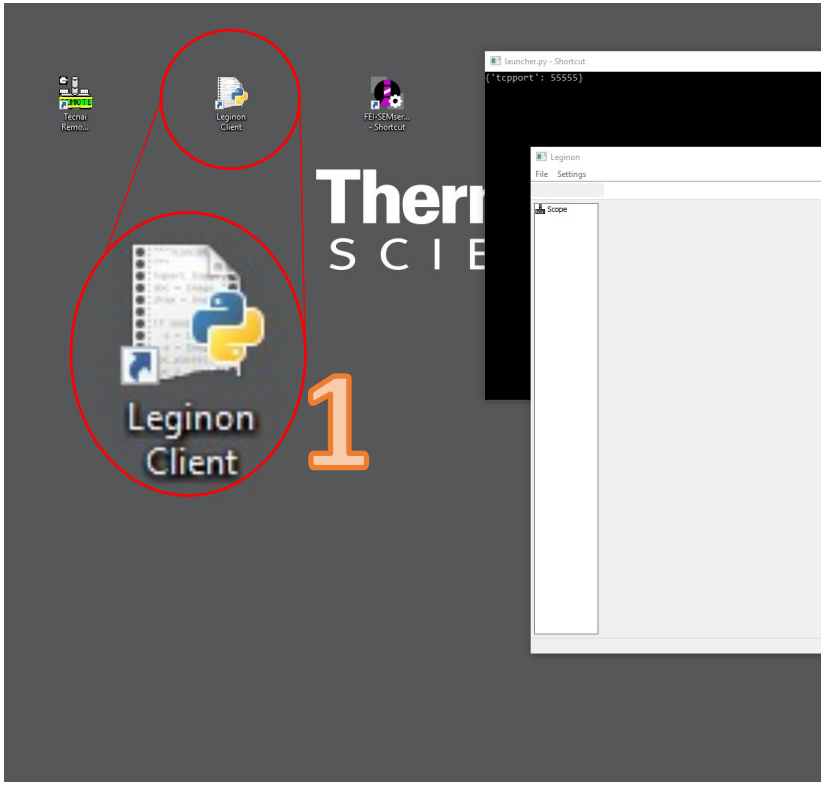
Leginon Protocol negative stain screening Fred Hutch Talos L120C

What you're starting with

- You have performed the "Talos Start Up Checklist" and have:
  - A cold, vacuum-stable microscope
  - A beam
  - Your sample inserted in RT single tilt holder

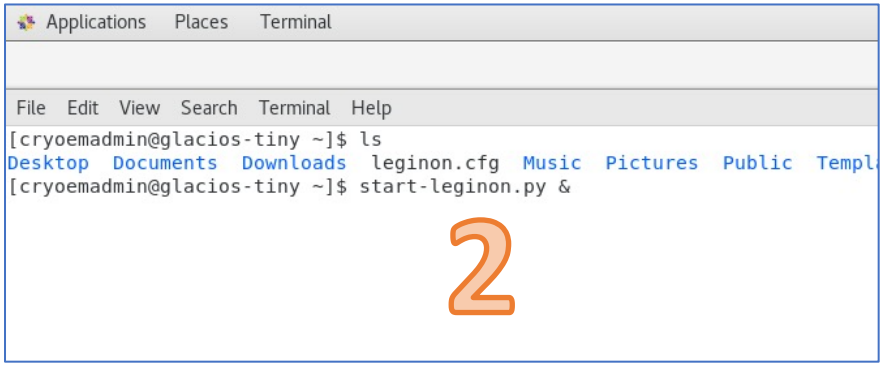
Start Leginon

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



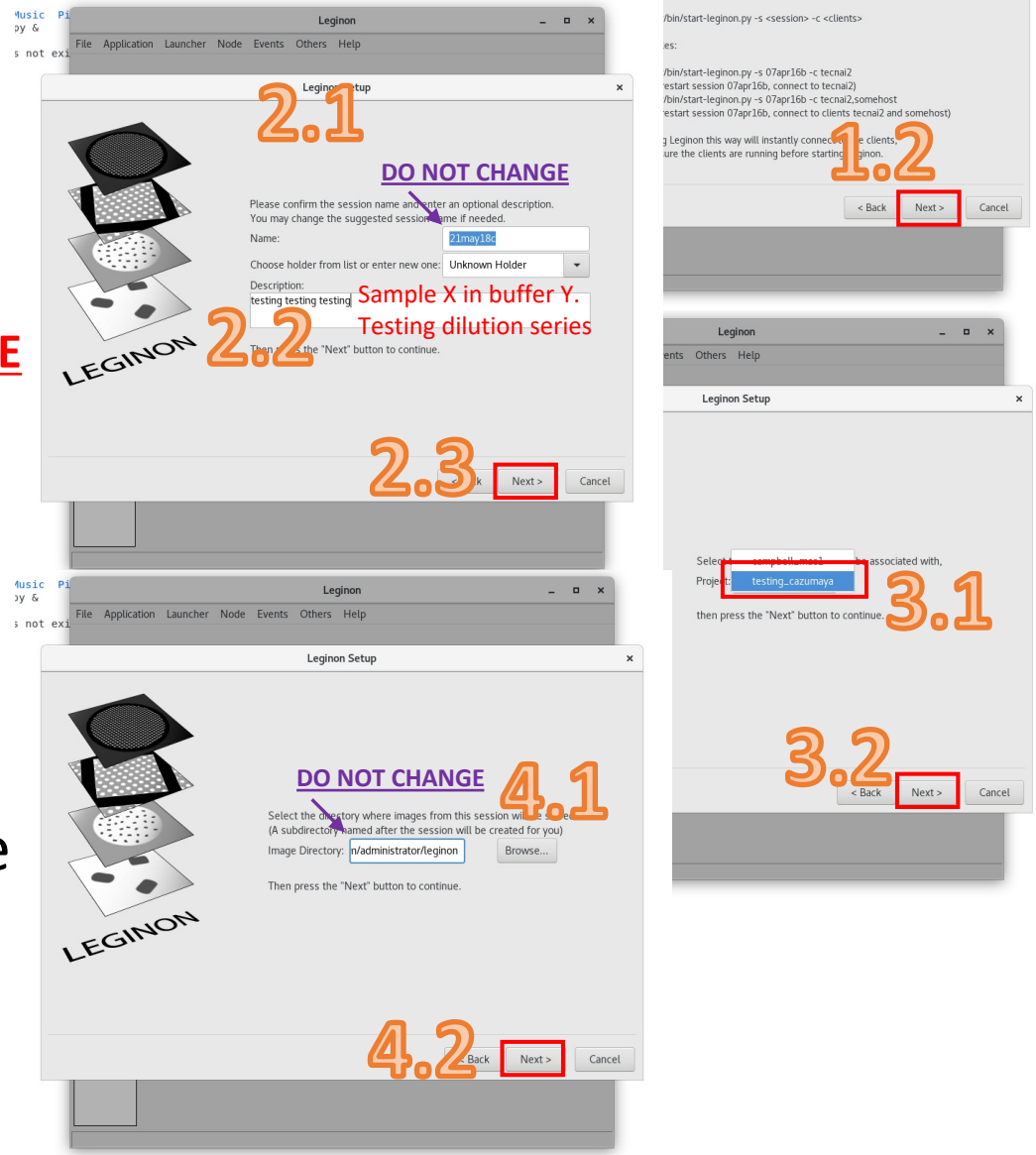
2. Login to Leginon computer
   1. Open terminal (right click on background
   2. start-leginon.py

Leginon-specific login and password



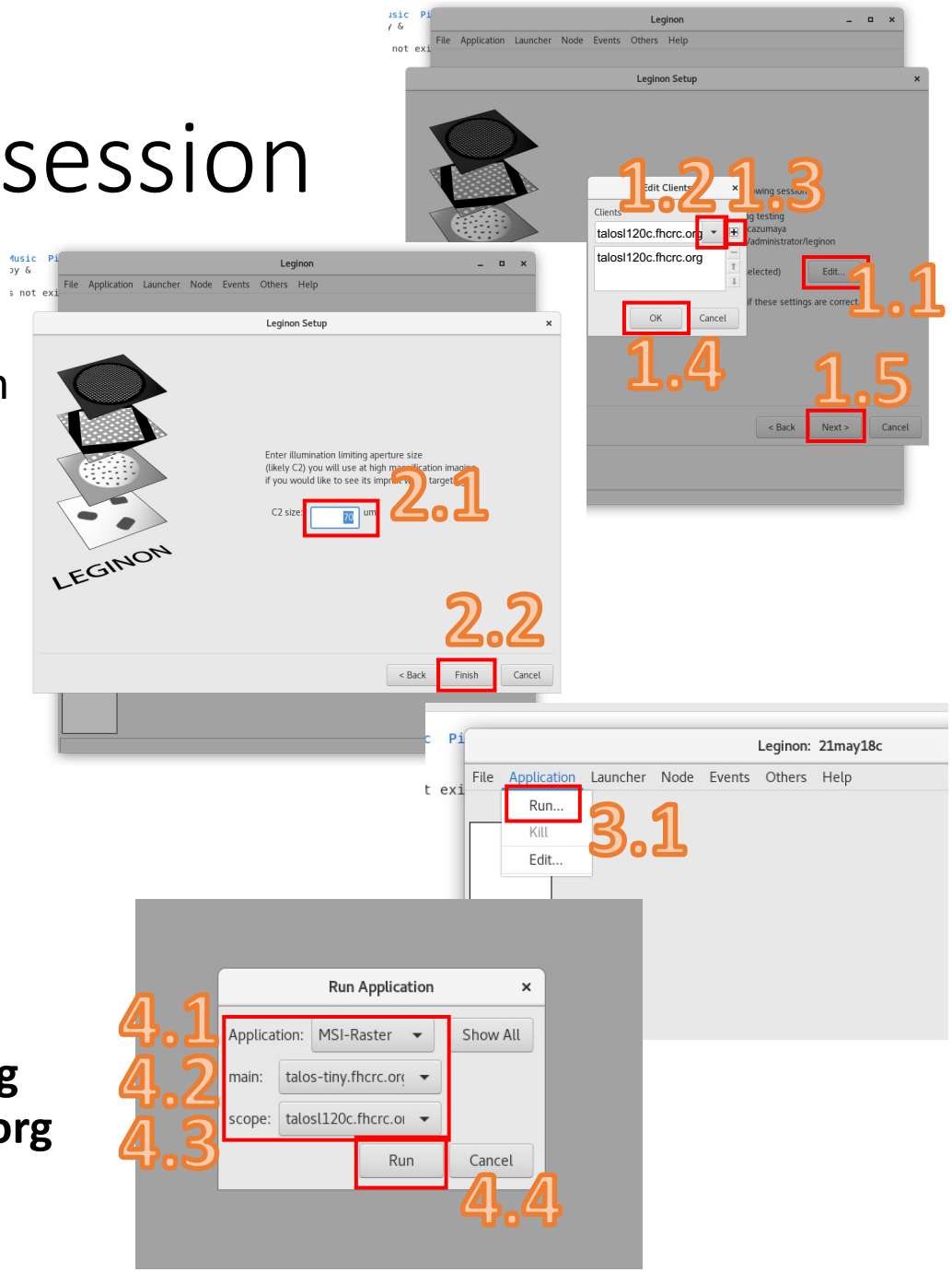
Create Leginon session

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

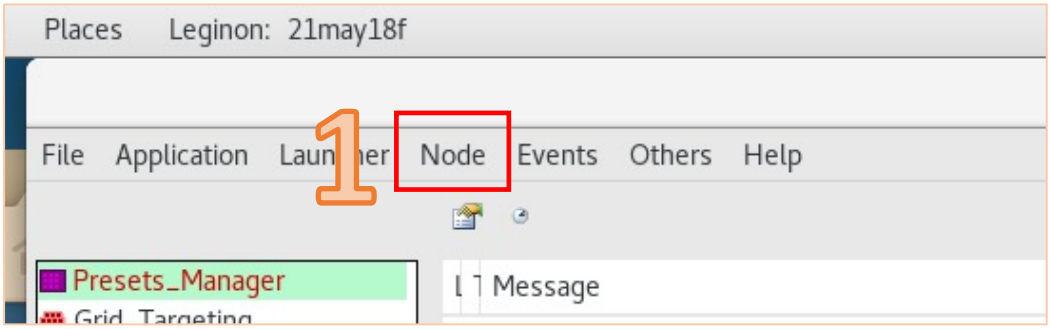
1. Add clients
    1. Edit
    2. talosl120c.fhcrc.org from dropdown
    3. +
    4. OK
    5. Next
2. Define C2 aperture
    1. 70um
    2. Finish
3. Start session
    1. Application -> Run
4. Choose application
    1. Application: MSI-Raster
    2. Main: **talos-tiny.fhcrc.org**
    3. Scope: **talosl120c.fhcrc.org**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

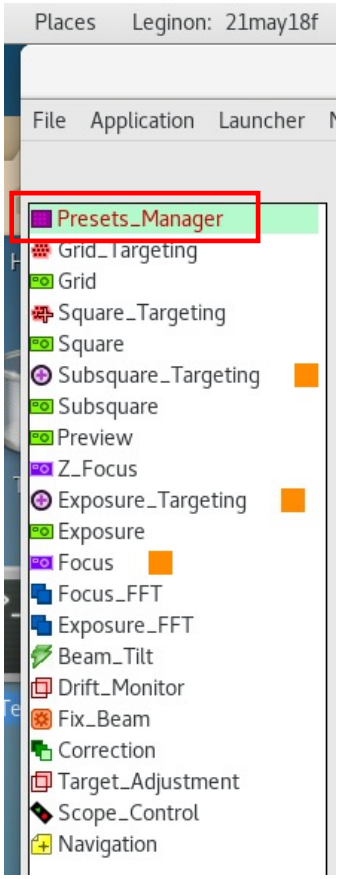


Setup session

1. Node -> Kill -> Preview

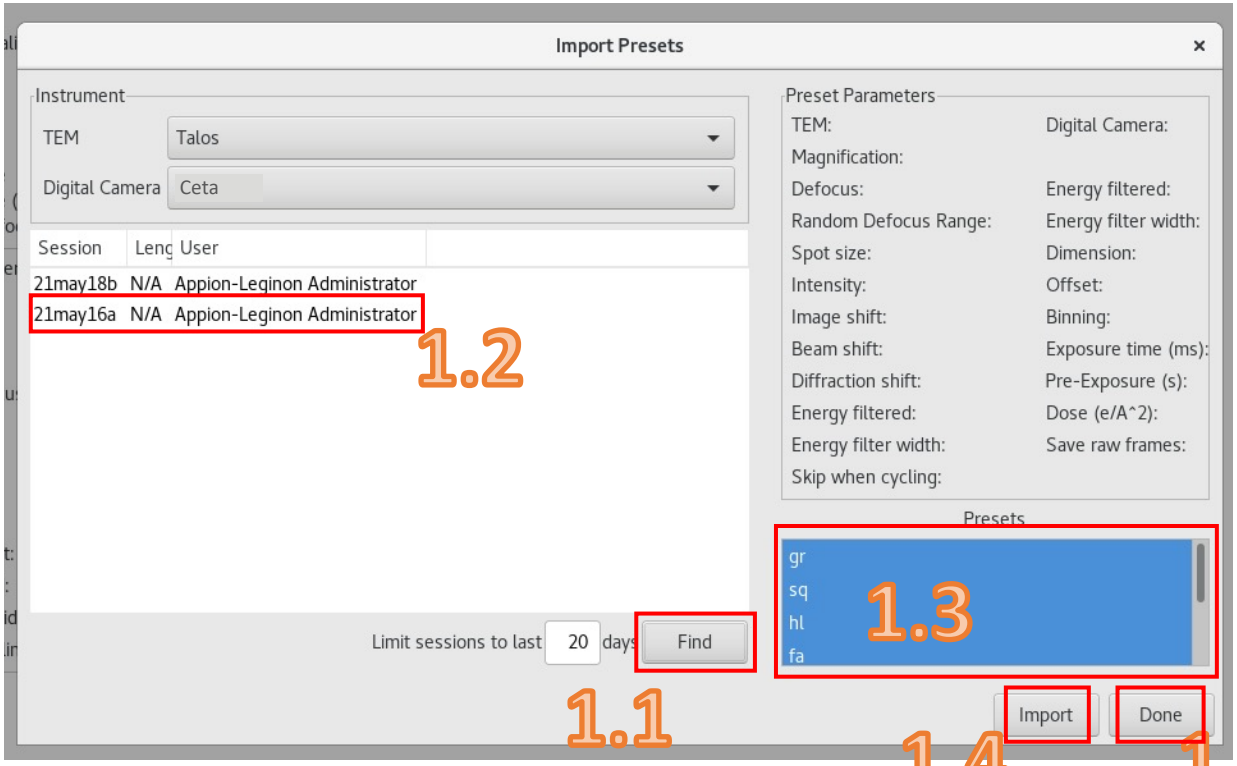
2. Import presets
    1. Presets Manager -> Blue dot icon



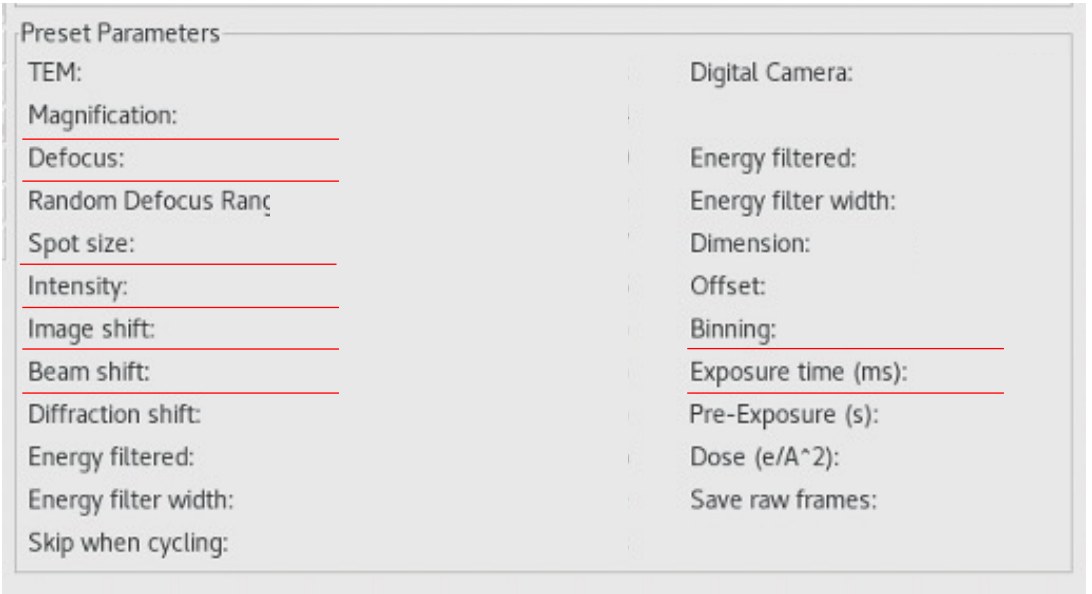


Setup session

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



Quick check presets



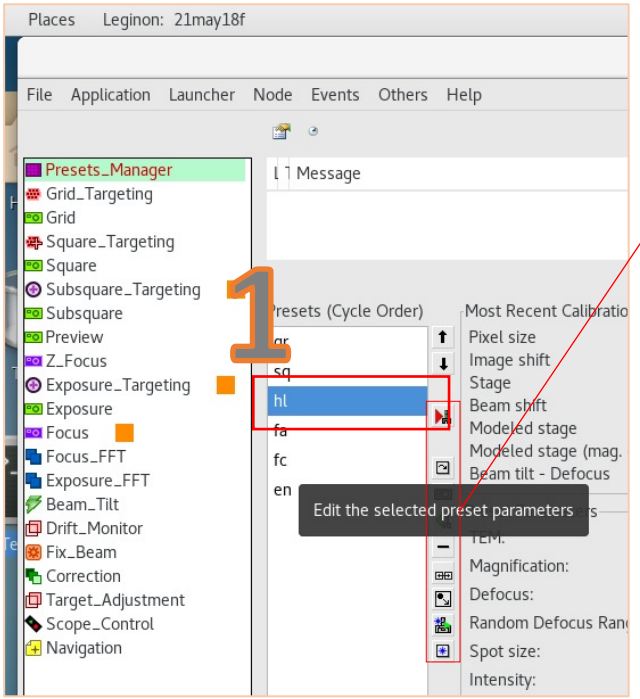
1. What to look at:
    1. Magnification
    2. Defocus
    3. Spot size
    4. Intensity
    5. Image shift
    6. Beam shift
    7. Binning
    8. Exposure time

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

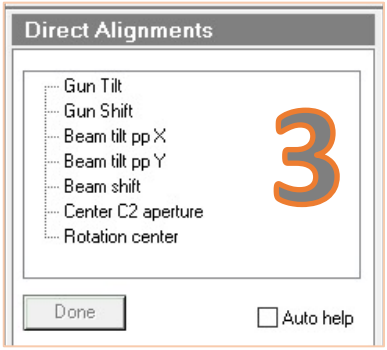
To change your presets

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



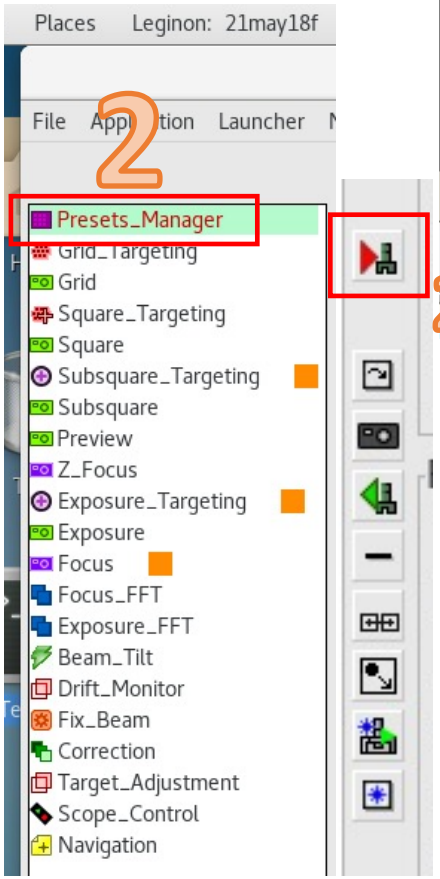
You should be near eucentric height when you adjust

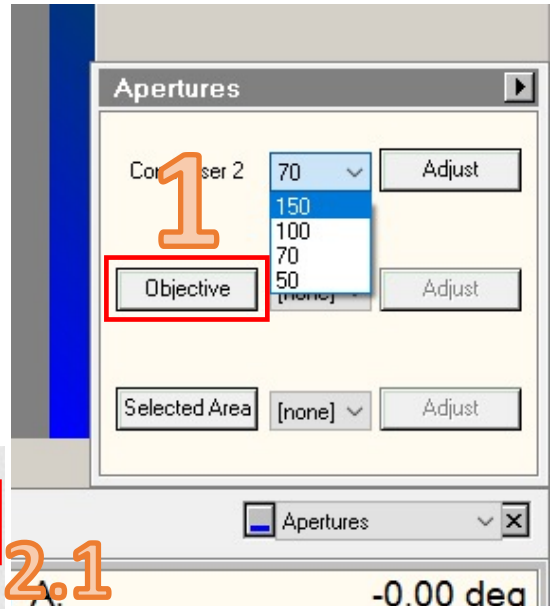
- Press eucentric focus before you center fa, fc, en
- ONLY adjust fa, fc, en beam shift with “Direct Alignments”
- ONLY adjust hl, sq, gr beam shift with roller ball
- Adjust beam with apertures you will use inserted
- Make sure defocus value is not changing when you Get from microscope
- Camera is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

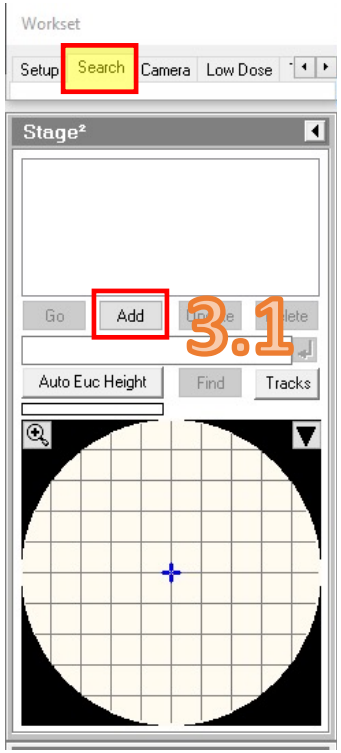


Find a good square

1. Start with Objective out (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. Mark squares of interest in “Search” tab on microscope TUI

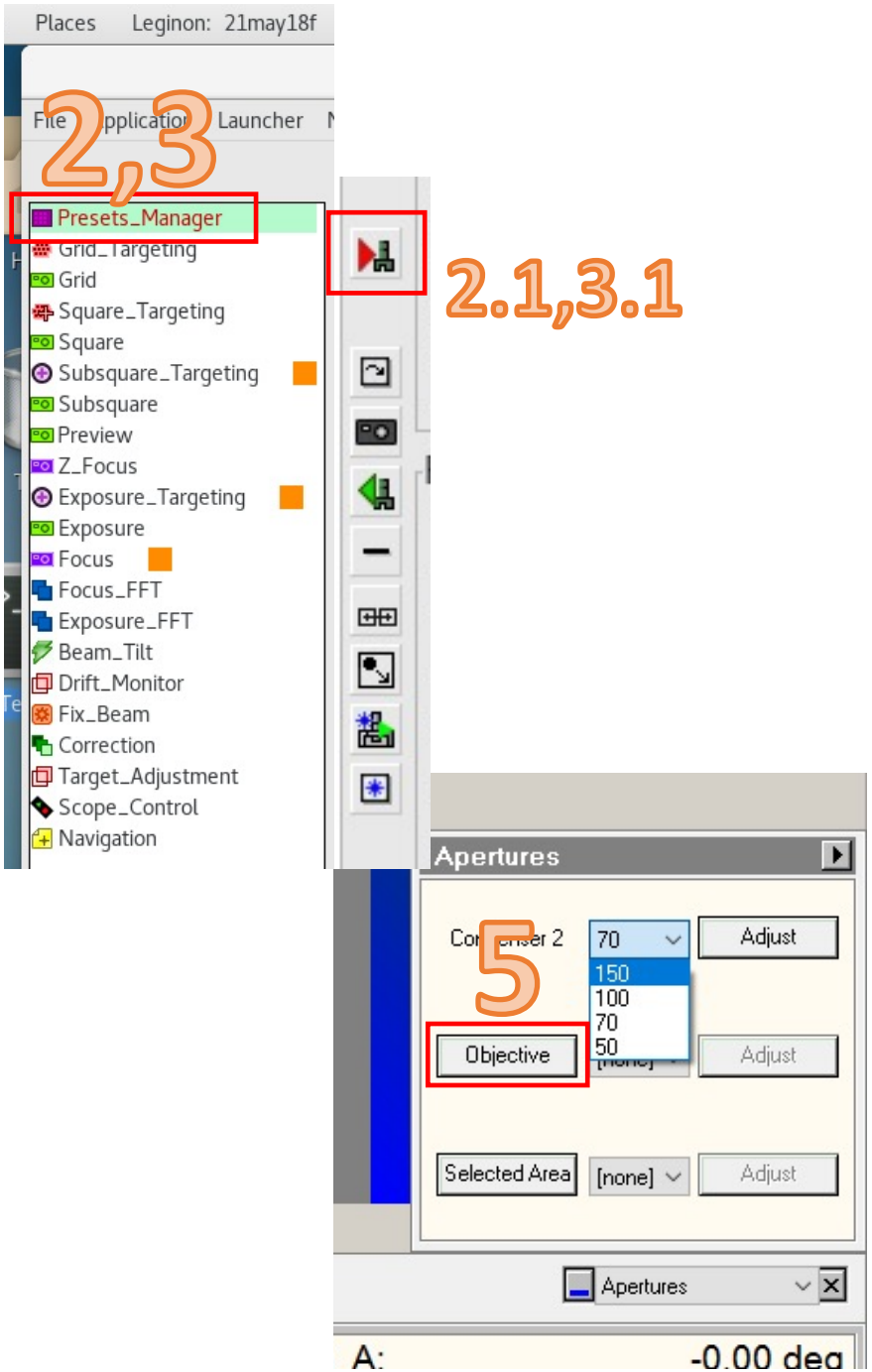






Zoom in on square

1. Go to Presets Manager
   1. Highlight sq and send to scope
2. Use joystick to center square
3. Go to Presets Manager
   1. Highlight hl and send to scope
4. Use joystick to center on good stain area
5. Click “Objective” in ”Apertures” to insert 100 um (will be yellow)

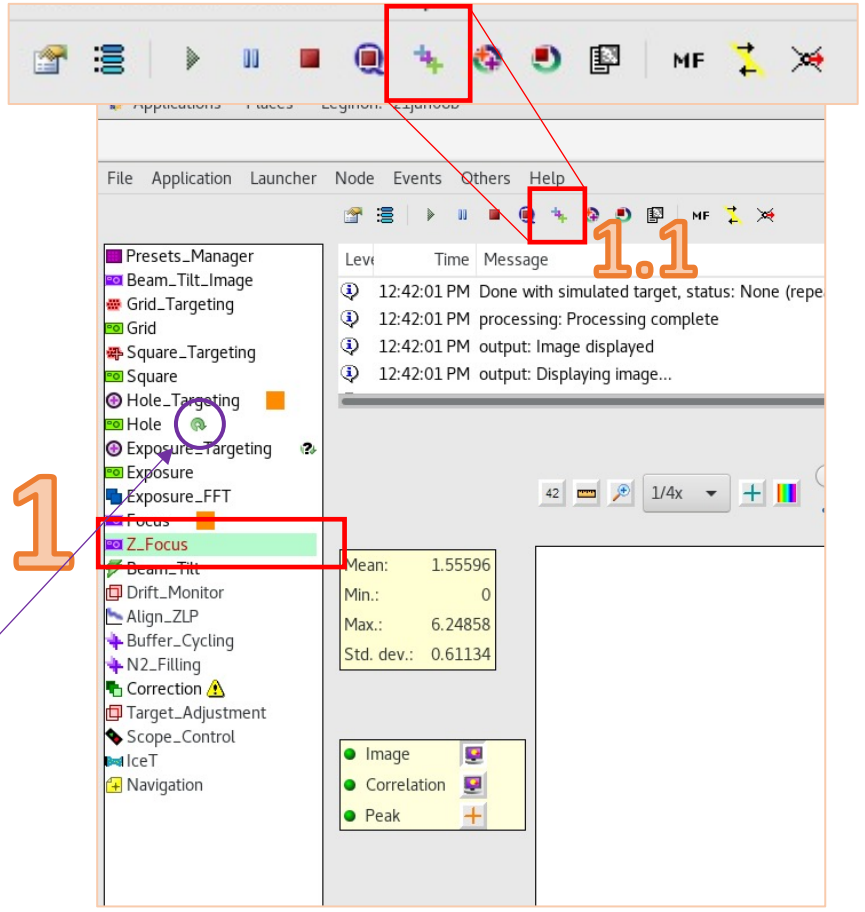


Simulate Z-focus

1. Go to Z-focus node
    1. Simulate target

This will run a wobbler in square magnification and hole magnification to refine the eucentric height

Wait for green arrows to stop turning before next step.

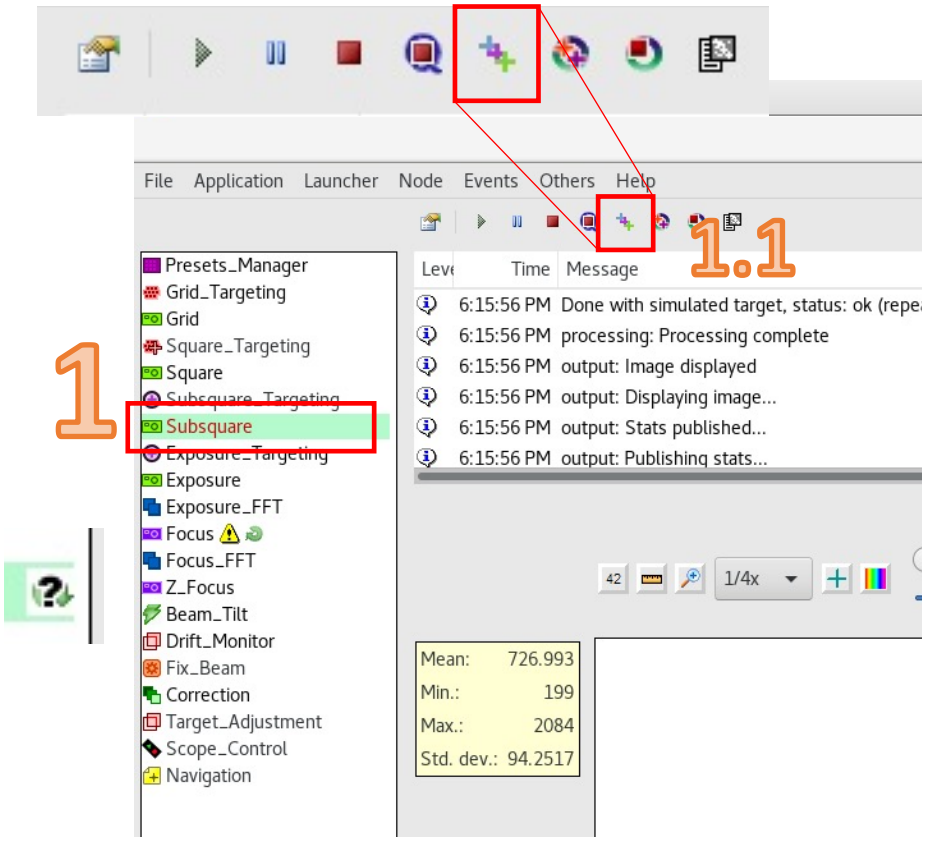


Simulate subsquare

1. Go to Subsquare
    1. Simulate target

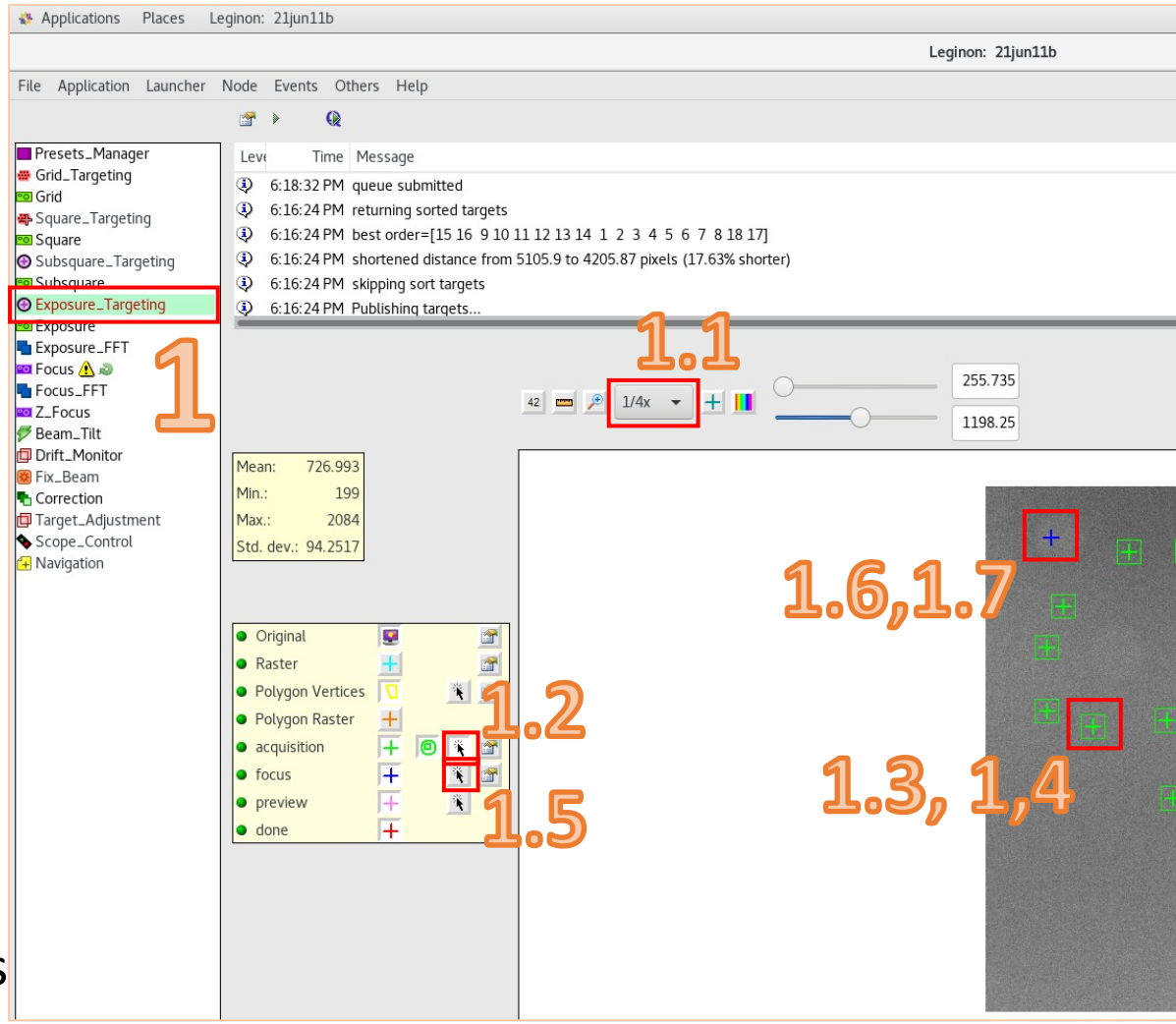
This will take a hole magnification image

Wait for ? next to “Exposure Targeting” before next step.



Remove raster and choose exposure and focus targets

1. Go to Exposure Targeting
    1. Zoom out (~1/4x)
    2. Select acquisition 
    3. Shift + right click on a target to remove all
    4. Left click to add targets where you want to image
    5. Select focus 
    6. Right click to remove focus spot if needed (do not focus on anything large and dark!)
    7. Left click to add focus spot

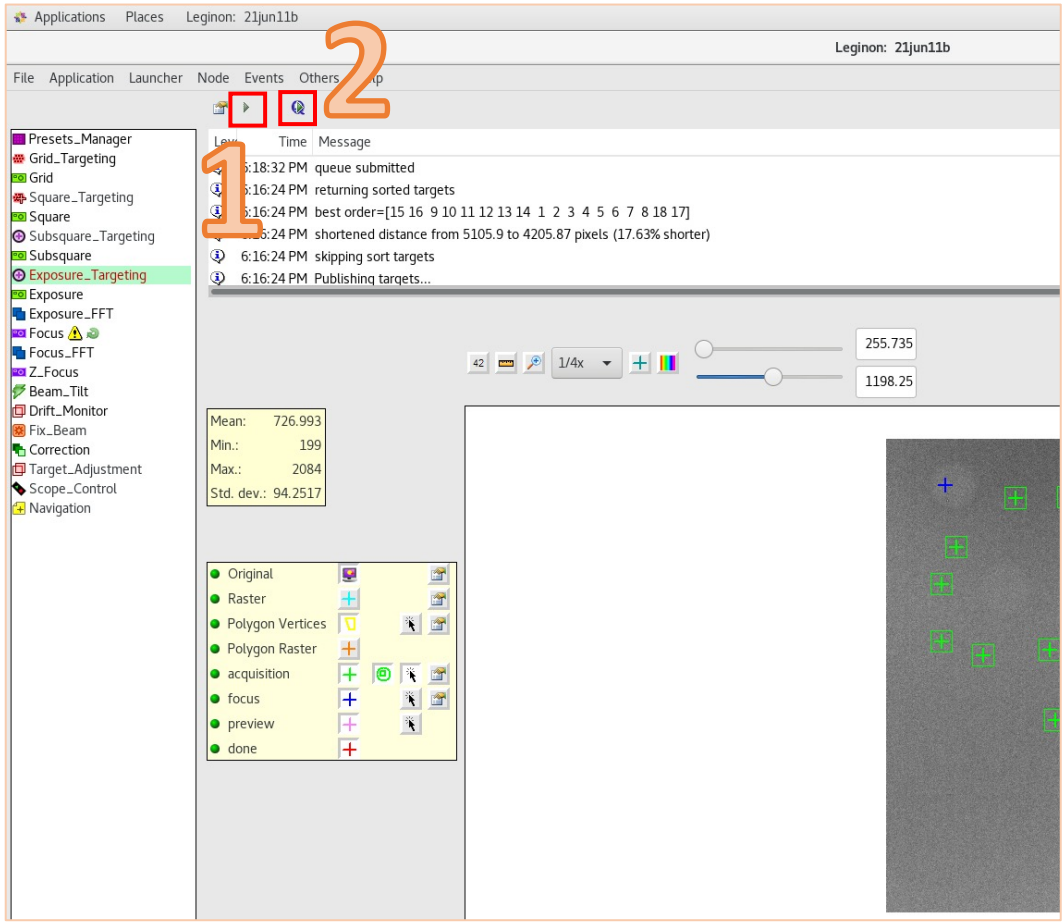


Submit exposure targets

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

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

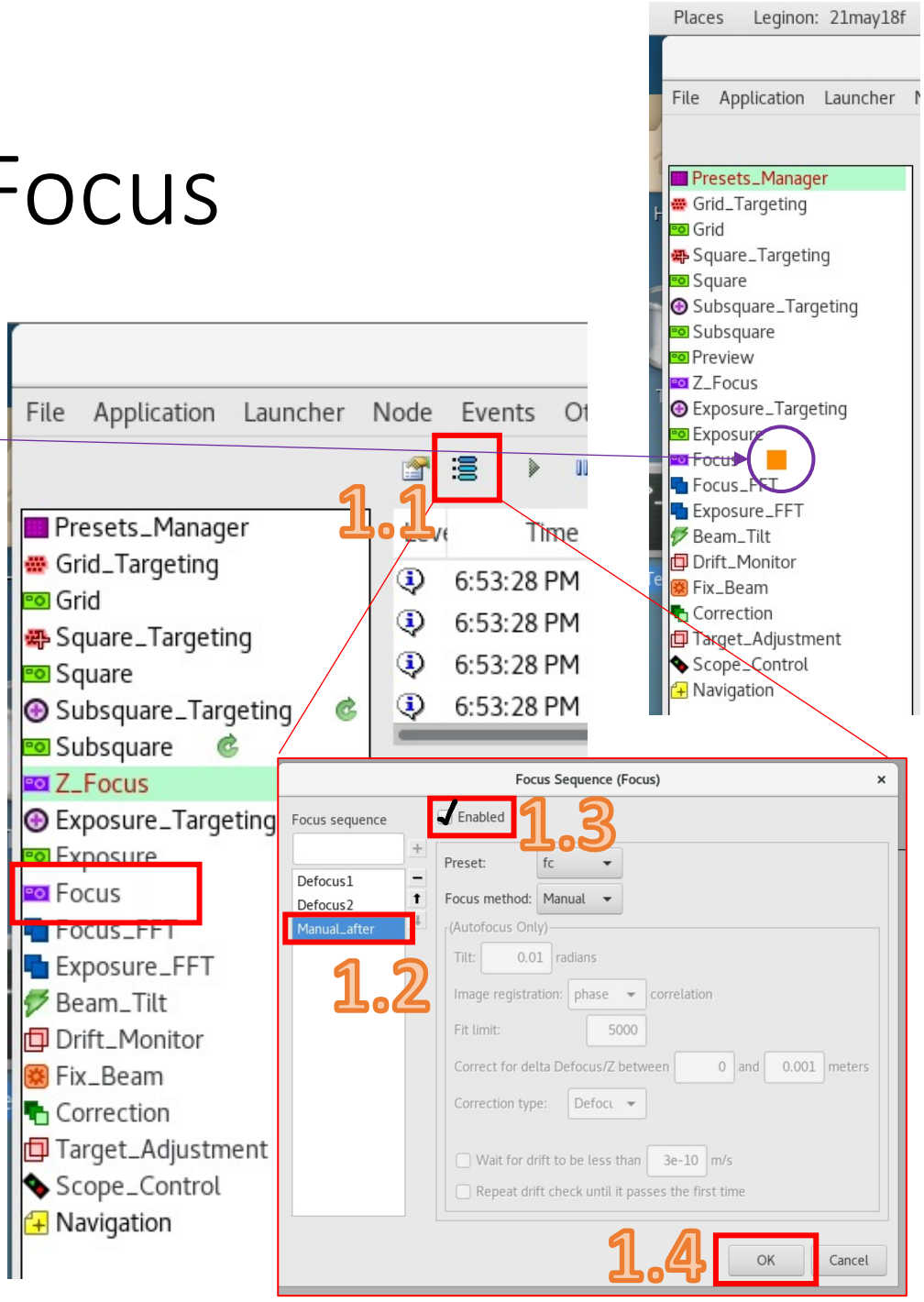
Focus sequence will start to run through “Target Adjustment”, “Drift Monitor”, and ”Focus”



Enable Manual Focus

Orange square means it is enabled.

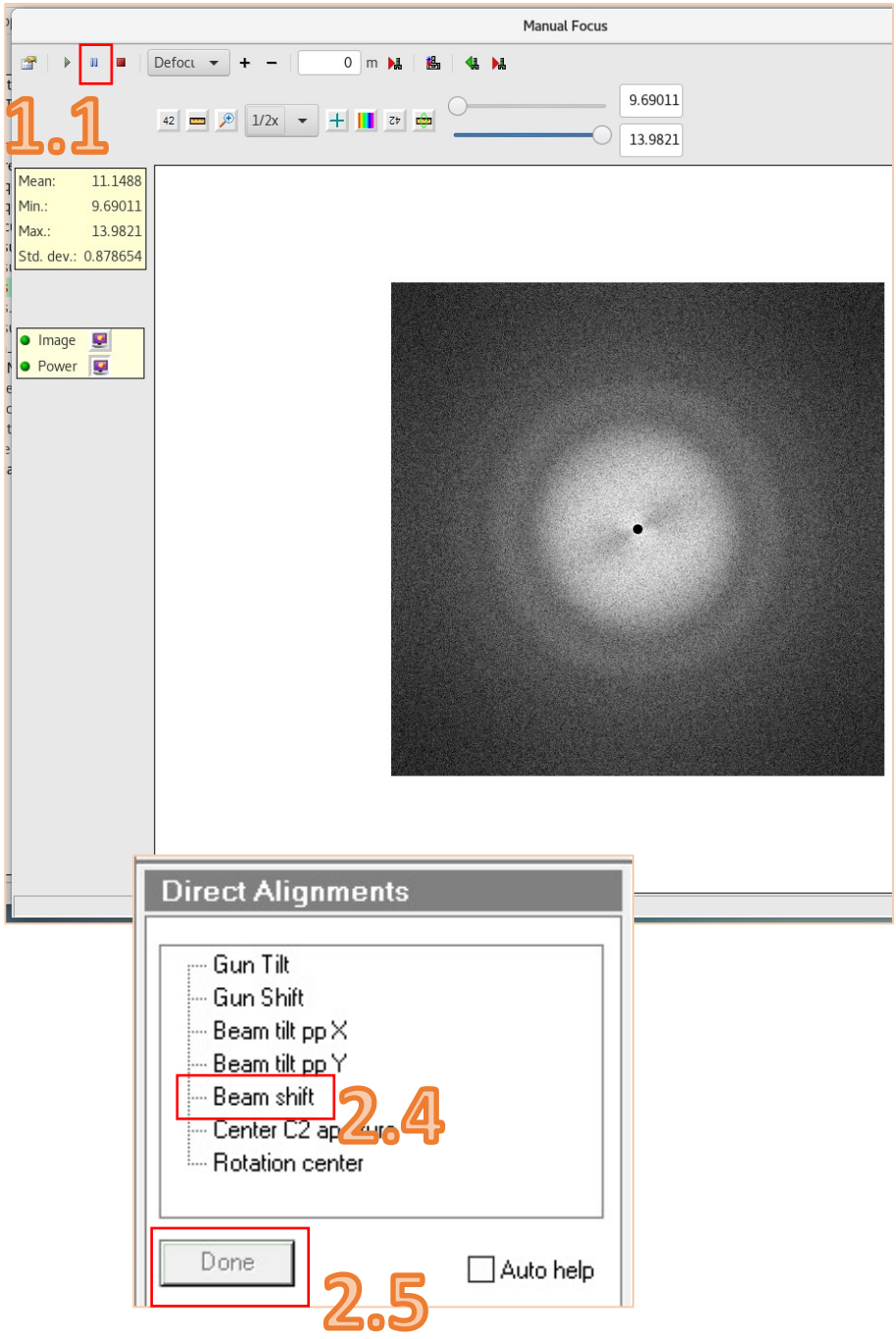
1. If not, Go to Focus
    1. Click blue bullet list
    2. Select Manual_after
    3. Check Enabled
    4. Ok



Manual Focus

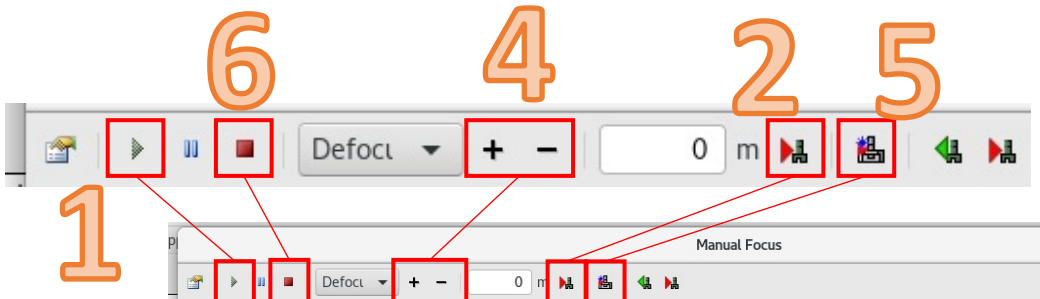
1. Manual focus window will pop up
    1. Pause (if you do not see ”normal” rings go to step 2, otherwise skip)
2. On microscope PC:
    1. Insert screen (handpanel R1)
    2. Press ”Eucentric focus” on handpanel
    3. Press “Reset defocus” on handpanel
    4. Click “Beam shift” in “Direct Alignments” and center beam with multifunction X and Y knobs
    5. Done

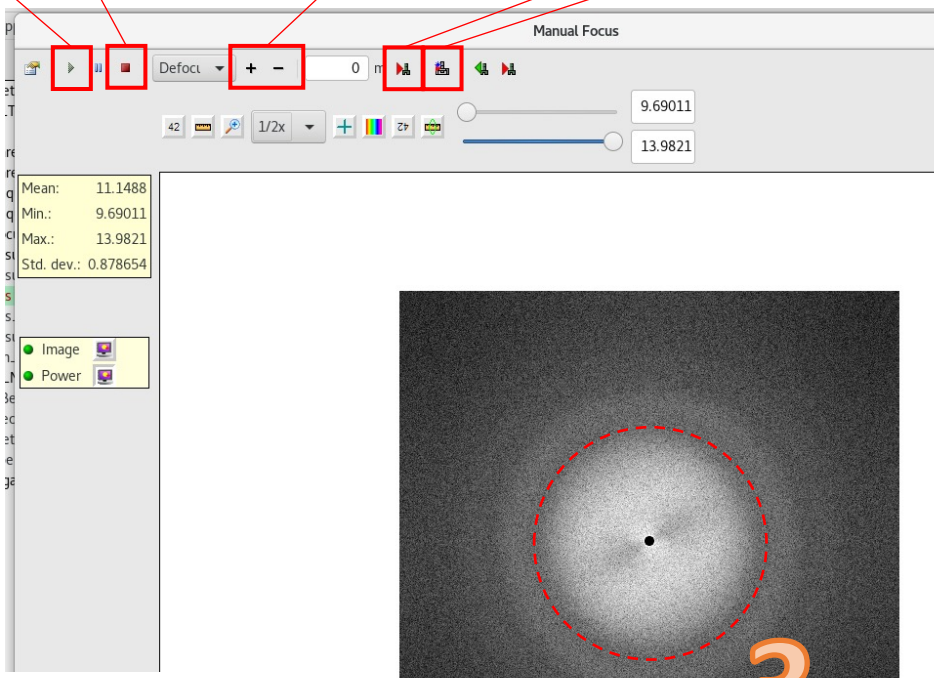
(Should only need to do this the first time you zoom in from gr)

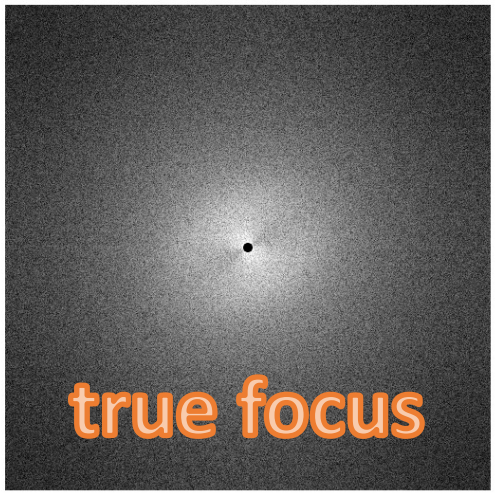


Manual Focus

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

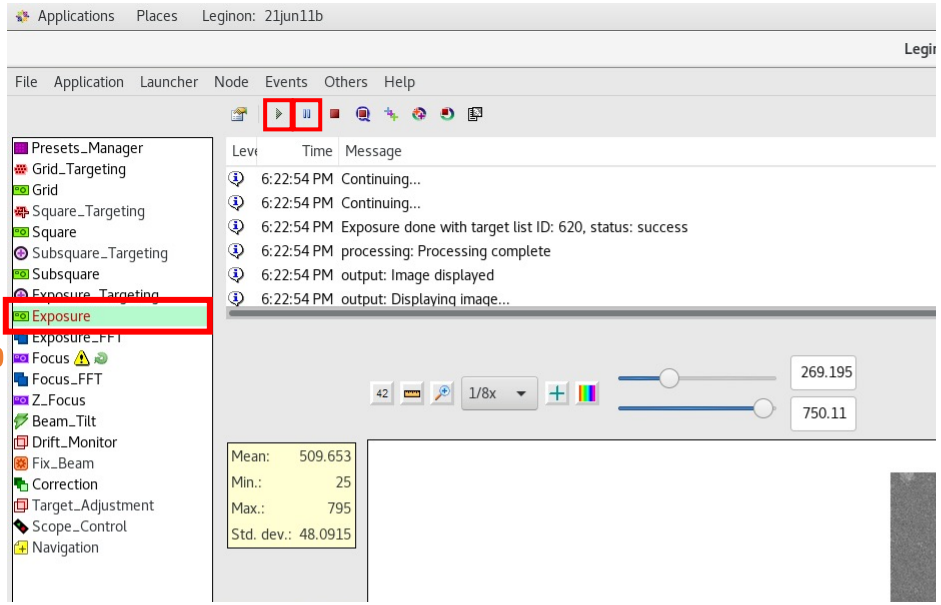


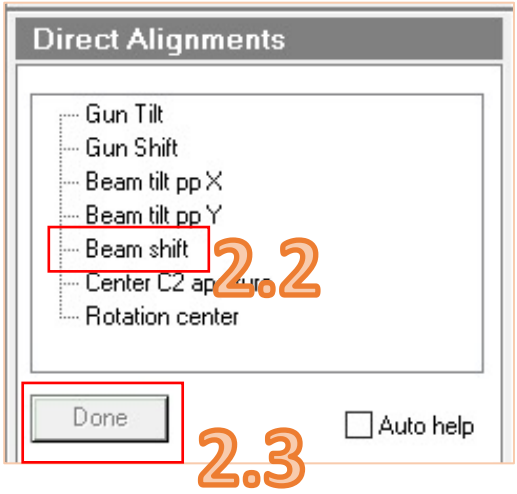




Monitor exposures

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

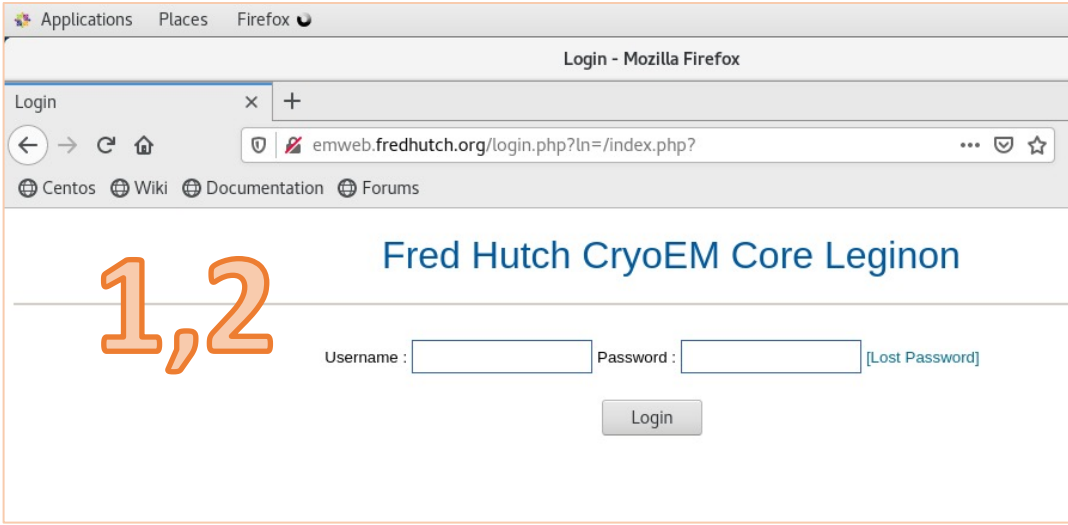




Monitor exposures

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
- More info on webserver on Teams channel

Setup collection on this grid (other Leginon protocol)

OR

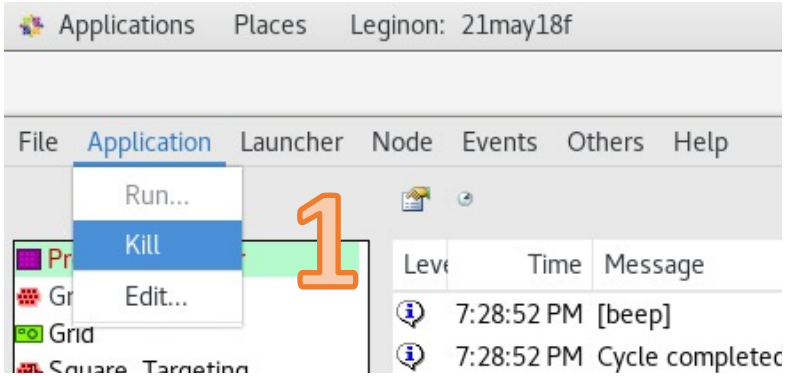
Screen more or another grid (repeat from slide 10)

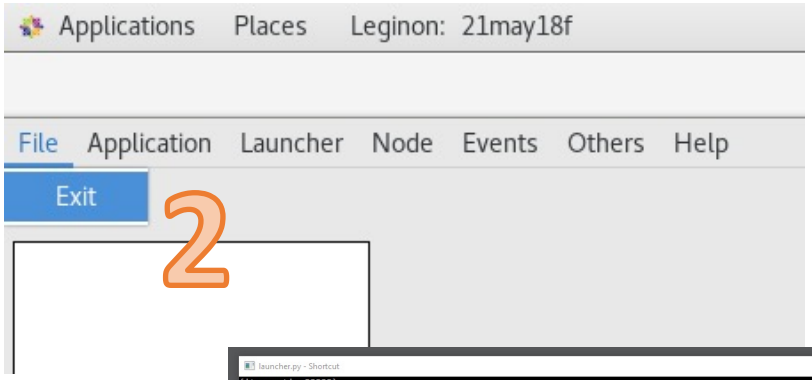
OR

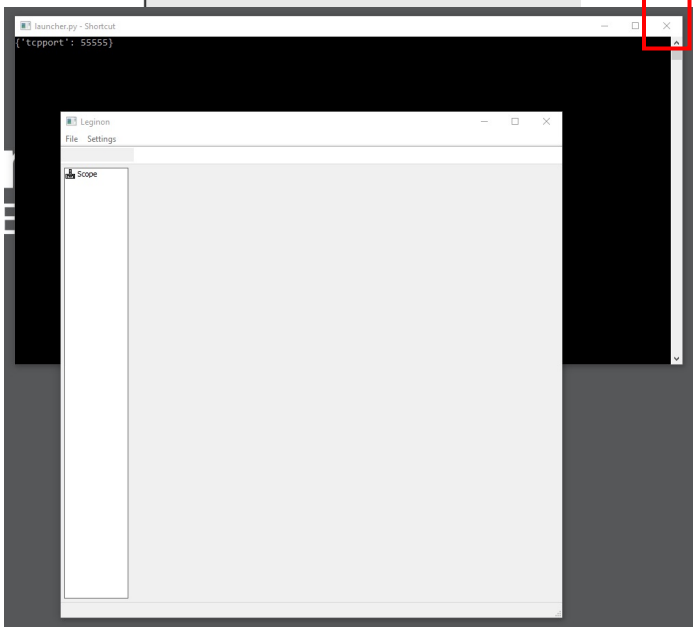
Shutdown (next slide).

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!

End iLab time and sign out!