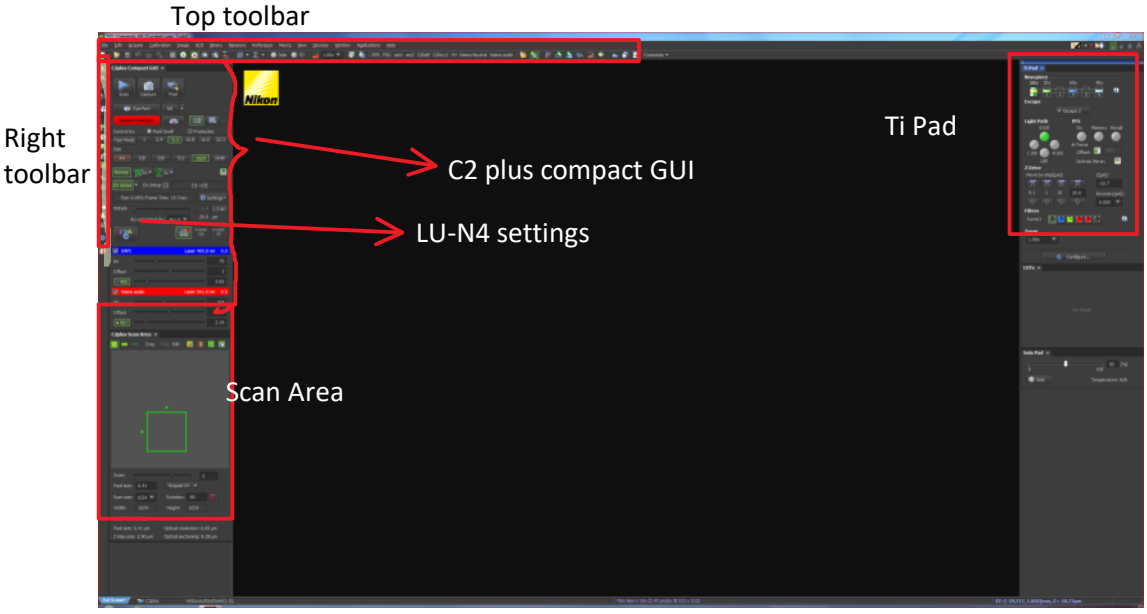
SOP Start Order

Monday, June 11, 2018 10:04 AM

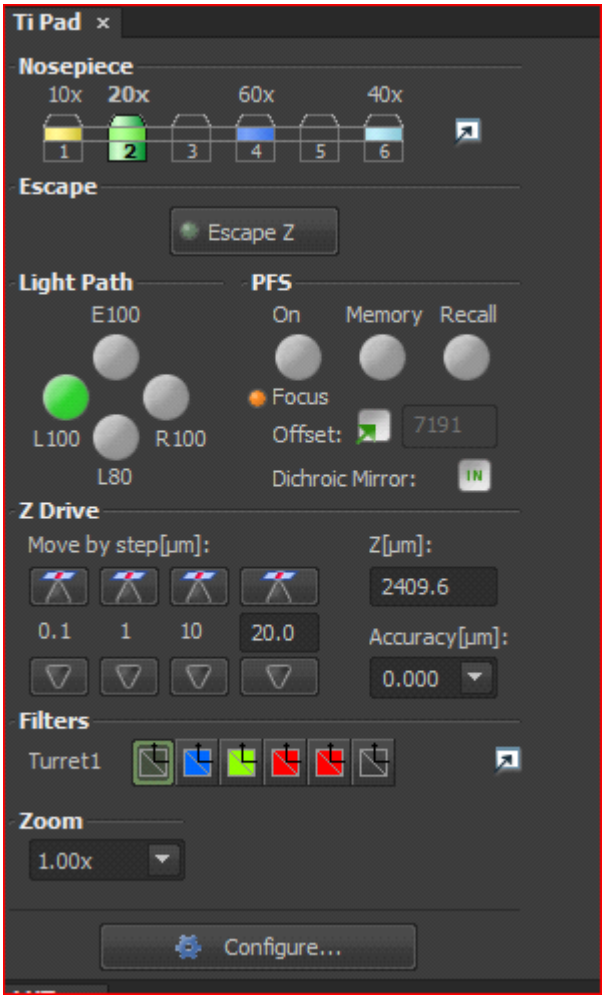
BOSTON UNIVERSITY
Proteomics and Imaging Facility

- For the LAB, always enter and exit through the main door via swipe card with your own ID
- For the Room, Please do not lock the door to LSE 450
- TURN ON Sequence
  - If you are using the environmental chamber (In Vivo Chamber), **15minutes before starting**
    - Turn on the control head
    - Turn on the O2/CO2 tank and pin valve
    - Change the stage insert only using the green screw driver on the shelf
    - Attach the gas inlet tubing to the chamber
  - Turn on power strip (under scope on shelf)
  - Turn on the PC power (button on front)
  - Turn on the monitor
  - Turn on Laser sled (switch on back, bottom left)
  - Turn key to On to engage lasers
  - Select the lasers you will be using for excitation using the illuminated push buttons on the front of the sled
  - Turn on scan unit big power button (next to scope)
  - Note: The red error light will be on as long as the
  - NOTE: When the microscope turns on the bright field light will be on
    - Button is on the left hand front side of scope base
  - Once the scan head lights are on and stable you can launch the NIS software

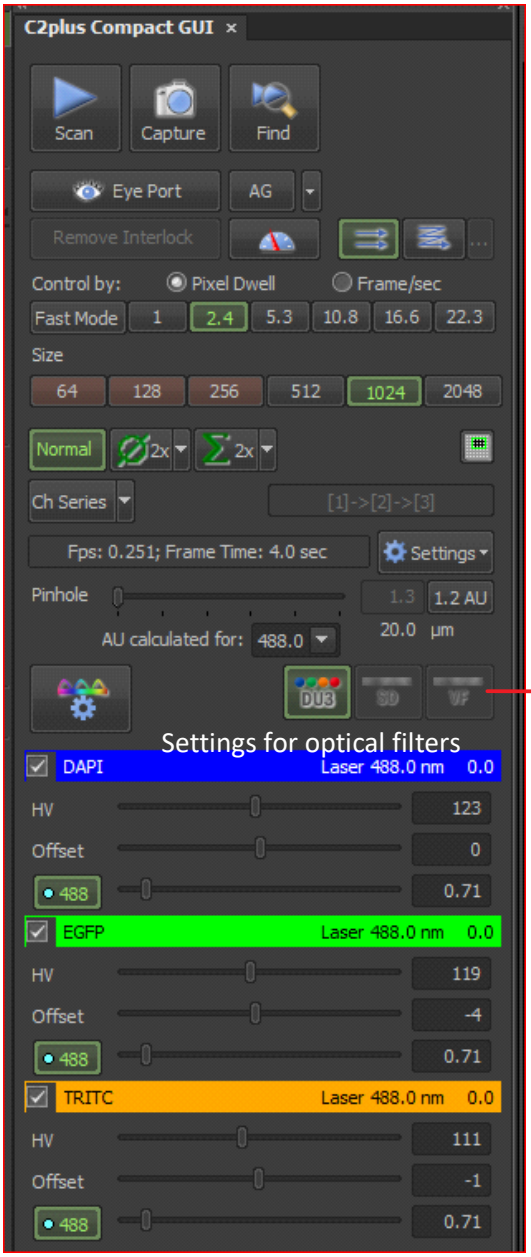
C2sI MARK UPS



Ti Pad


Ti Pad
Nosepiece
10x
20x
60x
40x
1
2
3
4
5
6
Escape
Escape Z
Light Path
E100
L100
R100
L80
PFS
On
Memory
Recall
Focus
Offset:
7191
Dichroic Mirror:
IN
Z Drive
Move by step[µm]:
0.1
1
10
20.0
Z[µm]:
2409.6
Accuracy[µm]:
0.000
Filters
Turret1
Zoom
1.00x
Configure...

C2Plus compact GUI



Scan-starts(stops) laser scanning
Capture will run a single capture
Find-

Eye Port -changes the light path between E100 and L100
Also inserts/removes interlock (when red NO laser emitted)
Also puts last filter cube in light path and turns on SOLA

Pixel Dwell-how long each pixel is excited/sampled
Size- number of pixels in the image

Scan Modes: Normal or Average (n)or Summation(n)

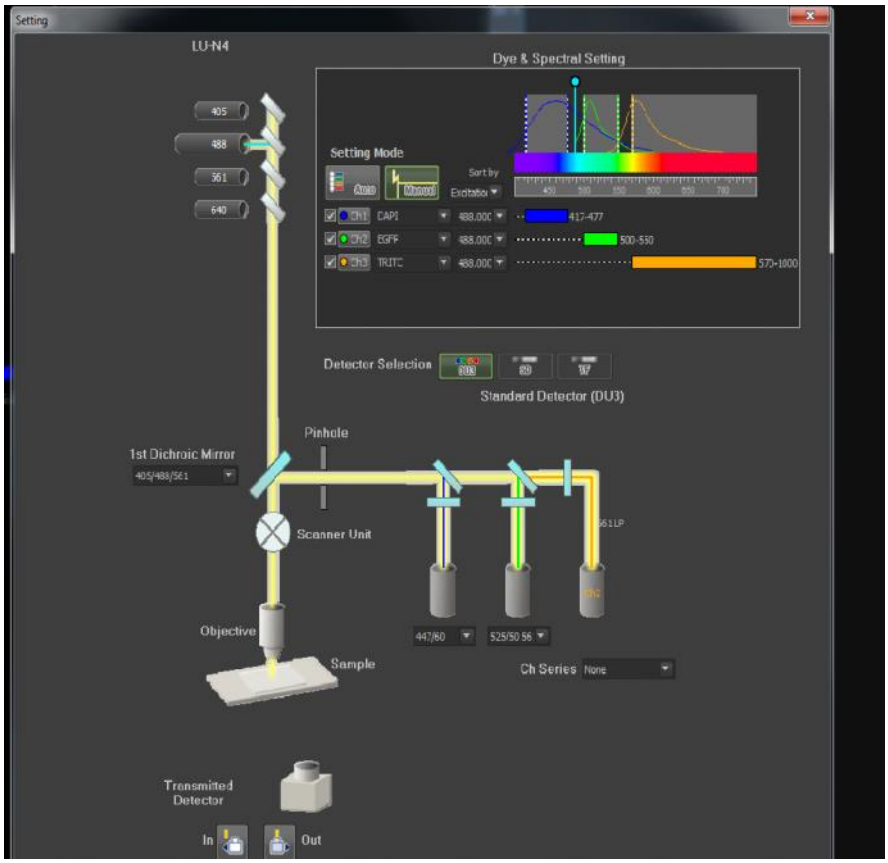
Channel series-each channel is independently scanned

Pinhole- should be set to the smallest value

See **Filter selection**

Check box uses that detector with
HV for voltage of the PMT, offset and laser intensity
AG button at top will attempt auto gain adjust

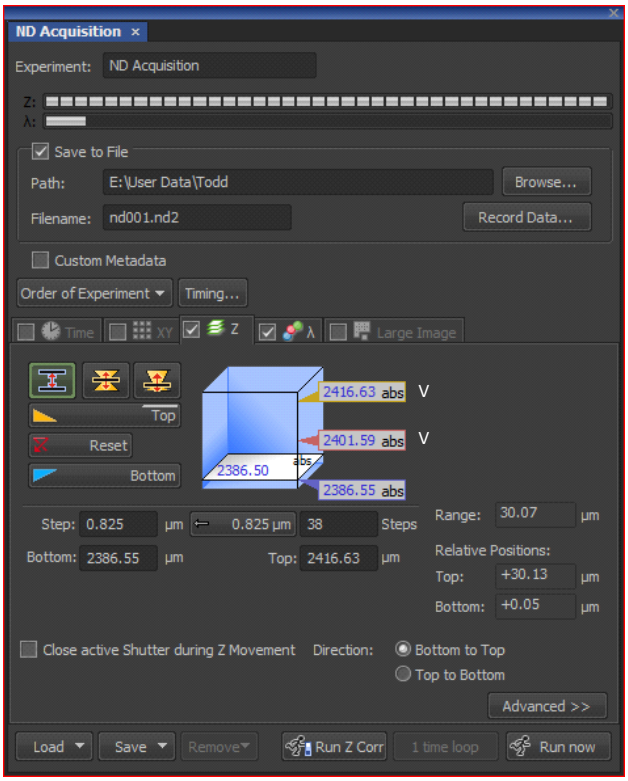
Filter selection



Select Auto (or Manual for advanced use)
Check the detector channel
Select dye from the drop down menu
Only 3 channels can be active at once.
For four channels, use a lambda scan with 2 optical configurations

Dye & Spectral window show the excitation and emission profiles in place

ND acquisition



M

M

V

V

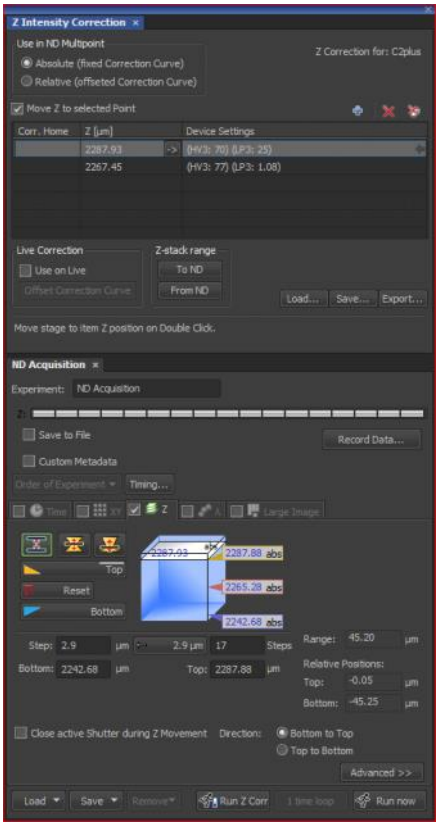
ND ACQ- allows you to add dimensionality to acquisitions
They can be :
Time- for timelapse live imaging
XY- for multiple areas on a slide
Z- optical section of a sample
λ- combination of multiple optical configurations
Large Image- stitch together multiple FOV to create a larger image

Save to file when checked enables the automatic saving of the resultant scan
NOTE: path is always your folder in User Data Folders on the DATA drive

This is a Z set up - Top and bottom are marked at left (M)
The tabs at left (V) allow you to visit those z points to check the quality of the image

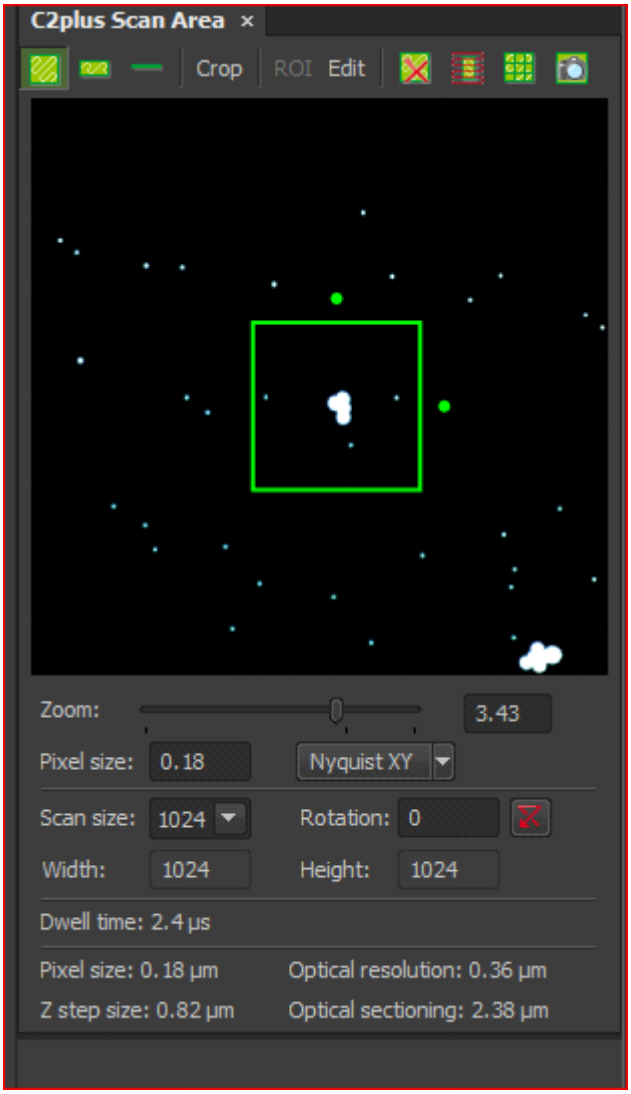
Step is the interval in z Button with arrow offers the optimal Nyquist step size

Run Now initiates the scan series with however many modalities are checked (Z, λ for this example



Z intensity correction enabled then found in Acquisition menu near the bottom
Looks like multiple sheets in icon bar

Scan area



Full FOV, Band scan, Line scan and crop

Box showing current active scan area (on full FOV)
Dots are rotation marks
NOTE: you cannot rotate an edge past the FOV

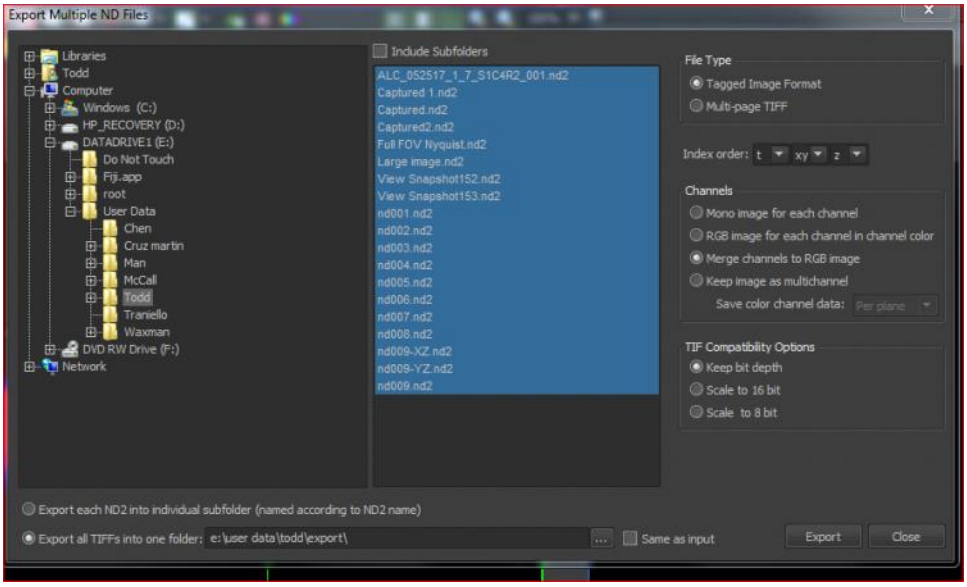
Zoom adjusted with slider, type number
or adjust green box
Nyquist XY will sample FOV at Nyquist criteria

Scan size can also be changed here

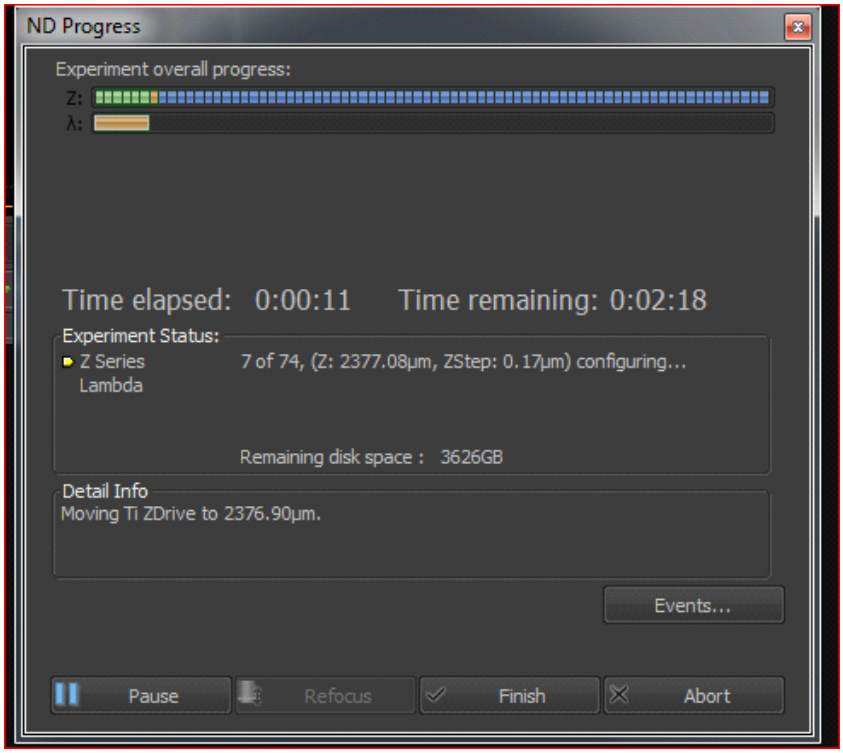
Resolution terms at the bottom are automatically calculated

Export

All ND2 files can be batch converted for easy viewing at the end of a session



Acquisition progress



Each dimension has its own bar with **n** units

Remaining time is displayed

Other info

Pause, finish ends after current cycle, abort quits acq.

Image window

LUT tools

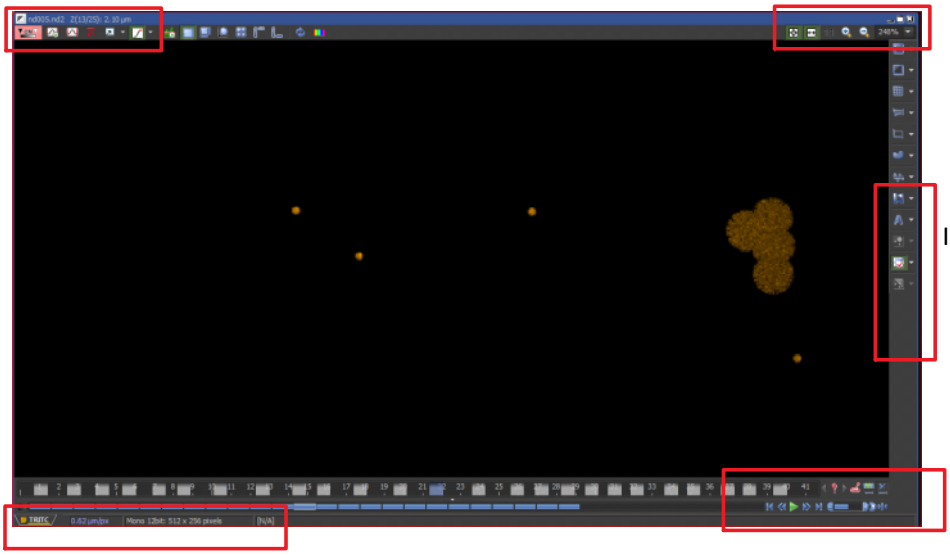


Image zoom and fit to window

Intensity, and overlays-rt click for properties

Image window status bar

Multidimensional playback (Z and or T)

LUTS

Tuesday, August 22, 2017 3:50 PM