Leginon Protocol collection negative stain Fred Hutch Talos L120C

What you're starting with

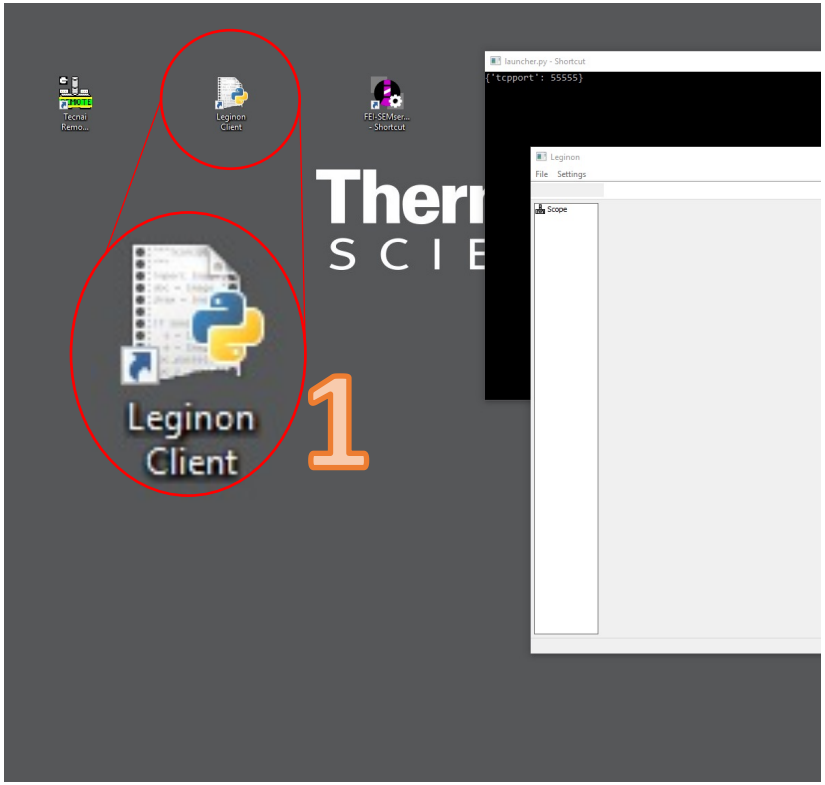
- You have performed the "Talos Start Up Checklist" and have:
  - A cold, vacuum-stable microscope
  - A beam

- You have screened your grid to confirm:
  - Good sample concentration
  - What good staining looks like at low/high magnification
  - Roughly how many images you would like to collect

Slides 4-10 have likely been done while you were screening.
Only redo these if you are starting a collection from the beginning of your session or you want to begin a new session for your collection

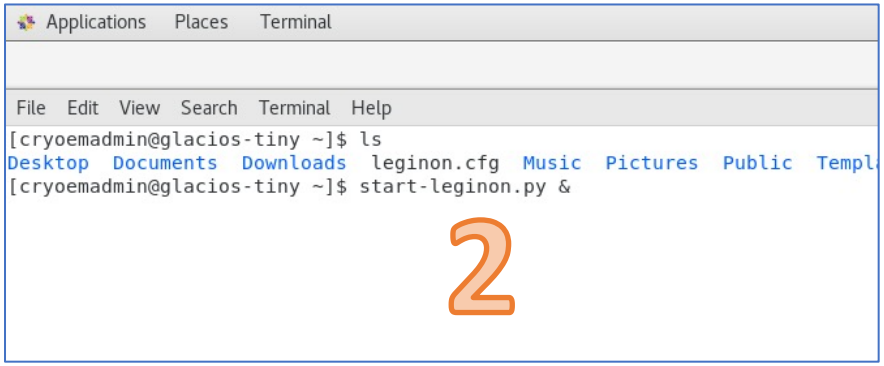
Start Leginon

1. Double click “Leginon Client” on the microscope computer to start
   1. Minimize TIA to find the icon on the right monitor’s desktop
   2. Two windows have to open before you start Leginon



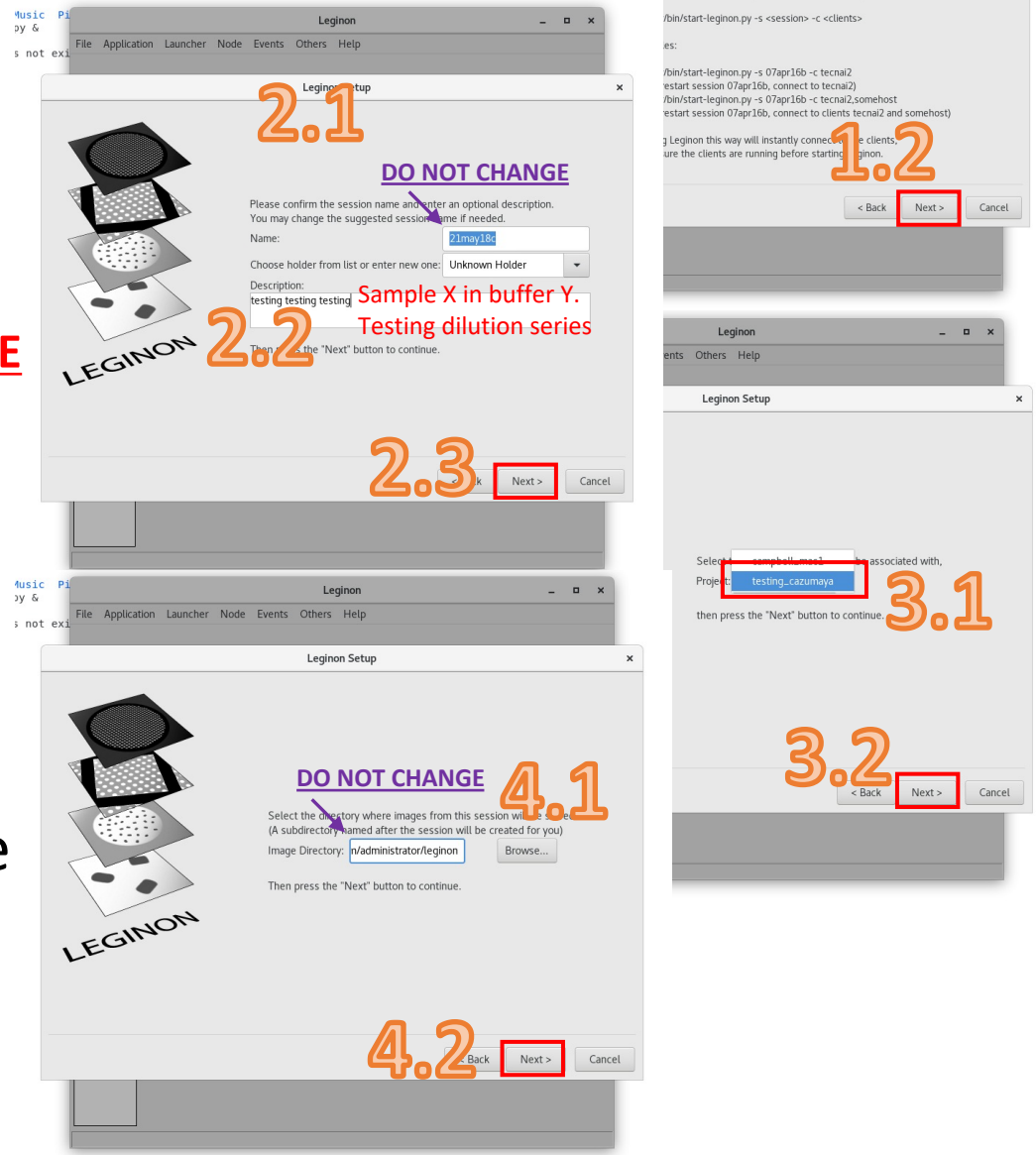
2. Login to Leginon computer
   1. Open terminal (right click on background
   2. start-leginon.py

Leginon-specific login and password



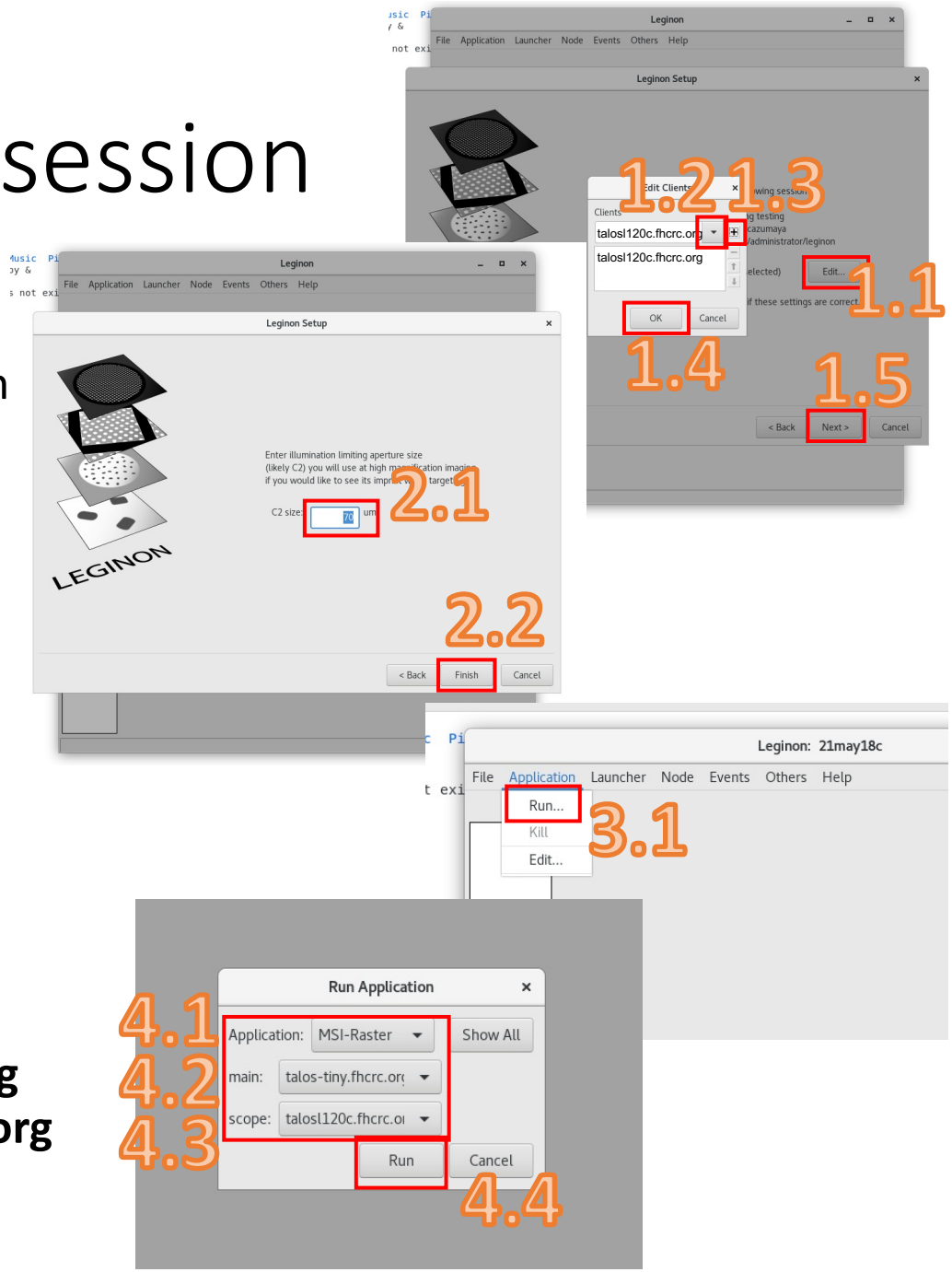
Create Leginon session

1. Choose session
    1. Create a new session
    2. Next
2. Define session
    1. Name: **DO NOT CHANGE**
    2. Description: for your whole session
    3. Next
3. Pick project
    1. Project: Pick from dropdown
    2. Next
4. Choose where to save
    1. Image directory: **DO NOT CHANGE**
    2. Next



Create Leginon session

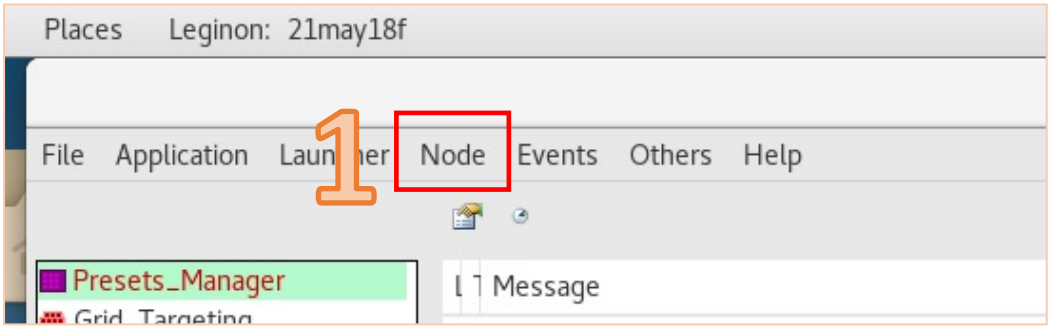
1. Add clients
    1. Edit
    2. talosl120c.fhcrc.org from dropdown
    3. +
    4. OK
    5. Next
2. Define C2 aperture
    1. 70um
    2. Finish
3. Start session
    1. Application -> Run
4. Choose application
    1. Application: MSI-Raster
    2. Main: **talos-tiny.fhcrc.org**
    3. Scope: **talosl120c.fhcrc.org**
    4. Run

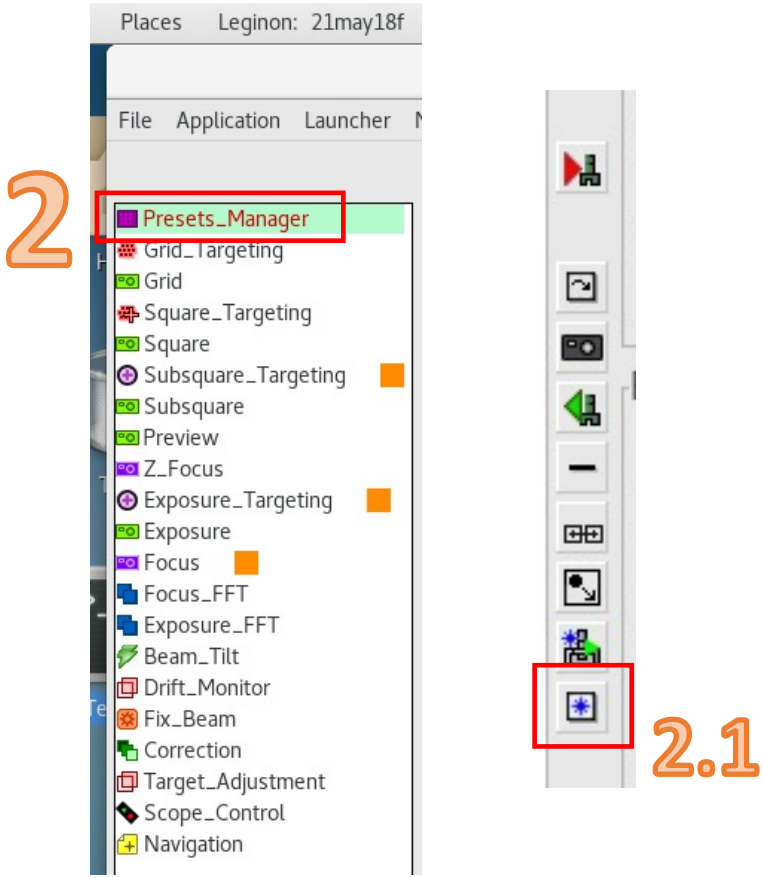


Setup session

1. Node -> Kill -> Preview

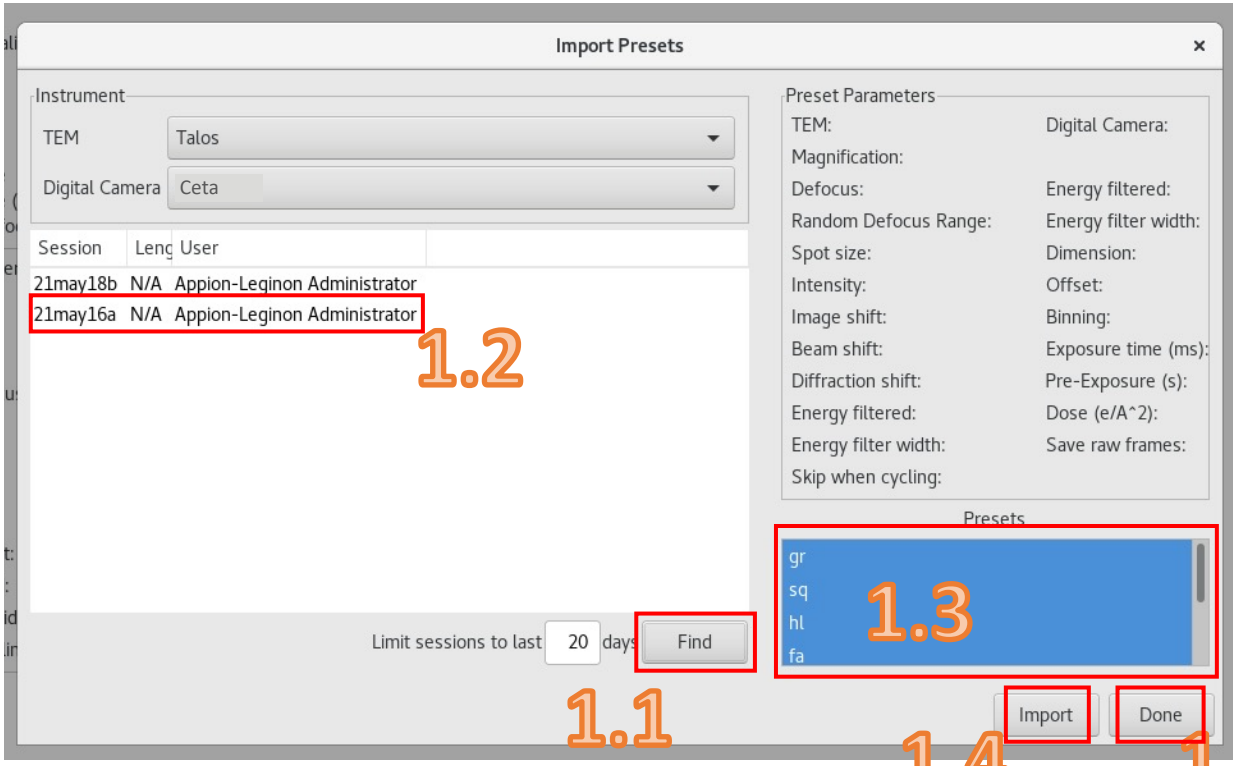
2. Import presets
    1. Presets Manager -> Blue dot icon



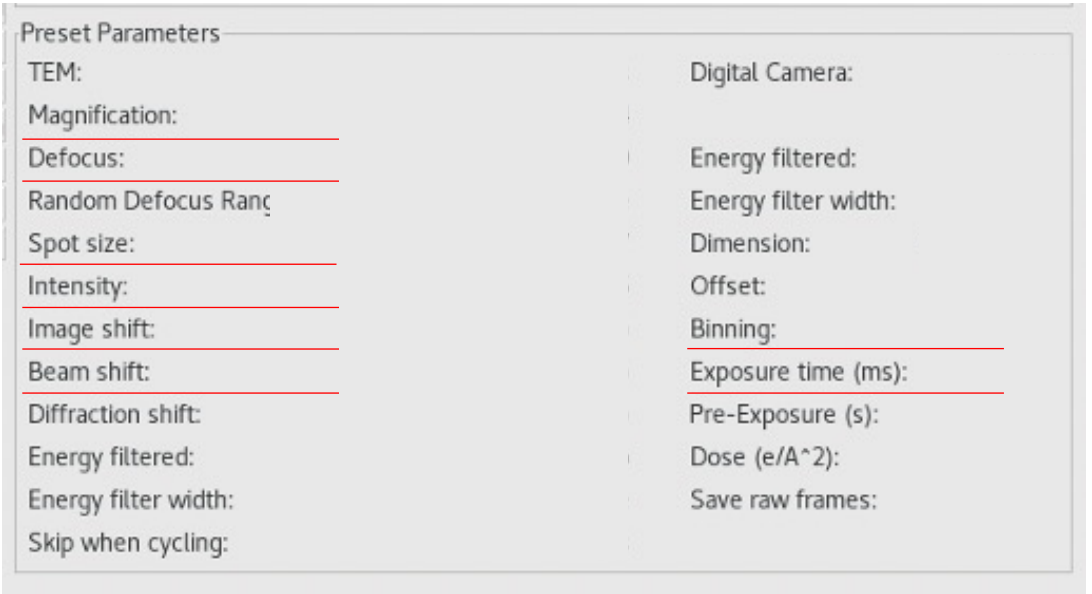


Setup session

1. Import presets
    1. Find
    2. Choose person you trust uses same settings
    3. Highlight all presets
    4. Import
    5. Done



Quick check presets



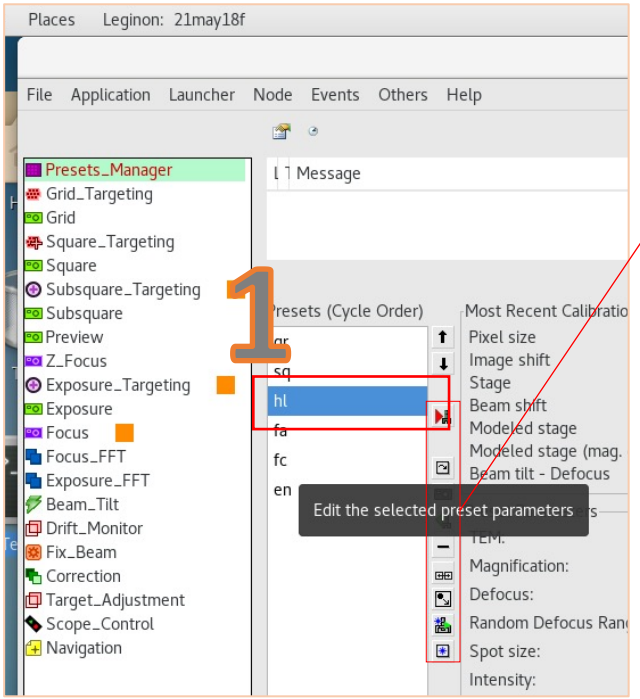
1. What to look at:
    1. Magnification
    2. Defocus
    3. Spot size
    4. Intensity
    5. Image shift
    6. Beam shift
    7. Binning
    8. Exposure time

Recommended Presets ->

	gr	sq	hl	fa	fc	en
1	84	1250	4300	same as en	same as en	you choose
2	-.002	-.002	-8e-05	-2e-06	-1e-06	-1.5e-06
3	7	7	7	6	6	6
4	~1.0	~0.88	~0.62	Cover the screen	Fill the large red circle on flu screen	Cover the screen
5	non-zero	non-zero	non-zero	0	0	0
6	non-zero	non-zero	non-zero	0	0	0
7	2x2	2x2	2x2	2x2	2x2	1x1
8	500	500	500	500	200	1000

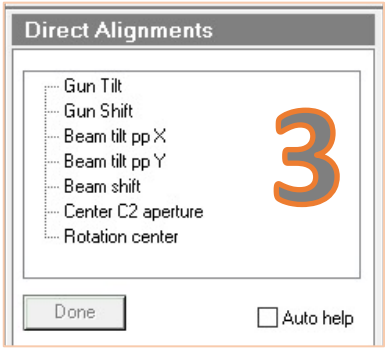
To change your presets

1. Select preset you want to alter
2. Click Send to microscope
3. Adjust on microscope
4. Click Get from microscope



You should be near eucentric height when you adjust

- Press eucentric focus before you center fa, fc, en
- ONLY adjust fa, fc, en beam shift with “Direct Alignments”
- ONLY adjust hl, sq, gr beam shift with roller ball
- Adjust beam with apertures you will use inserted
- Make sure defocus value is not changing when you Get from microscope
- Camera is ~a big as fluscreen at high mags



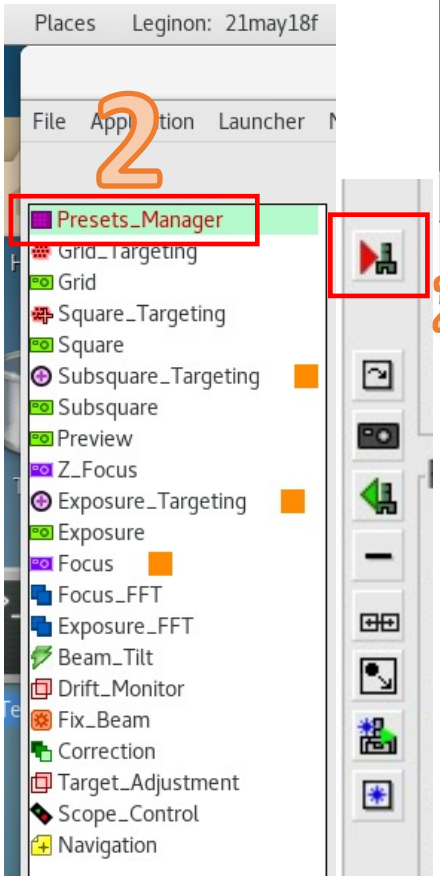
Find good square(s)

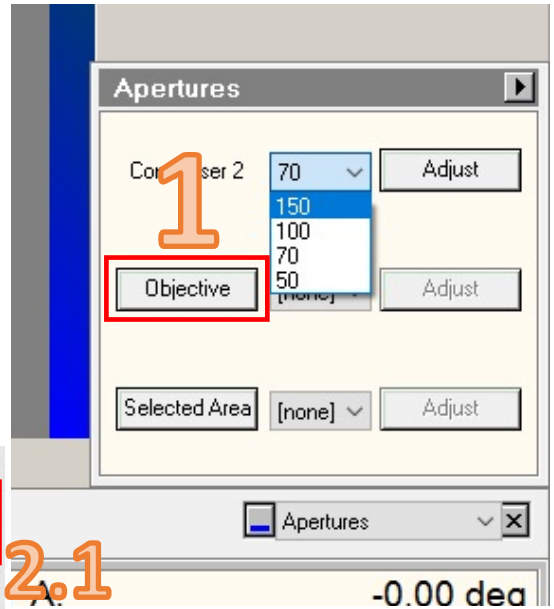
1. Click ”Objective” on microscope “Apertures” to remove (will turn grey)

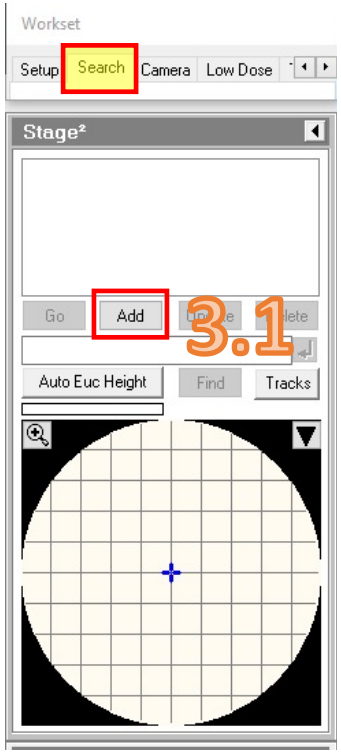
2. Go to Presets Manager
   1. Highlight gr and send to scope

3. Insert screen on microscope (handpanel R1)

4. Use joystick to navigate around and choose a square
   1. Click “Add” to mark squares of interest in “Search” tab on microscope TUI

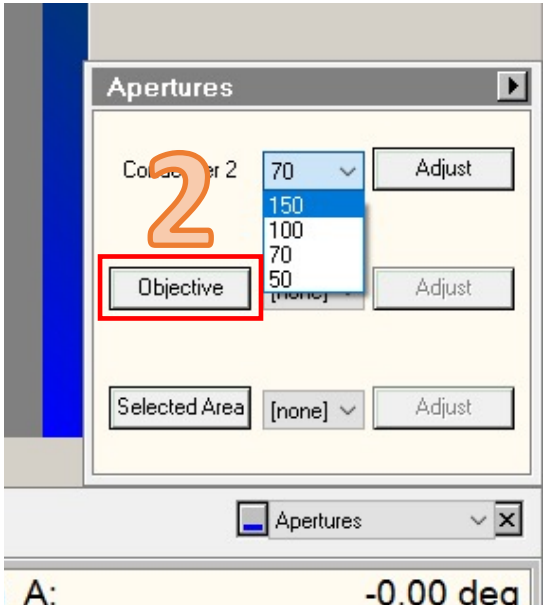


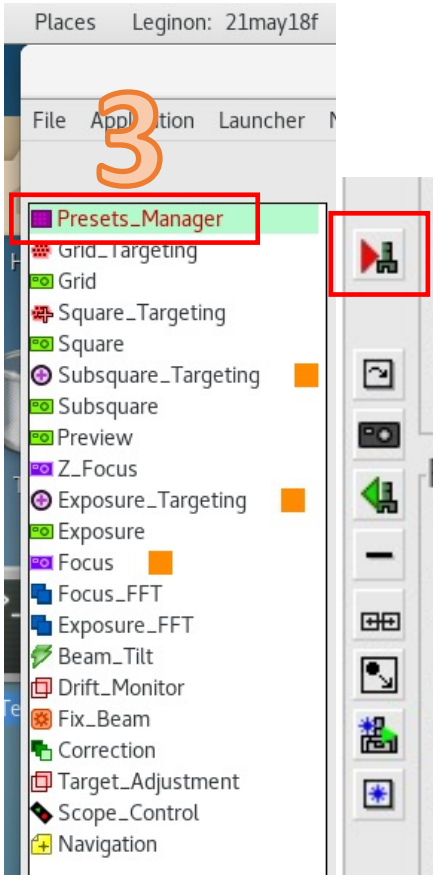




Find alignment square

1. Use joystick to navigate to a clear feature you can find in en, hl, and sq
2. Click “Objective” in ”Apertures” to insert (will be yellow)
3. Send sq to the microscope and center on feature
4. Send hl to the microscope and center on feature
5. Send en to the microscope and center on feature

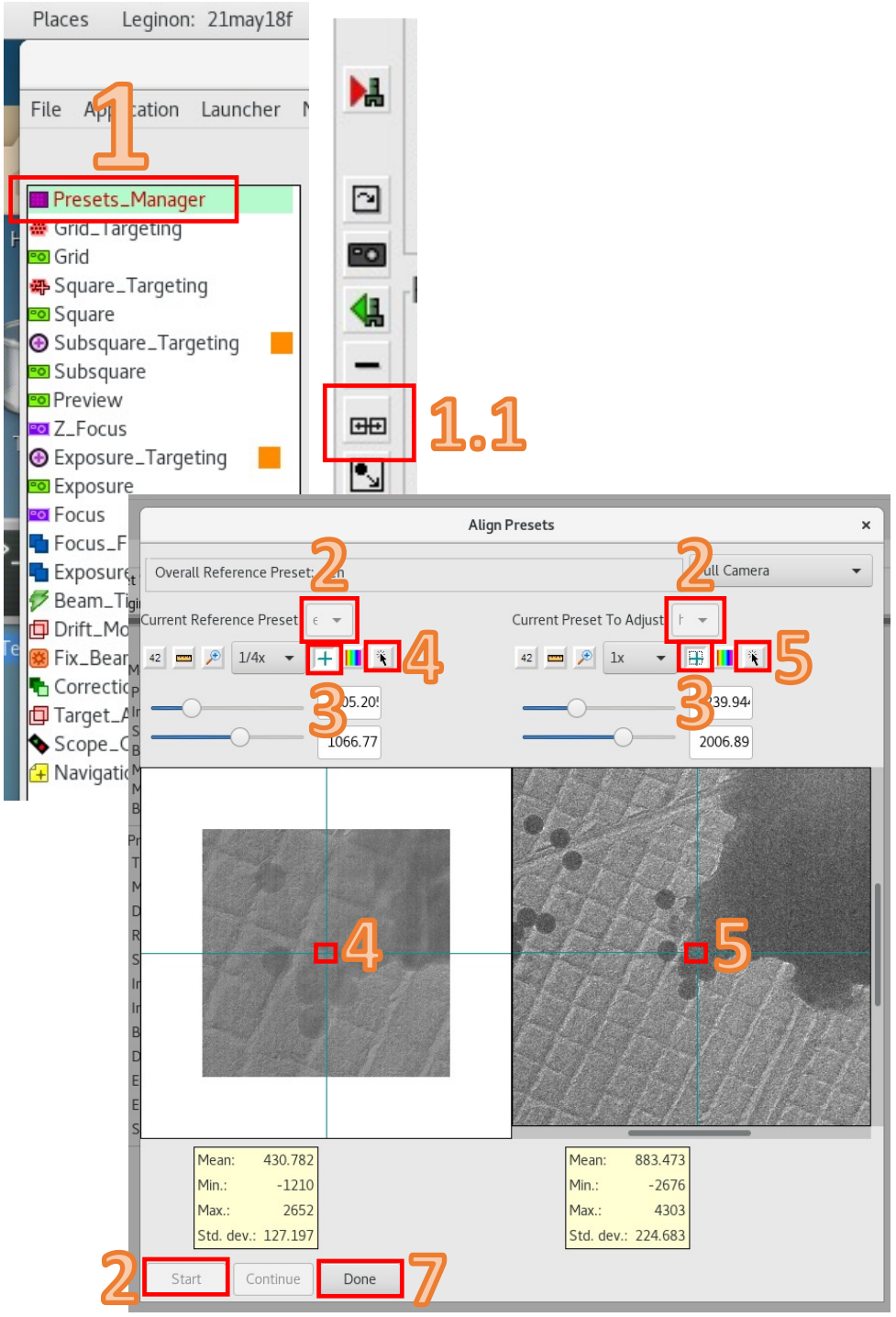




3, 4, 5

Align en and hl

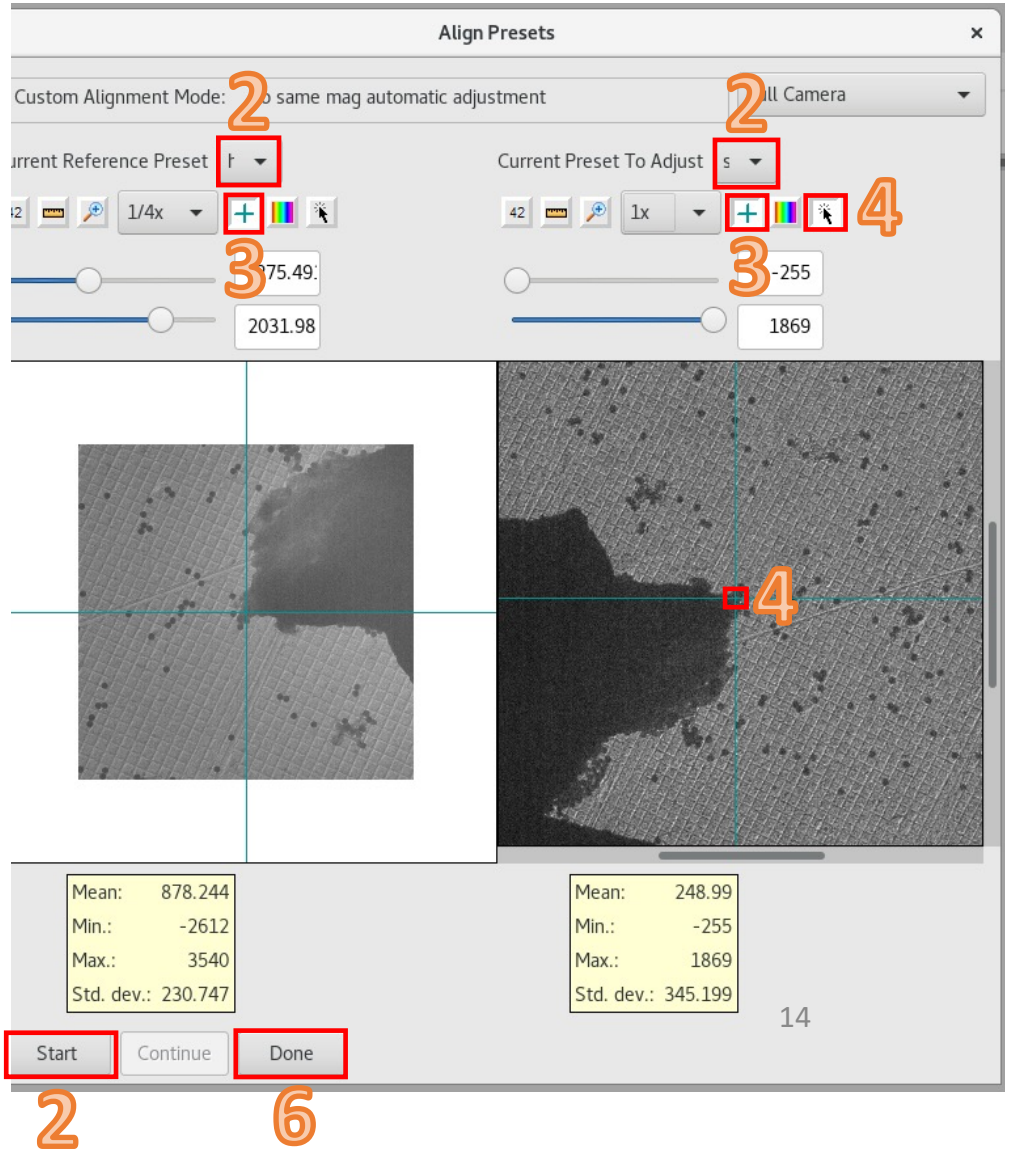
1. In Presets Manager:
    1. Click 'Align Presets'
2. Select en on left and hl on right and click Start
    1. Wait for images to populate
3. Turn on crosshair for both views
4. Turn on clicker and center feature in en by clicking on image
    1. Wait for scope to react (it's moving the stage)
5. Turn on clicker and center feature in hl by clicking on image
    1. Wait for scope to react (it's moving the beam/image)
6. Click on hl image again if it doesn't get there the first time
7. Click Done when they are centered



Align hl and sq

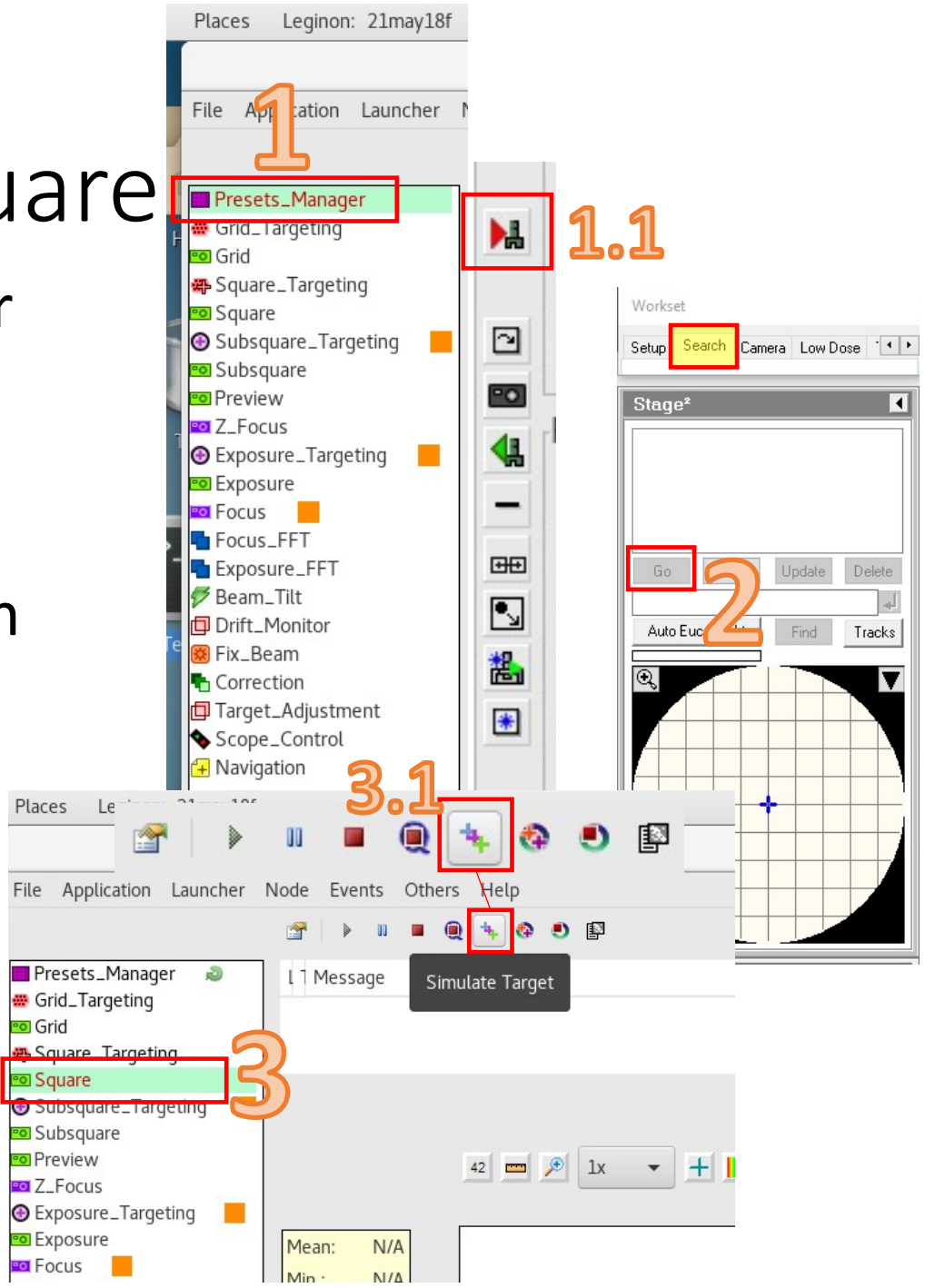
There is a 180deg rotation when moving from hl to sq so choose a feature with directionality!!

1. In Presets Manager:
   1. Click 'Align Presets'
2. Select hl on left and sq on right and click Start
   1. Wait for images to populate
3. Turn on crosshair for both views
4. Turn on clicker and center feature in sq by clicking on image
   1. Wait for scope to react (it's moving the beam)
5. Click on sq image again if it doesn't get there the first time
6. Click Done when they are centered



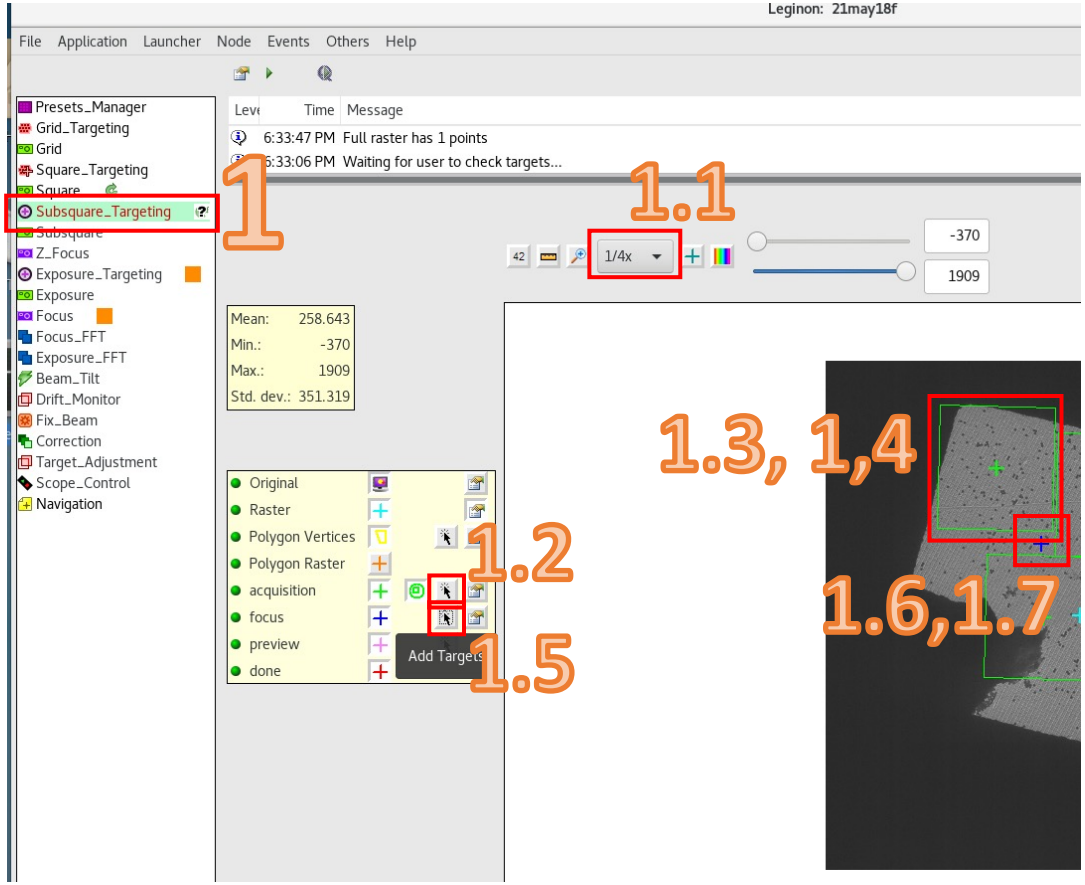
Go to good square

1. Go to Presets Manager
    1. Highlight sq and send to scope
2. ”Go” to good square from saved positions in Search tab
3. Go to Square node
    1. “Simulate Target”
4. Repeat 2&3 for all good squares for collection



Add Subsquare and Z-focus targets

1. Go to Subsquare Targeting
    1. Zoom out (~1/4x)
    2. Select acquisition
    3. Right click on a target to remove
    4. Left click to add targets where you want to image
    5. Select focus
    6. Right click to remove Z-focus spot if needed (do not focus near grid bar)
    7. Left click to add Z-focus spot



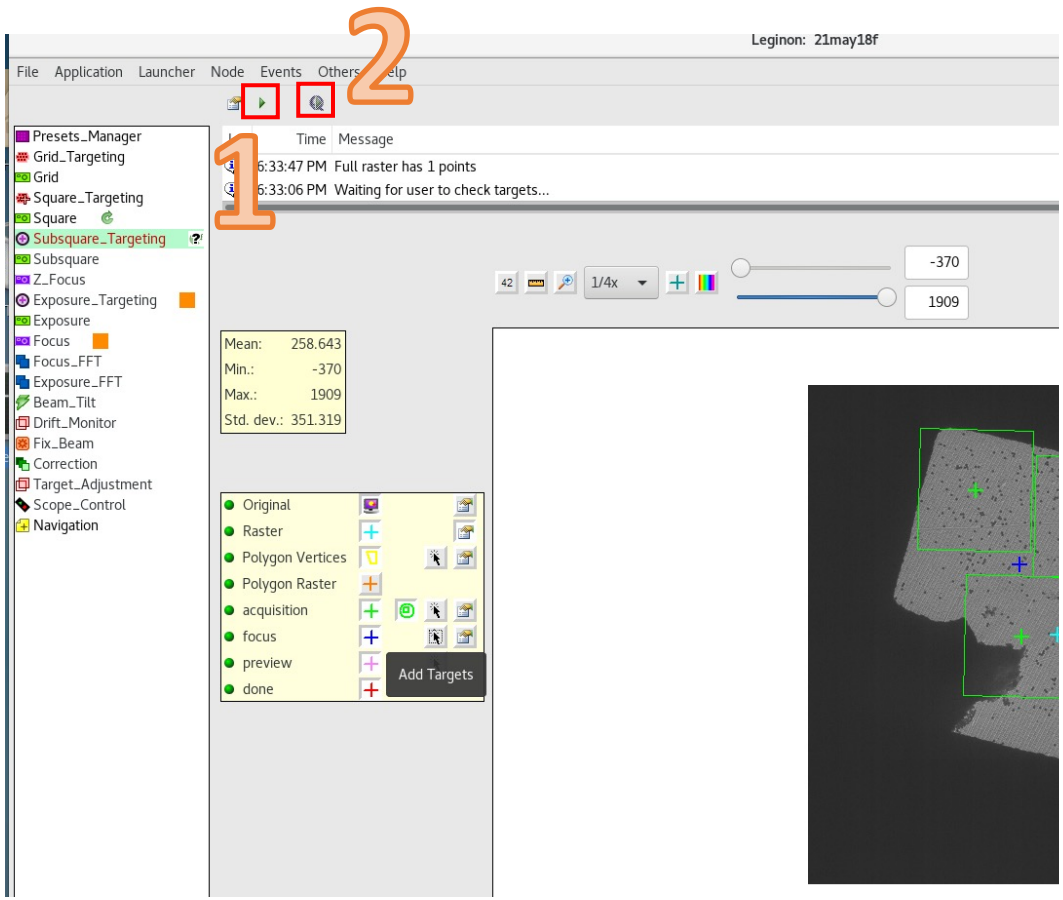
Green boxes will be approximate images
Dark blue + is Z-focus spot (put in middle)

Submit subsquare targets

1. Click “play” button to add targets to put targets in queue

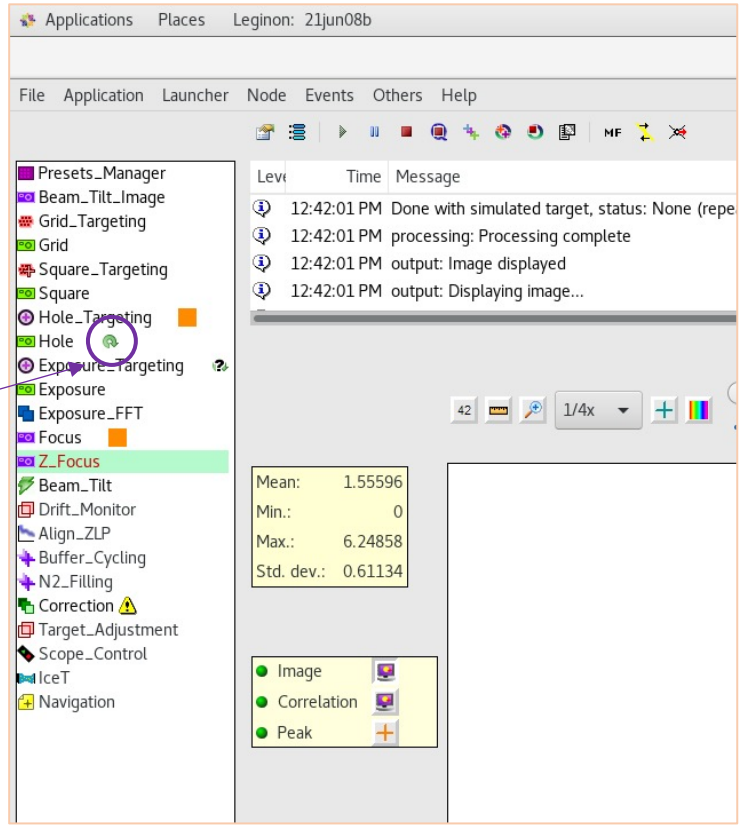
2. Click “Qplay” button to submit the queue for collection

Focus sequence will start to run through “Target Adjustment”, “Z-Focus”, and ”Subsquare”



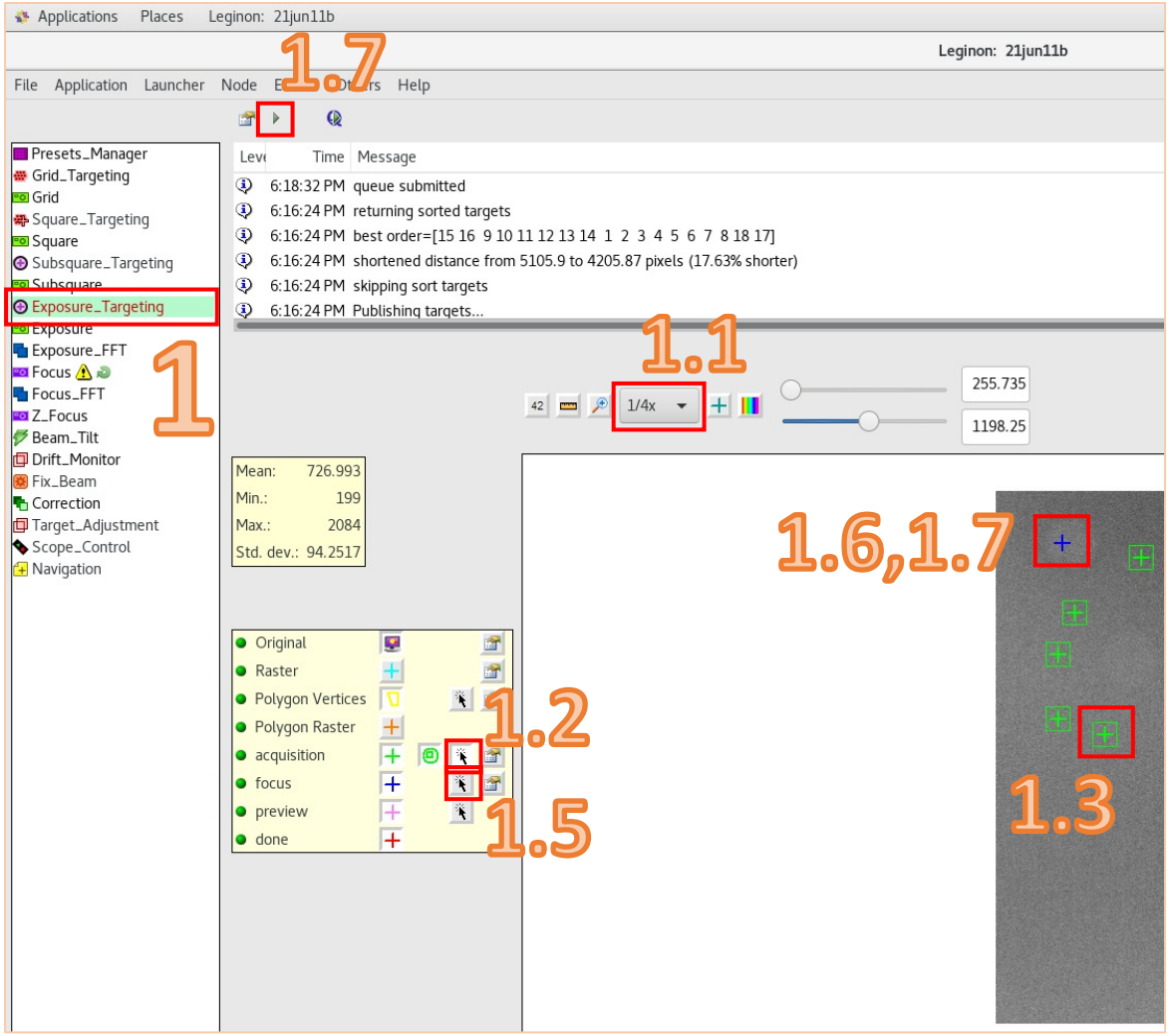
Do not submit exposure queue before subsquare queue finishes

Wait for green arrows by Z-focus and Hole to stop turning before submitting Exposure queue!!



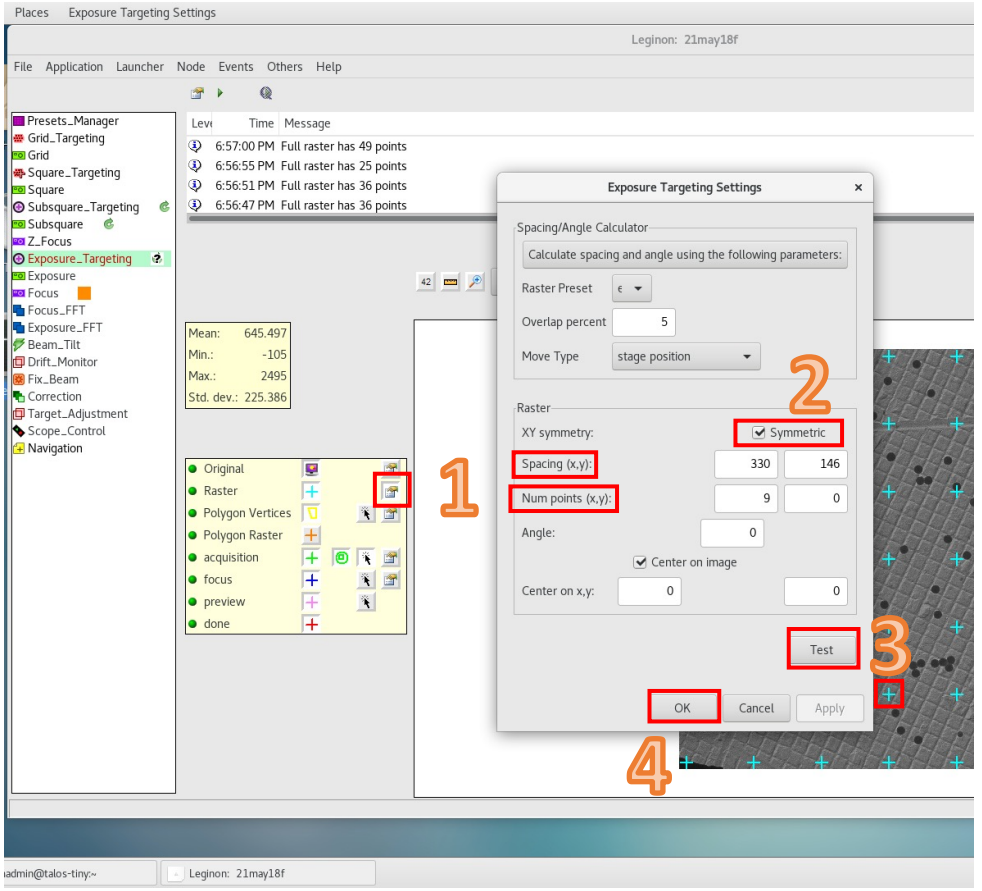
Accept or move exposure and focus targets

1. Go to Exposure Targeting
    1. Zoom out (~1/4x)
    2. Select acquisition 
    3. Right click on a target to remove any you don't need
    4. Select focus 
    5. Right click to remove focus spot if needed (do not focus on anything dark!)
    6. Left click to add focus spot
    7. Click "play" button to add targets to put targets in queue



Alter your raster spacing (usually just one time)

1. Click pointer finger by ‘raster’ to adjust spacing and number of points to overlap the amount you want (usually none)
    1. Right -> is not typical – suggestions on Teams
2. Having ‘symmetric’ checked it will only read in X values
3. ‘Test’ to see how it will look – light blue +
4. ‘Ok’ to accept for future subsquares

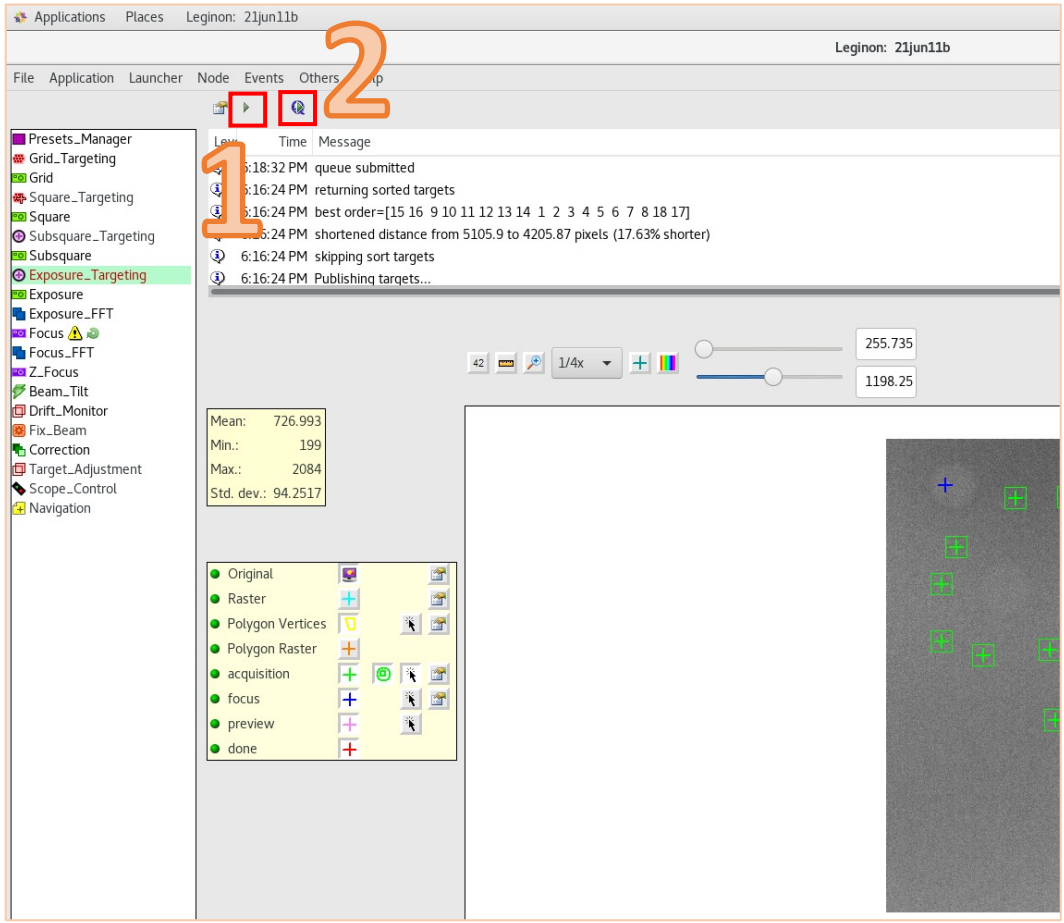


Submit exposure targets

Subsquares queue finished? At least one square of exposure targets queued?

1. Click “Qplay” button to submit the queue for collection

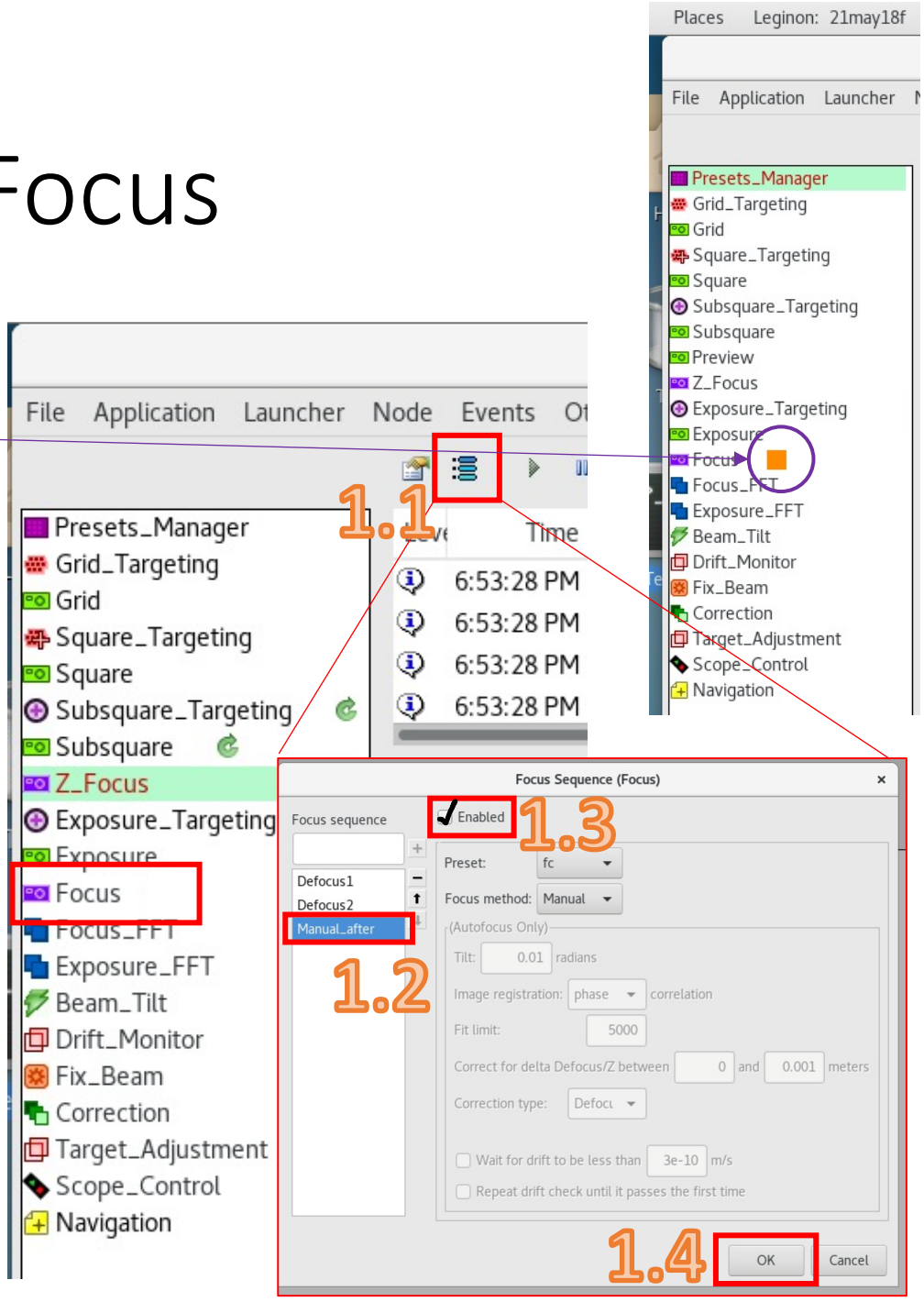
Focus sequence will start to run through “Target Adjustment”, “Drift Monitor”, ”Focus”, and “Exposure”



Enable Manual Focus

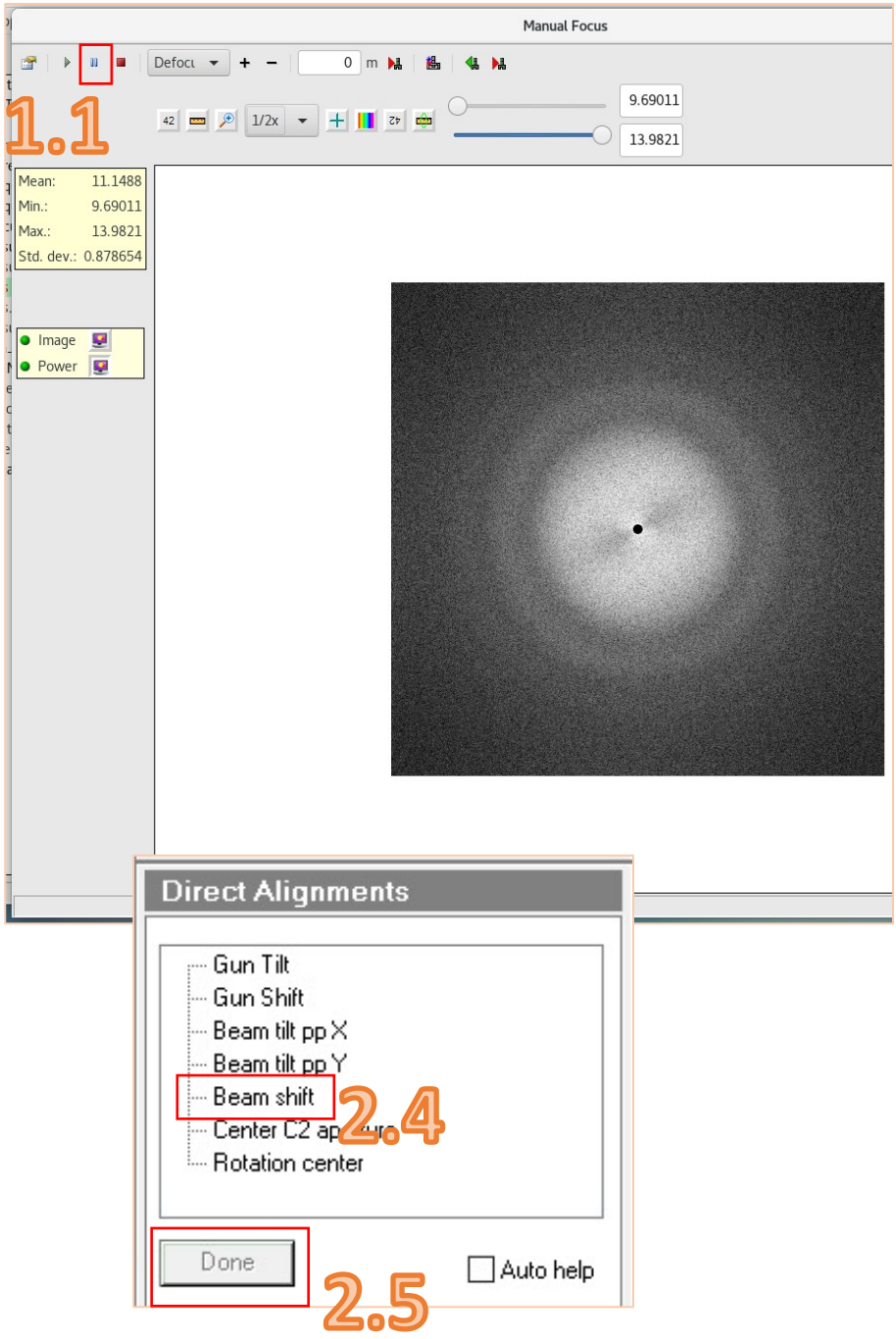
Orange square means it is enabled.

1. If not, Go to Focus
    1. Click blue bullet list
    2. Select Manual_after
    3. Check Enabled
    4. Ok



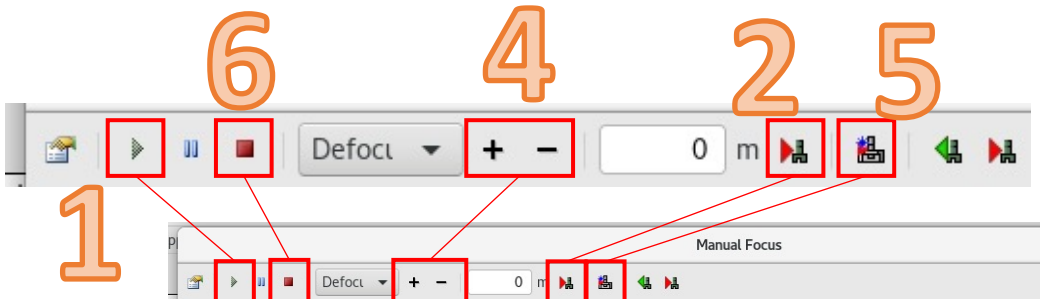
Manual Focus

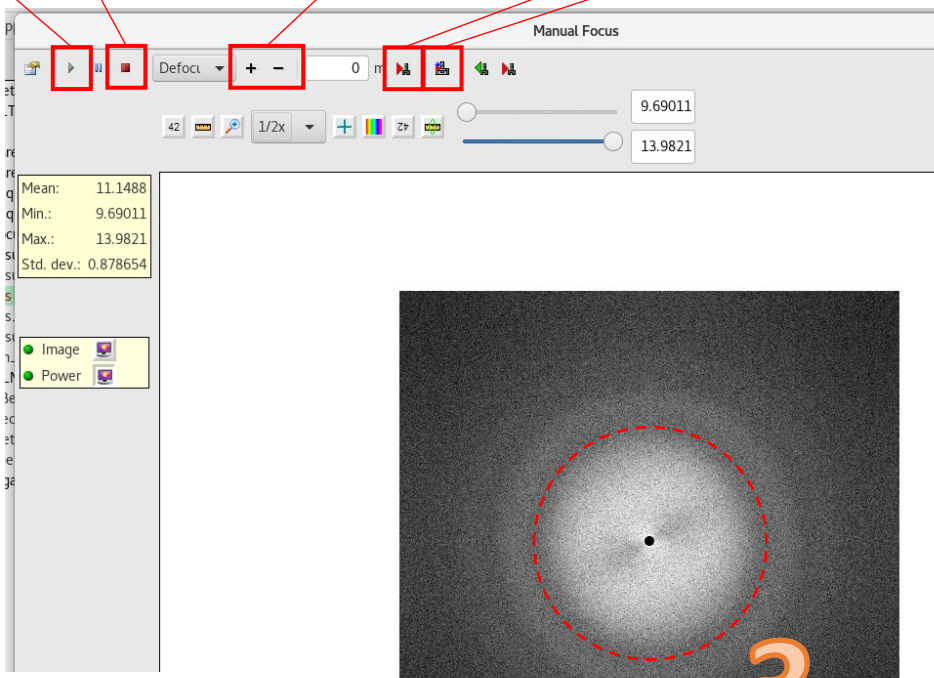
1. Manual focus window will pop up
    1. Pause
2. On microscope PC:
    1. Insert screen (handpanel R1)
    2. Press ”Eucentric focus” on handpanel
    3. Press “Reset defocus” on handpanel
    4. Click “Beam shift” in “Direct Alignments” and center beam with multifunction X and Y knobs
    5. Done

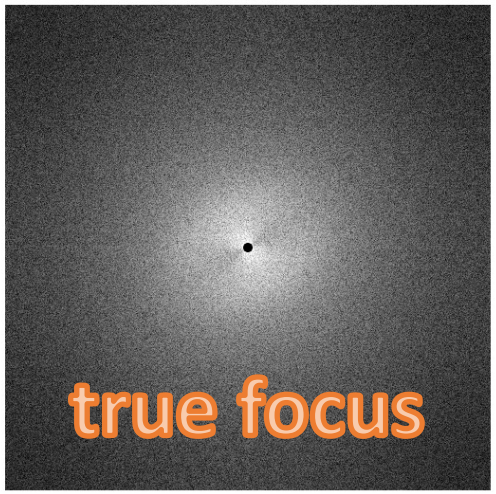


Manual Focus

1. (In Manual Focus) Play
2. Send 0 to microscope
3. If not at true focus (no thon rings): click on first zero of the FFT
4. Click + or - to get to true focus
5. Click Reset defocus
6. Stop

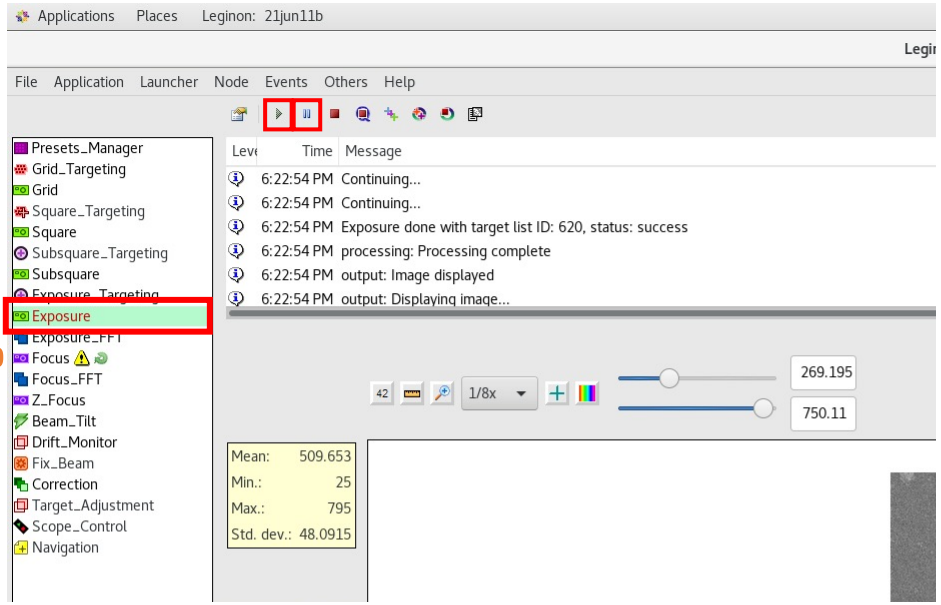


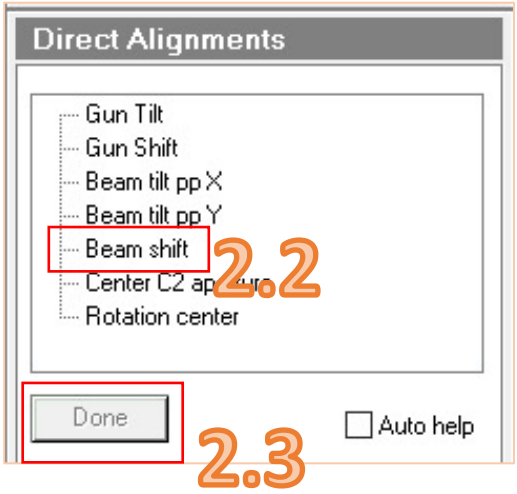




Monitor exposures

1. Go to Exposure
    1. If you can see the edge of the beam: Pause
2. On Microscope computer
    1. Insert screen (handpanel R1)
    2. Click “Beam shift” in “Direct Alignments” and center beam with multifunction X and Y knobs
    3. Done
3. Back in Exposure: Play

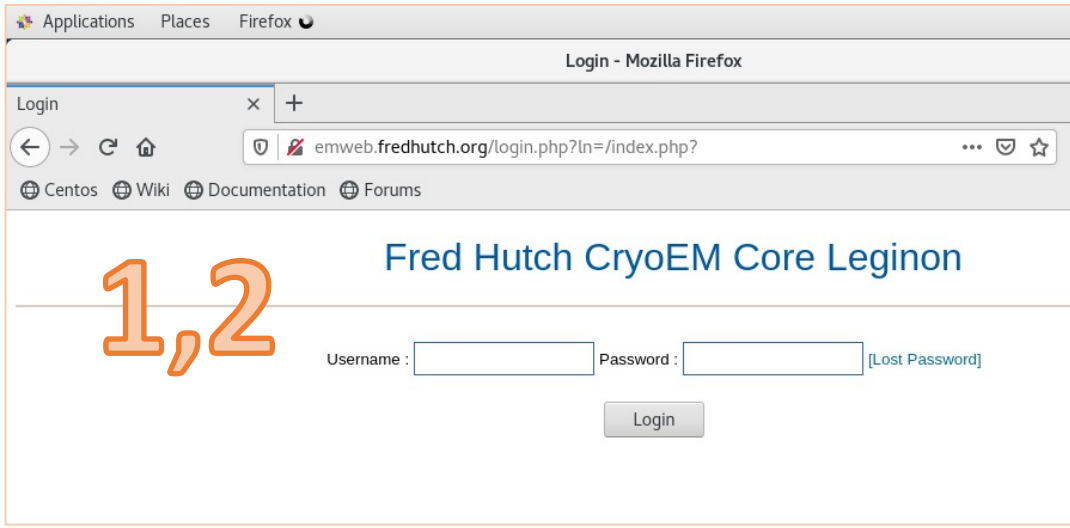




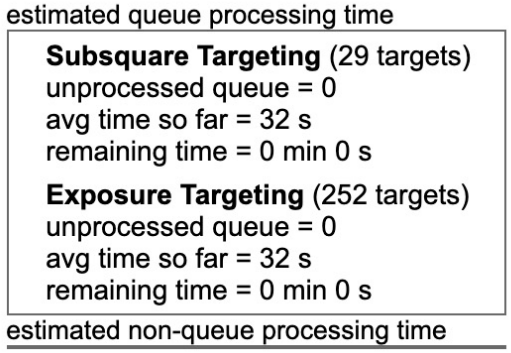
Monitor exposures

1. Open the internet on the Leginon computer or anywhere you are on VPN
   1. emweb.fredhutch.org

2. Sign in with your leginon username and password



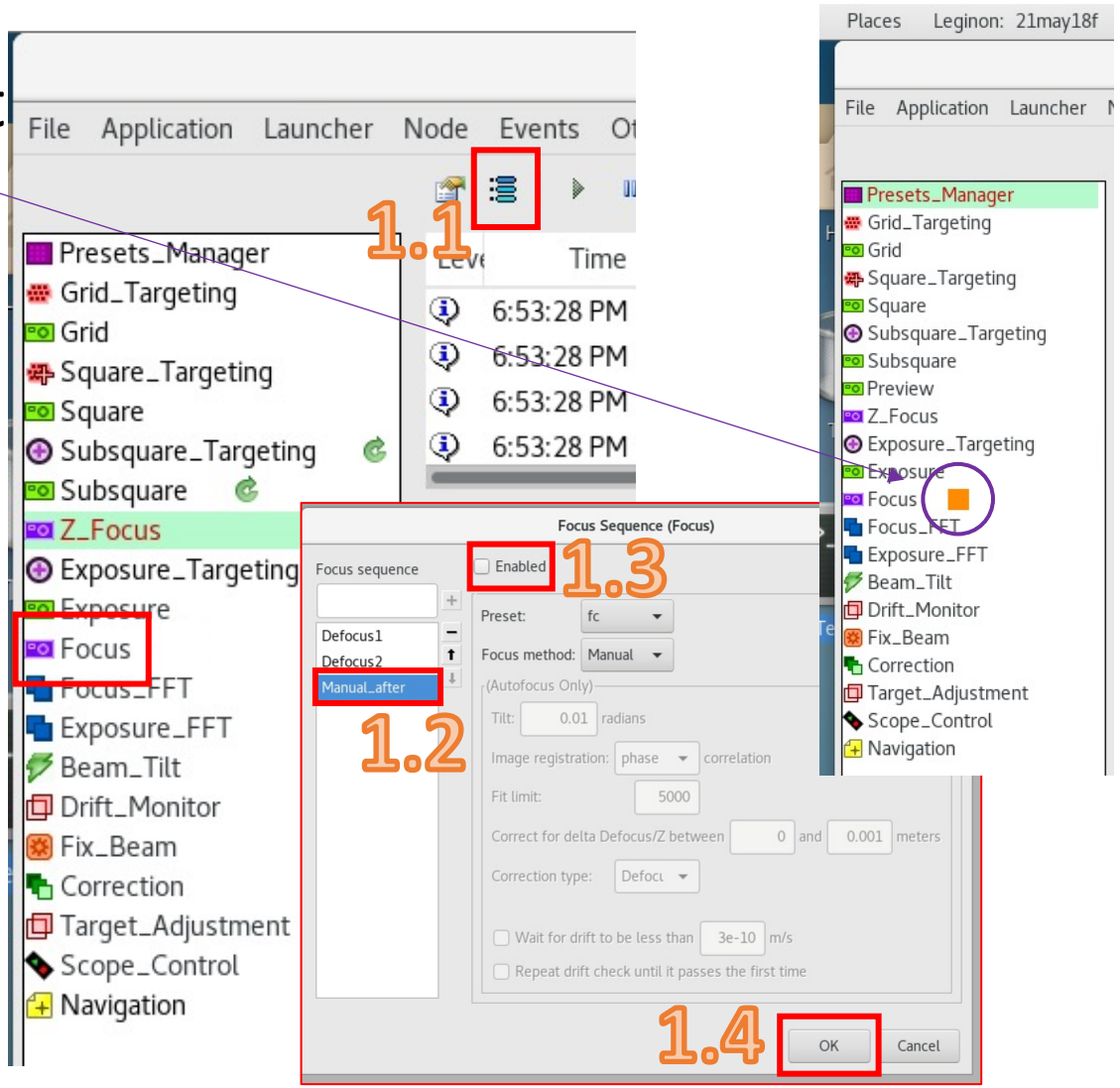
- View images in image viewer or 3-way image viewer
- Compare between sessions in 2-way image viewer
- Use LOI to determine how much time is left in your collection
  - More info on webserver on Teams channel



Disable Manual Focus after first subsquare (or two if it's being difficult)

Orange square means it is enabled.

1. Go to Focus
    1. Click blue bullet list
    2. Select Manual_after
    3. Uncheck Enabled
    4. Ok



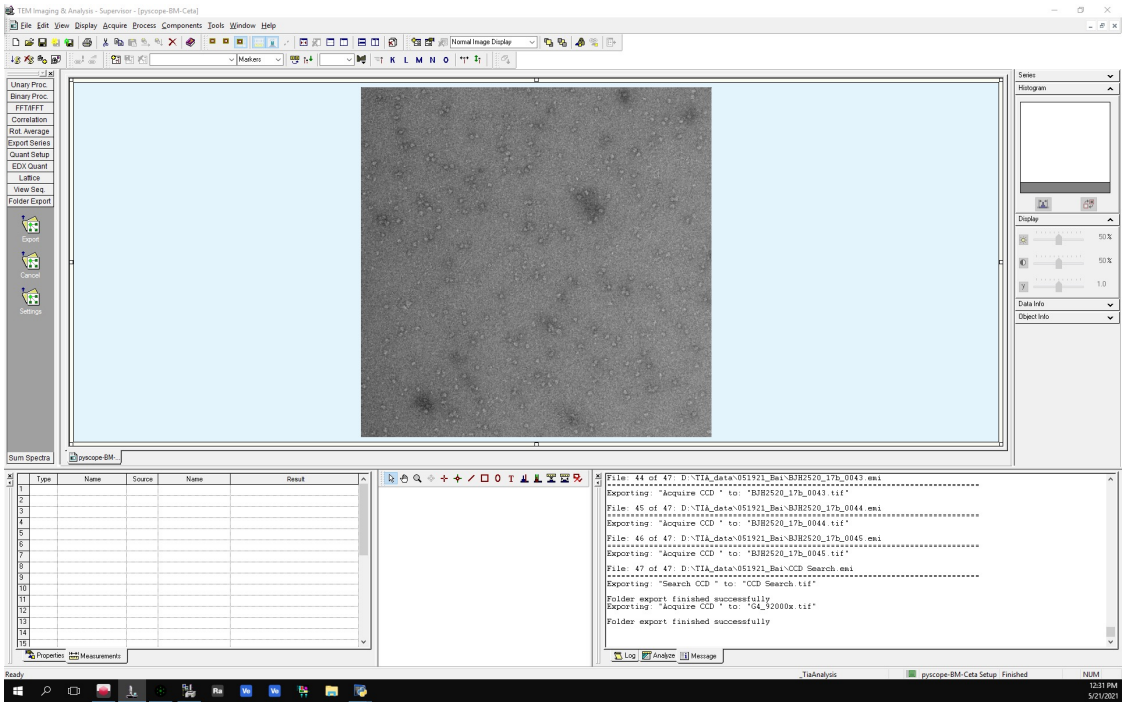
Do not leave the room unless:

- Autofocus is working (can send zero to scope successfully)
- Manual focus is disabled (no orange box)
- The beam is centered (monitor exposures)
- Your images look good

- If you would like remote access to Leginon computer, discuss with Caleigh.
- You cannot move the beam remotely!
- Make sure you have door access to the suite and the Talos room

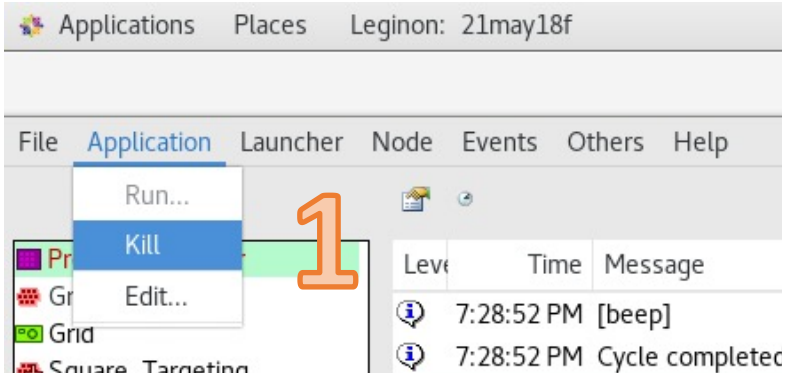
When you leave the room:

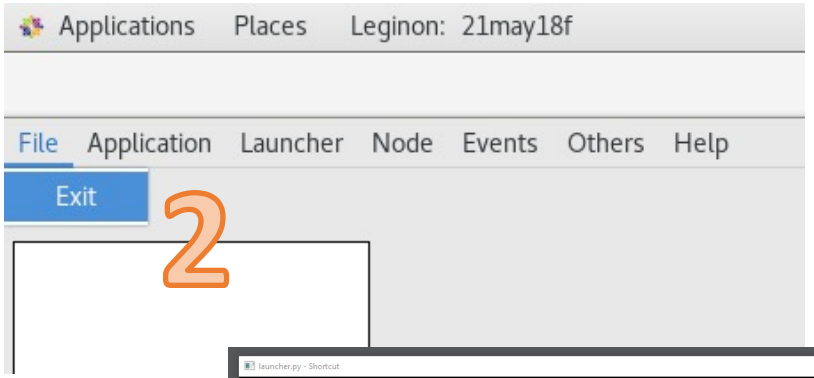
- Leave TIA window up when you leave the room and Leginon is running so people can see the microscope is occupied and running

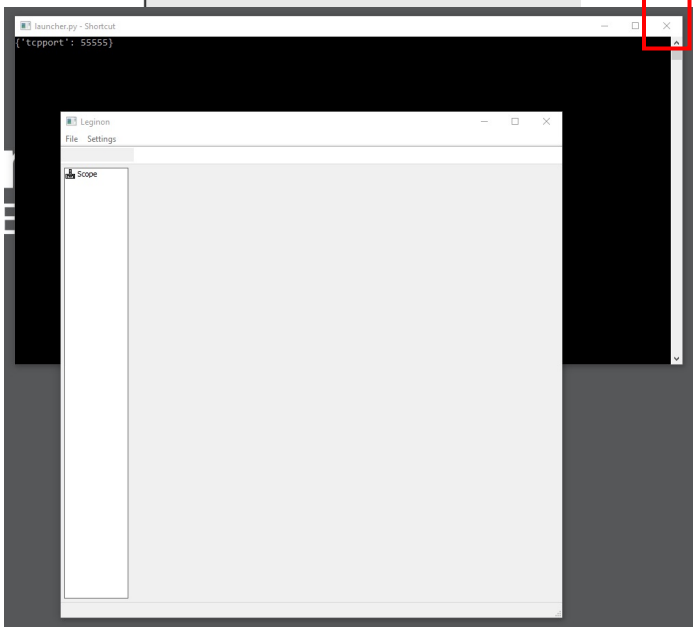


Shutdown Leginon

1. Application -> Kill
2. File -> Exit
3. Logout of computer
    1. Power logo ->
       Username -> Sign out
4. Close client on microscope computer







Do shutdown Talos check list!

!! Most important: Close the column valves and turn the filament off !!

End iLab time and sign out!