

**Leginon Protocol**

**negative stain**

**screening**

**Fred Hutch**

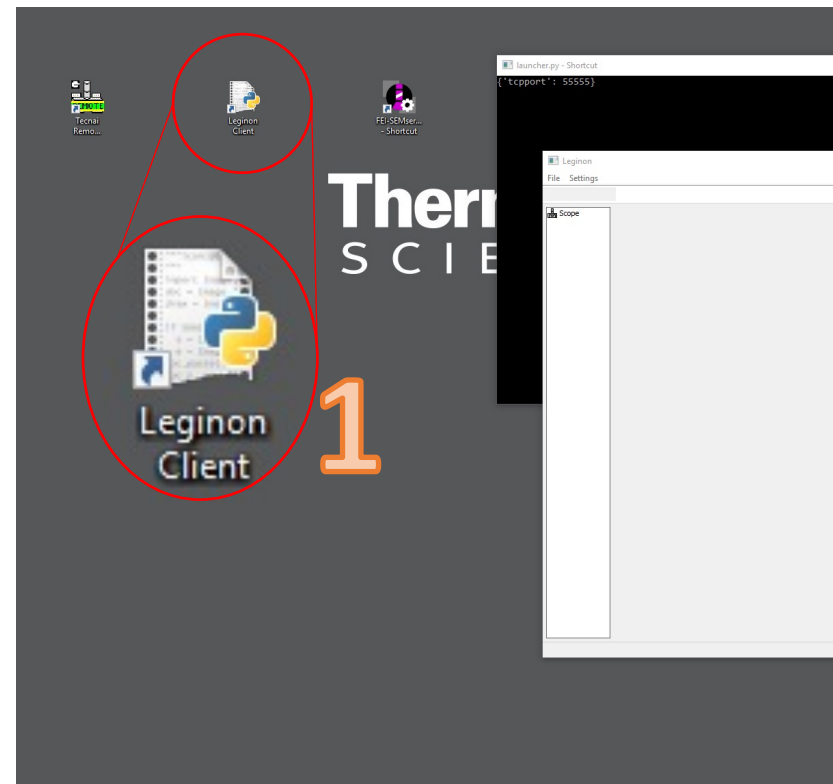
**Talos L120C**

# 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



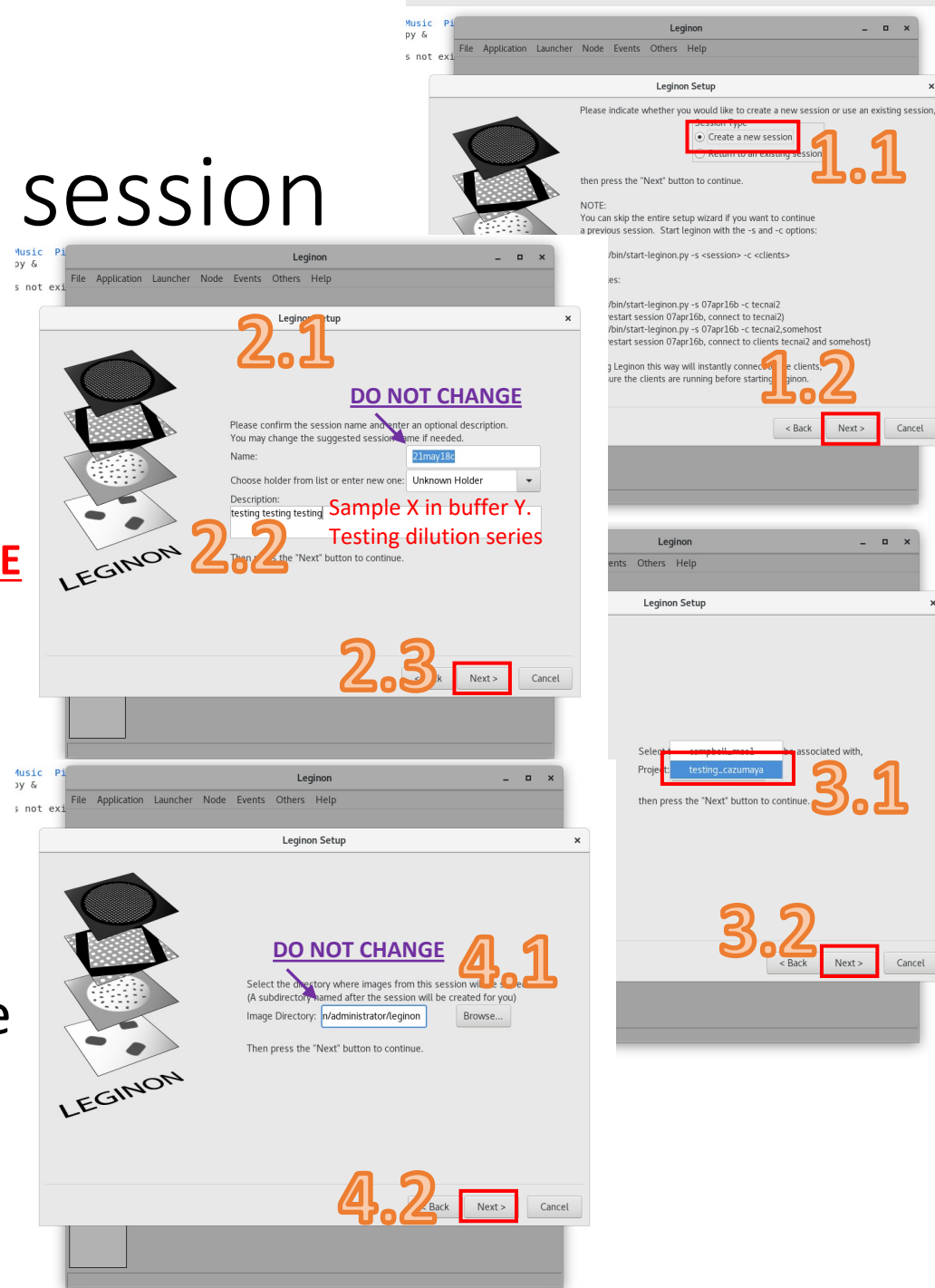
## Leginon-specific login and password

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

```
Applications Places Terminal
File Edit View Search Terminal Help
[cryoemadmin@glacios-tiny ~]$ ls
Desktop Documents Downloads leginon.cfg Music Pictures Public Templates
[cryoemadmin@glacios-tiny ~]$ start-leginon.py &
```

# Create Leginion 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. talos120c.fhcrc.org from dropdown
3. +
4. OK
5. Next

## 2. Define C2 aperture

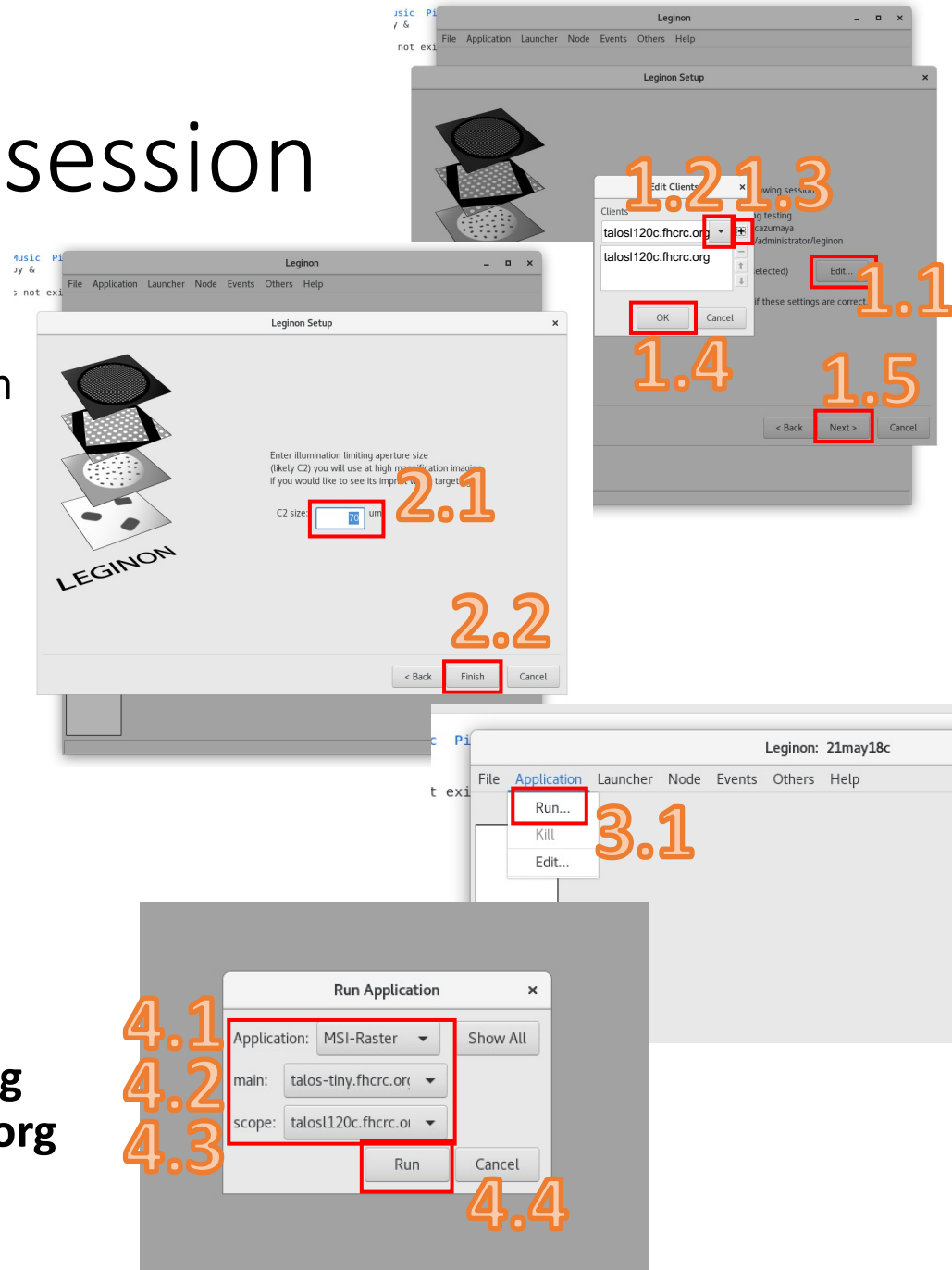
1. 70um
2. Finish

## 3. Start session

1. Application -> Run

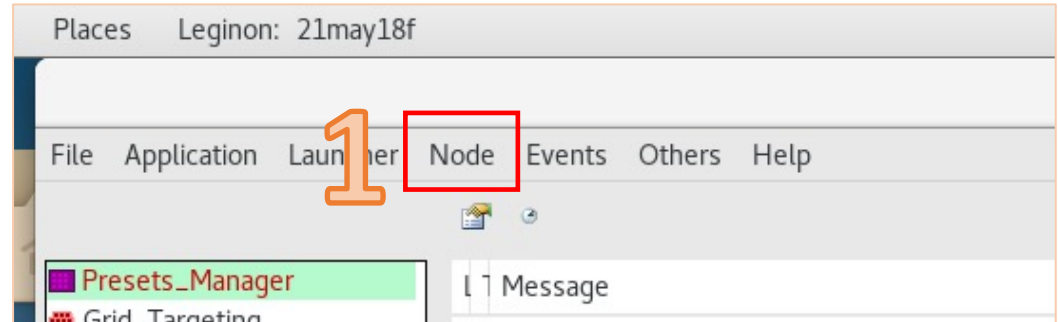
## 4. Choose application

1. Application: MSI-Raster
2. Main: **talos-tiny.fhcrc.org**
3. Scope: **talos120c.fhcrc.org**
4. Run



# Setup session

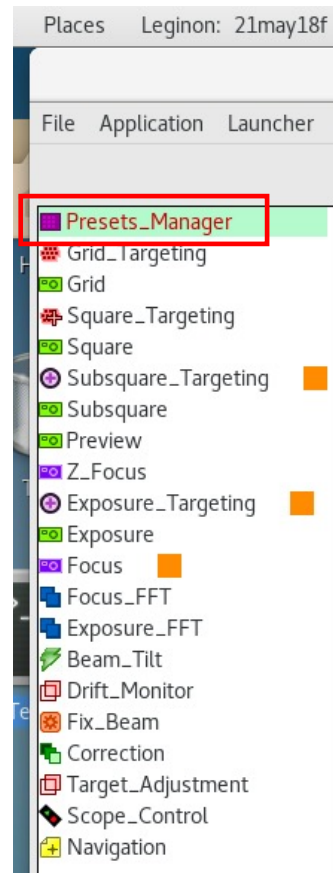
1. Node -> Kill -> Preview



2. Import presets

1. Presets Manager -> Blue dot icon

2

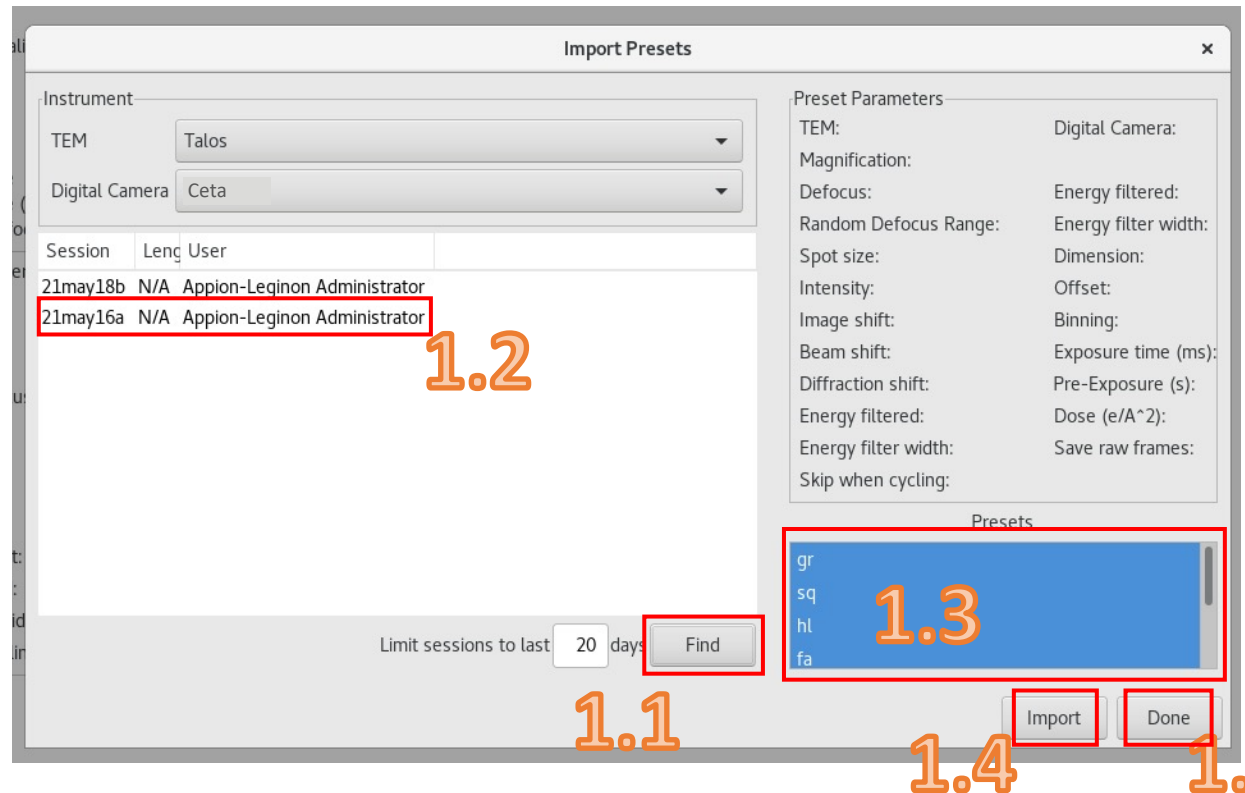


2.1

# 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 ->

### Preset Parameters

TEM:

Magnification:

Defocus:

Random Defocus Range:

Spot size:

Intensity:

Image shift:

Beam shift:

Diffraction shift:

Energy filtered:

Energy filter width:

Skip when cycling:

Digital Camera:

Energy filtered:

Energy filter width:

Dimension:

Offset:

Binning:

Exposure time (ms):

Pre-Exposure (s):

Dose (e/A<sup>2</sup>):

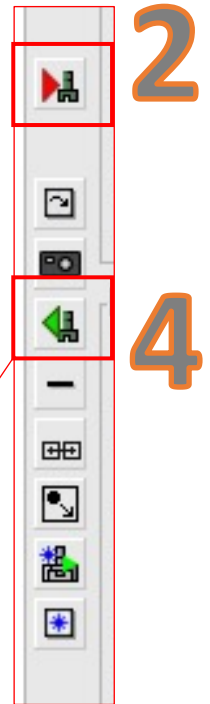
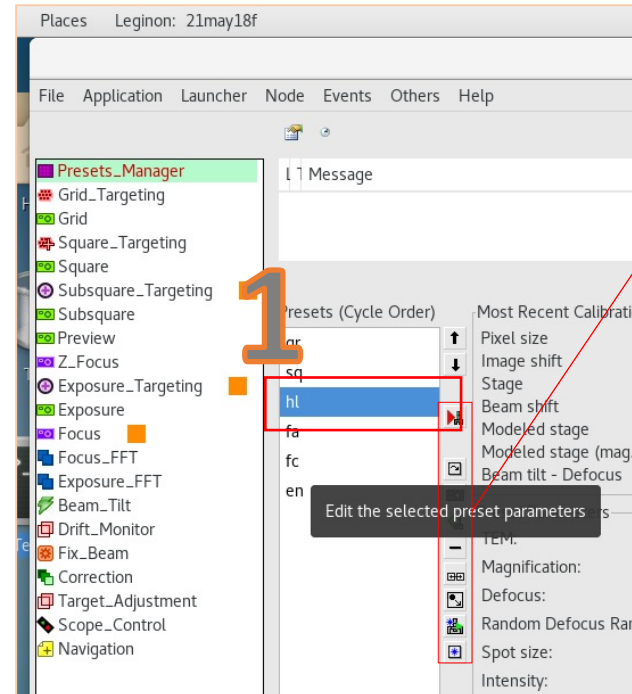
Save raw frames:

	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



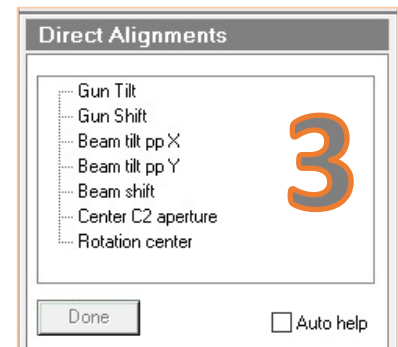
# To change your presets

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



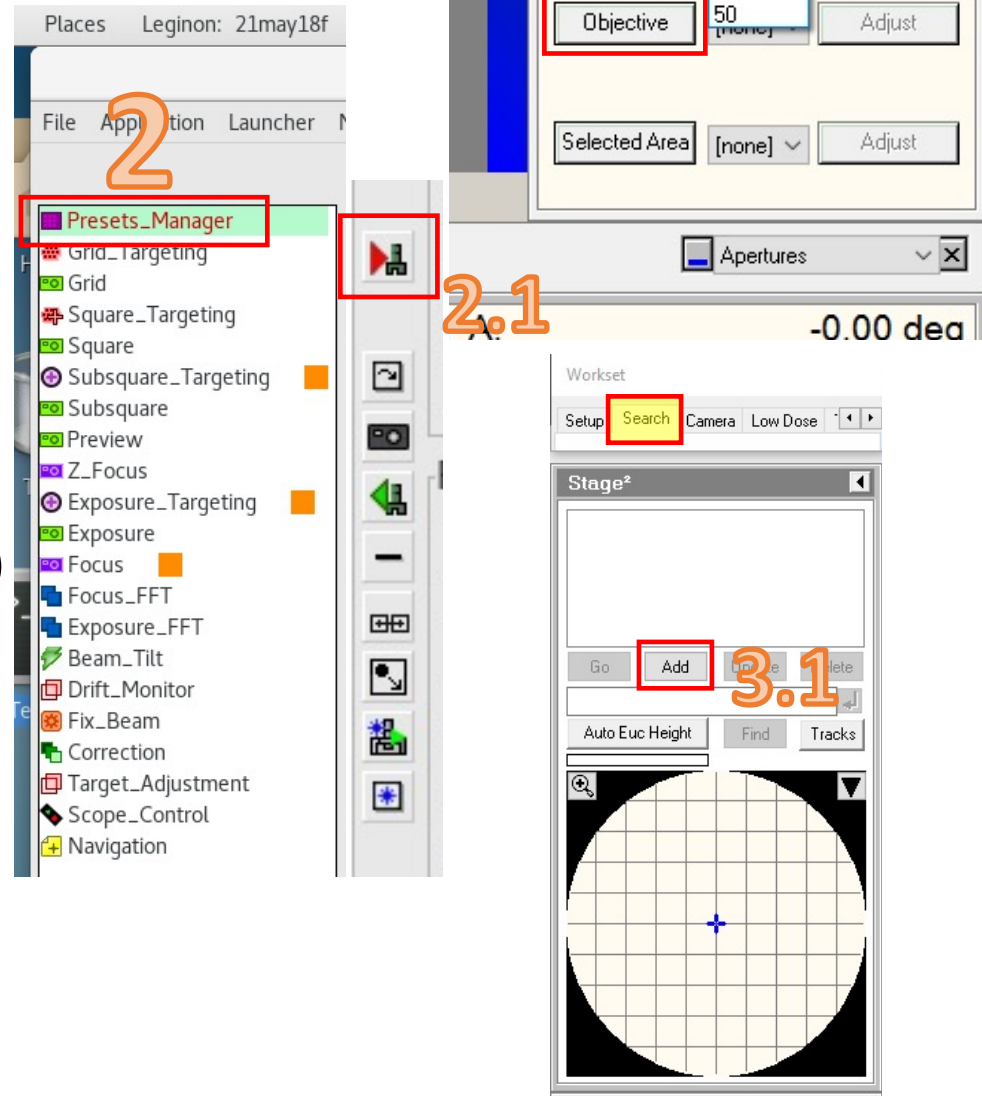
## You should be near eucentric height when you adjust

- 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



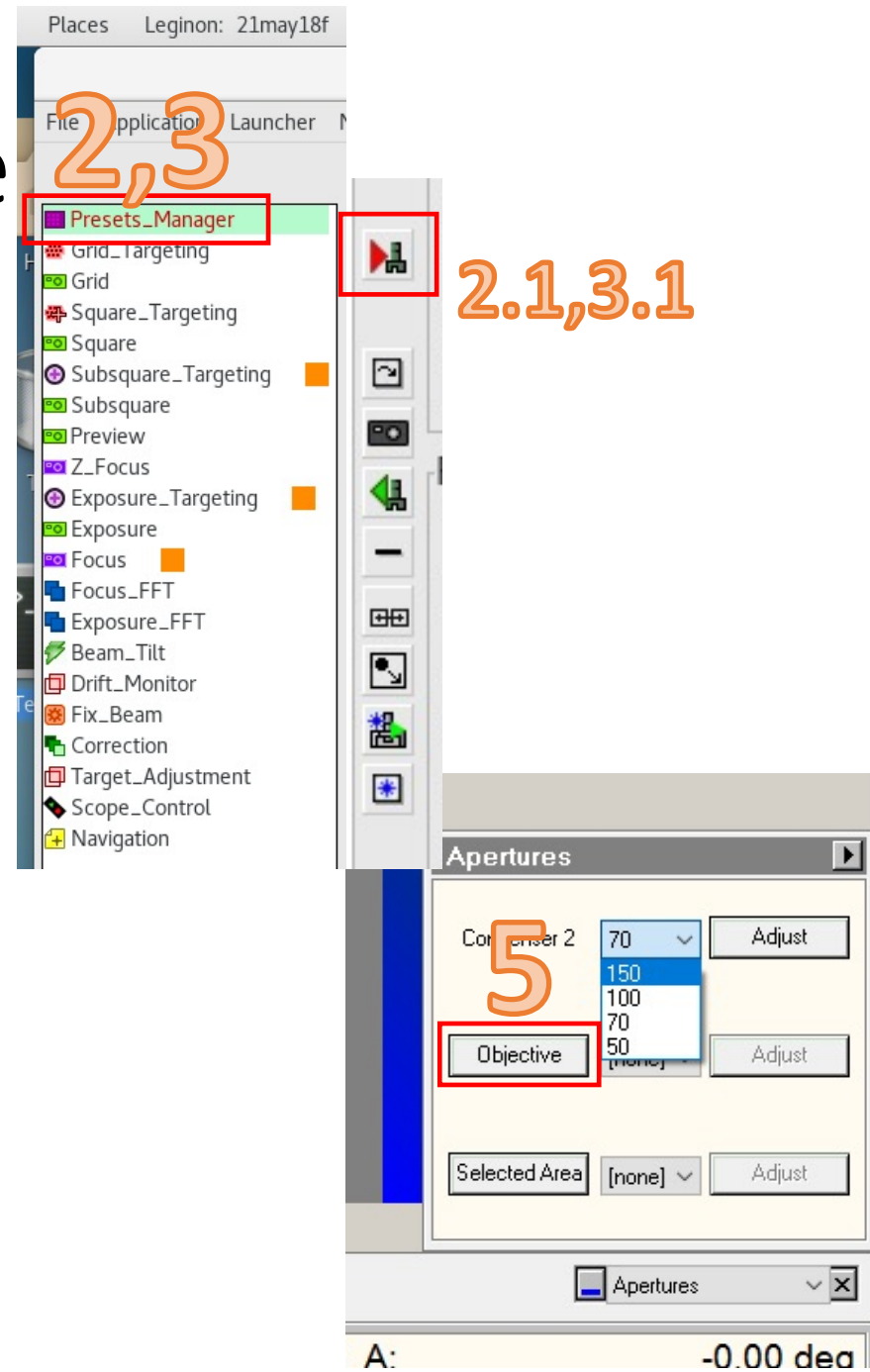
# Find a good square

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)

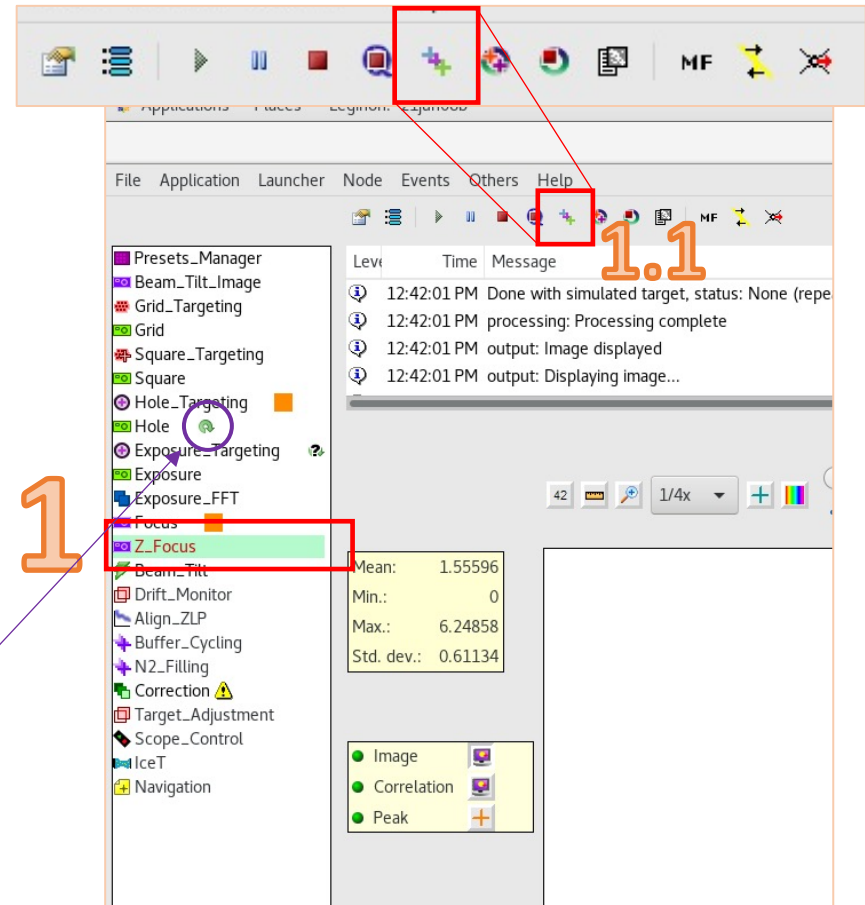


# Simulate Z-focus

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.

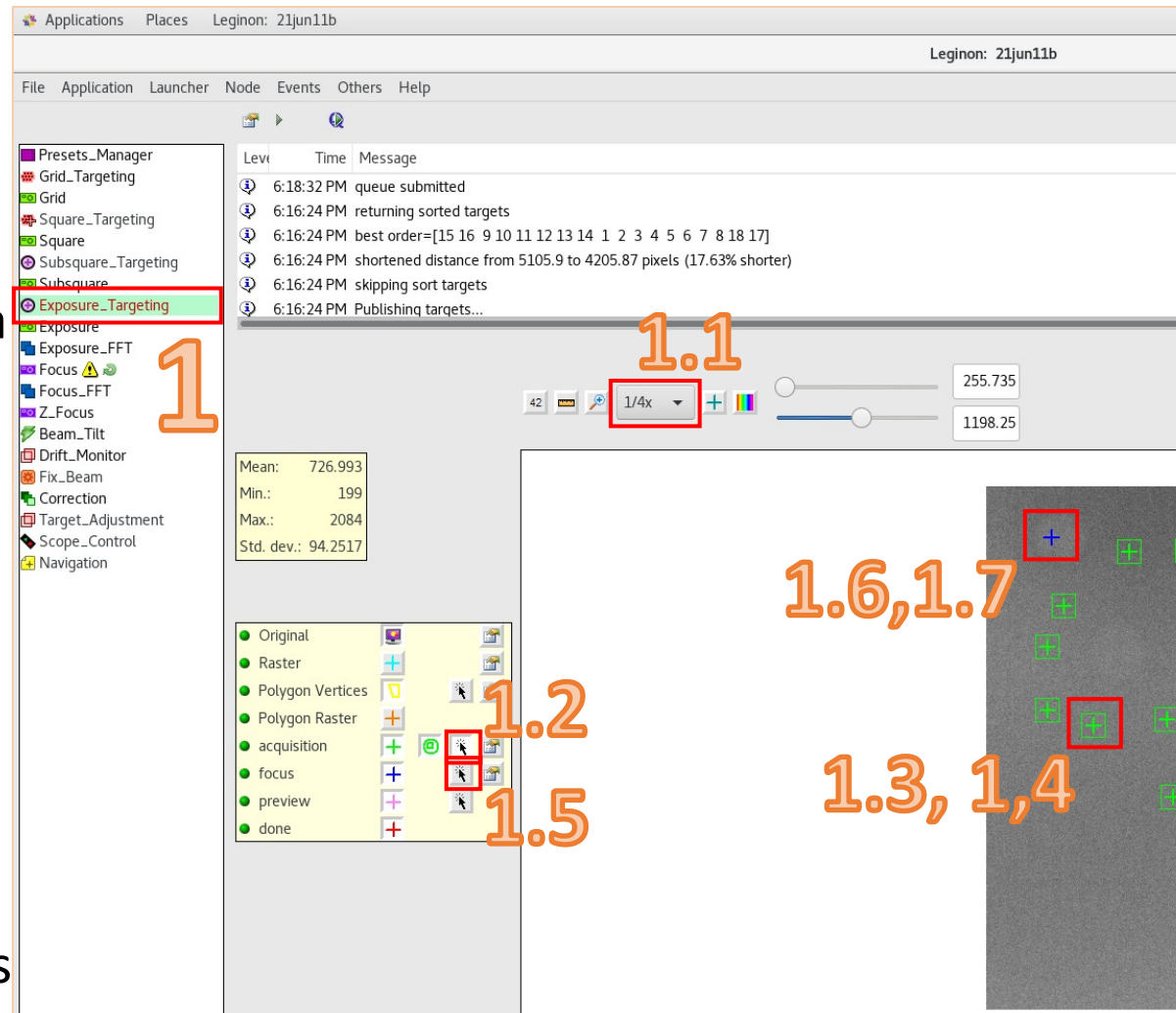
The screenshot shows a software interface with a toolbar at the top. A red box highlights a magnification icon (a plus sign with a crosshair) in the toolbar. Below the toolbar is a menu bar with options: File, Application, Launcher, Node, Events, Others, Help. Below the menu bar is a node list on the left and an events log on the right. The node list includes: Presets\_Manager, Grid\_Targeting, Grid, Square\_Targeting, Square, Subsquare\_Targeting, Subsquare (highlighted in green), Exposure\_Targeting, Exposure, Exposure\_FFT, Focus, Focus\_FFT, Z\_Focus, Beam\_Tilt, Drift\_Monitor, Fix\_Beam, Correction, Target\_Adjustment, Scope\_Control, and Navigation. The events log shows a table with columns: Level, Time, and Message. The messages include: Done with simulated target, status: ok (repe...), processing: Processing complete, output: Image displayed, output: Displaying image..., output: Stats published..., and output: Publishing stats... A red box highlights a magnification icon in the events log, and a large orange '1.1' is next to it. A purple arrow points from the text 'Wait for ? next to "Exposure Targeting"' to a question mark icon in the node list. A large orange '1' is next to the 'Subsquare' node in the node list.

Level	Time	Message
	6:15:56 PM	Done with simulated target, status: ok (repe...
	6:15:56 PM	processing: Processing complete
	6:15:56 PM	output: Image displayed
	6:15:56 PM	output: Displaying image...
	6:15:56 PM	output: Stats published...
	6:15:56 PM	output: Publishing stats...

Mean: 726.993  
Min.: 199  
Max.: 2084  
Std. dev.: 94.2517

# Remove raster and choose exposure and focus targets

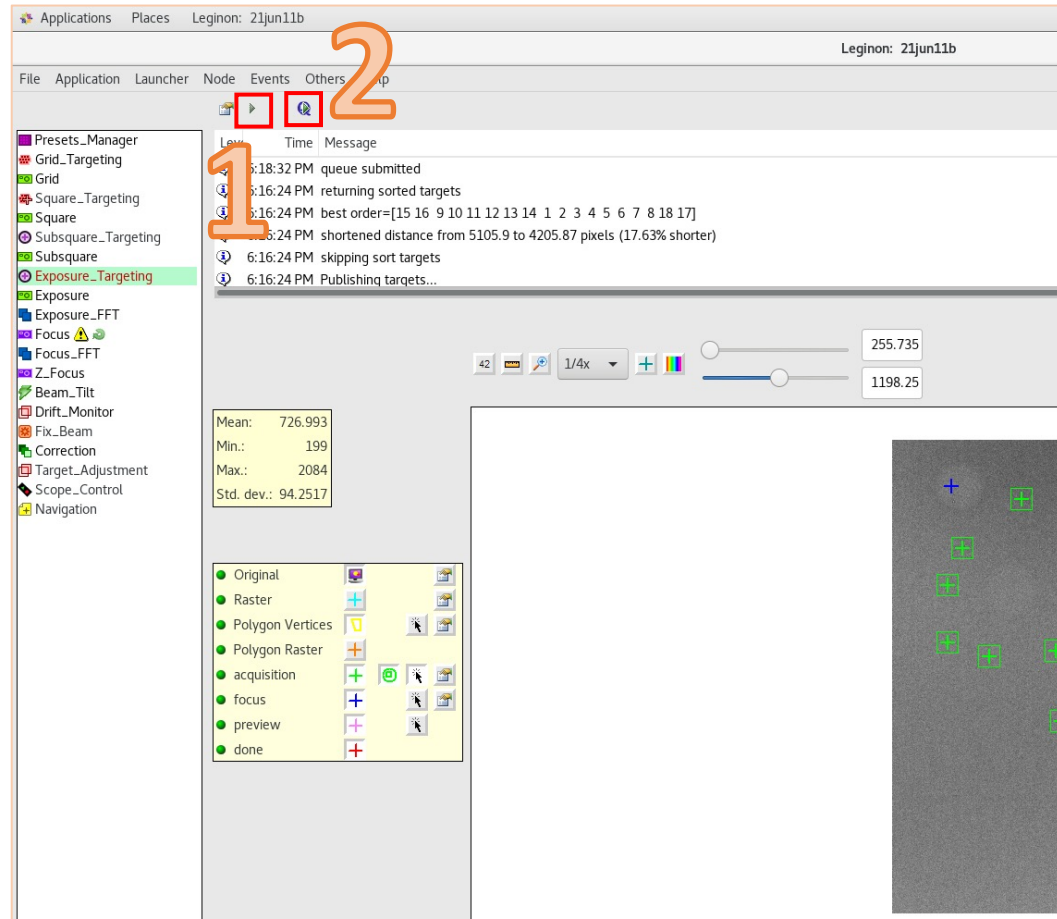
1. Go to Exposure Targeting
  1. Zoom out ( $\sim 1/4x$ )
  2. Select acquisition 
  3. Shift + right click on a target to remove all
  4. Left click to add targets where you want to image
  5. Select focus 
  6. Right click to remove focus spot if needed (do not focus on anything large and dark!)
 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

1

The screenshot shows a software interface with a sidebar on the left containing a list of menu items. The 'Focus' item is highlighted with a red box. A red box also highlights a blue bullet list icon in the top right of the sidebar. A red box highlights the 'Focus' item in the 'Focus Sequence (Focus)' dialog. The 'Focus Sequence (Focus)' dialog is open, showing a list of focus sequences with 'Manual\_after' selected. The 'Enabled' checkbox is checked, and the 'Focus method' is set to 'Manual'. The 'OK' button is highlighted with a red box.

1.1

1.2

1.3

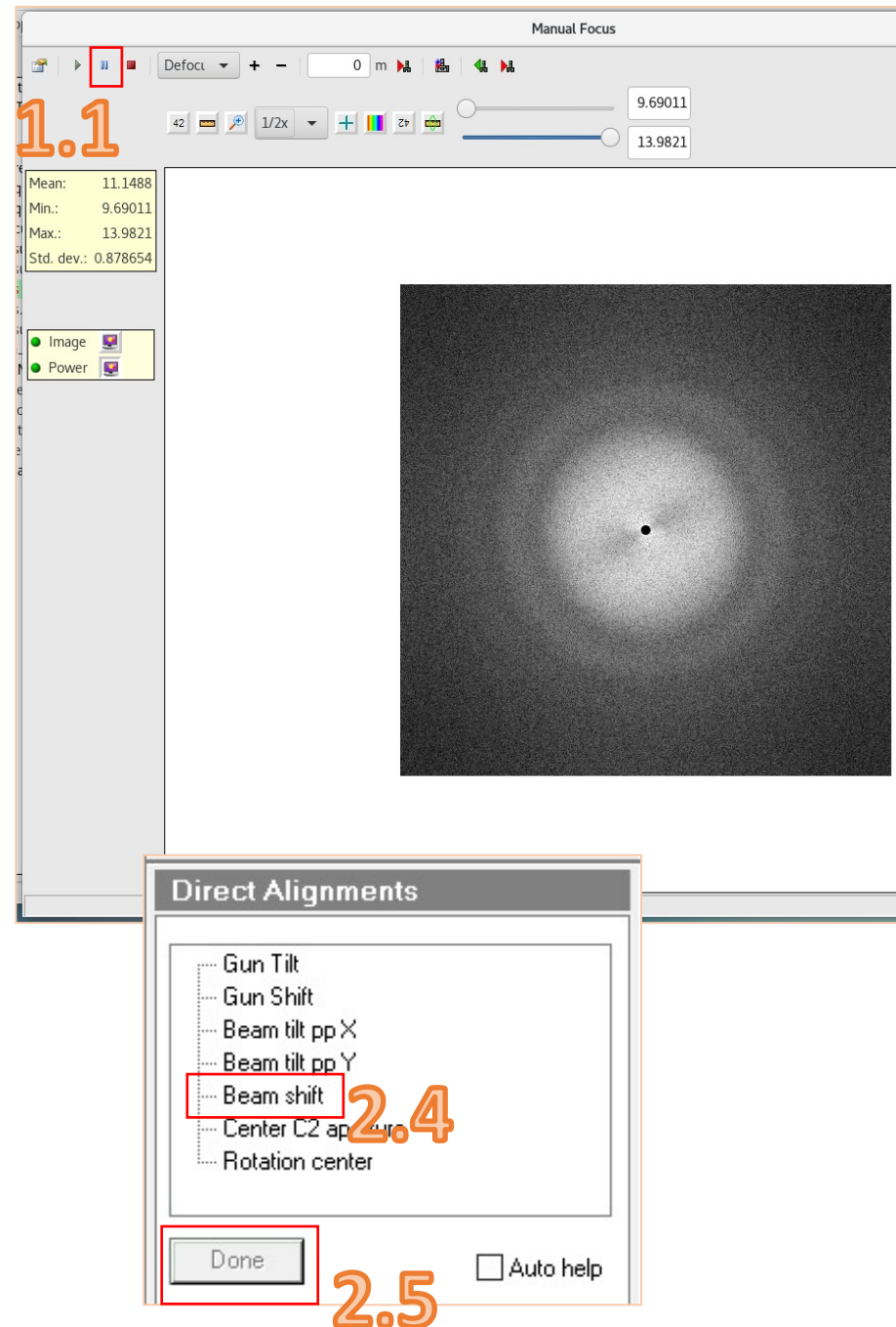
1.4



# Manual Focus

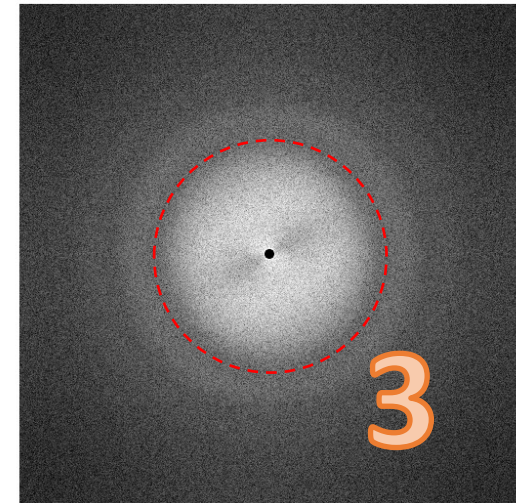
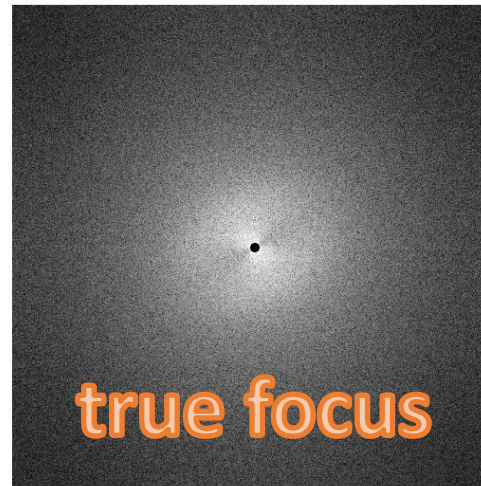
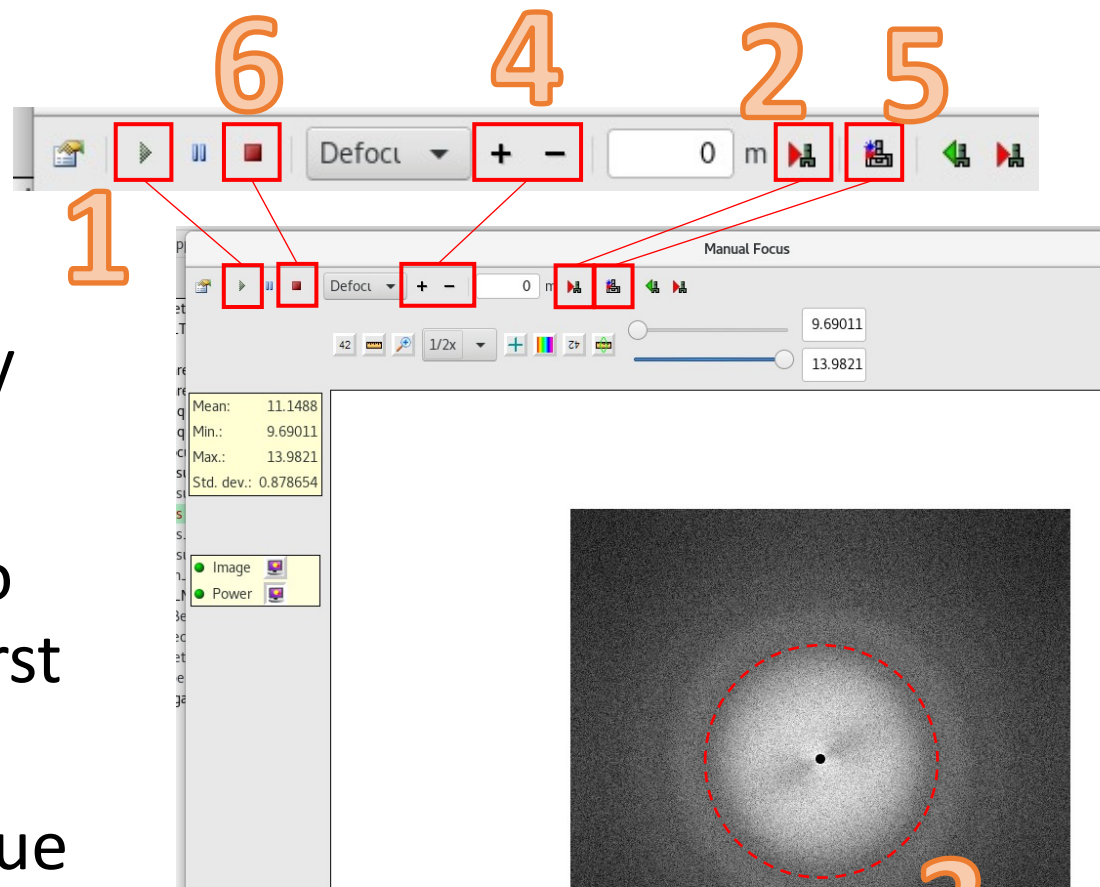
1. Manual focus window will pop up
  1. Pause (if you do not see "normal" rings go to step 2, otherwise skip)
2. On microscope PC:
  1. Insert screen (handpanel R1)
  2. Press "Eucentric focus" on handpanel
  3. Press "Reset defocus" on handpanel
  4. Click "Beam shift" in "Direct Alignments" and center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

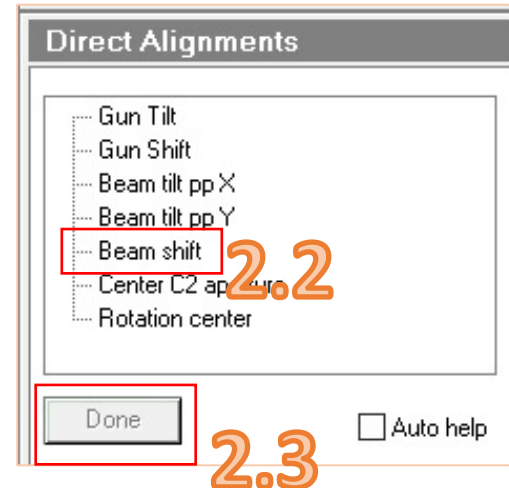
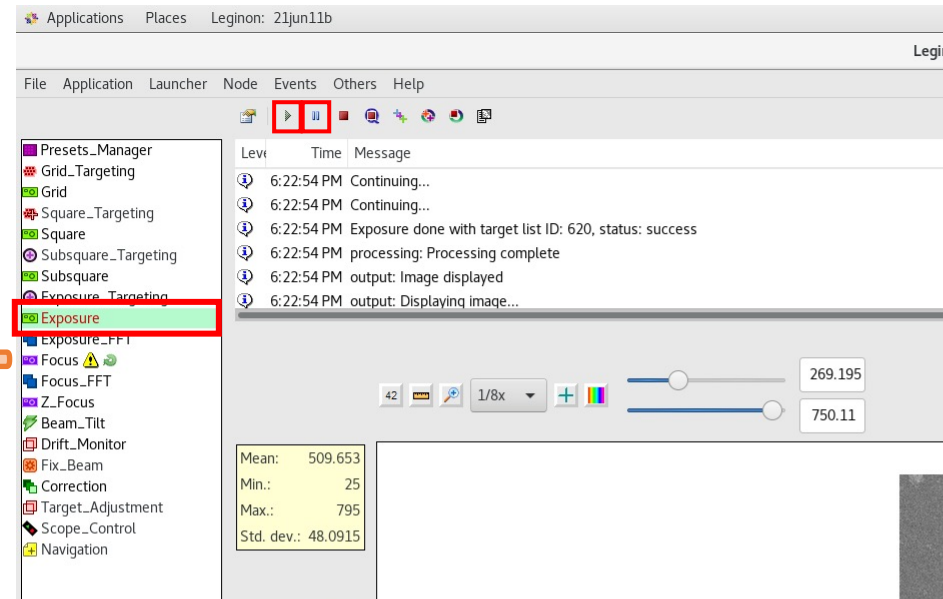


# Monitor exposures



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

1



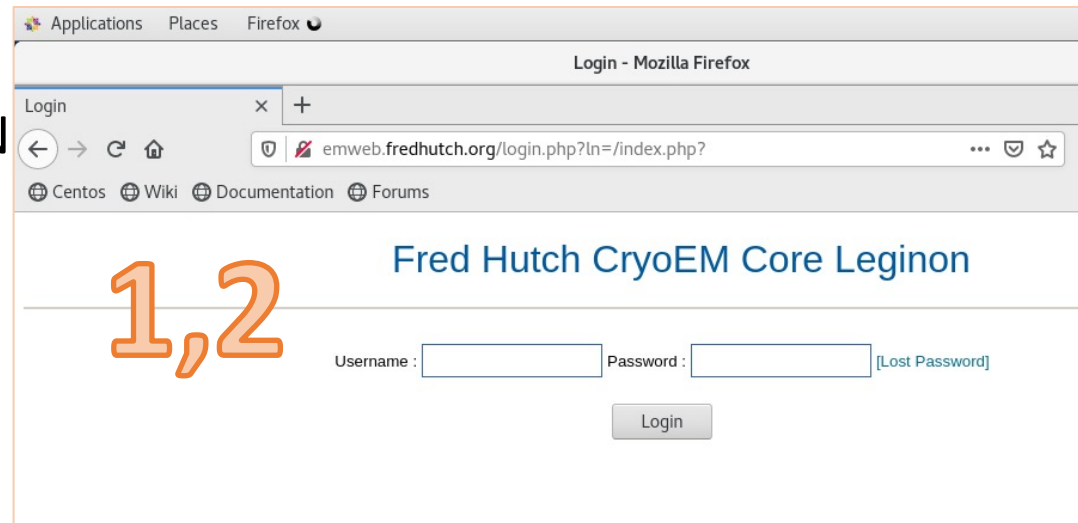
# Monitor exposures

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 Legimon protocol)

OR

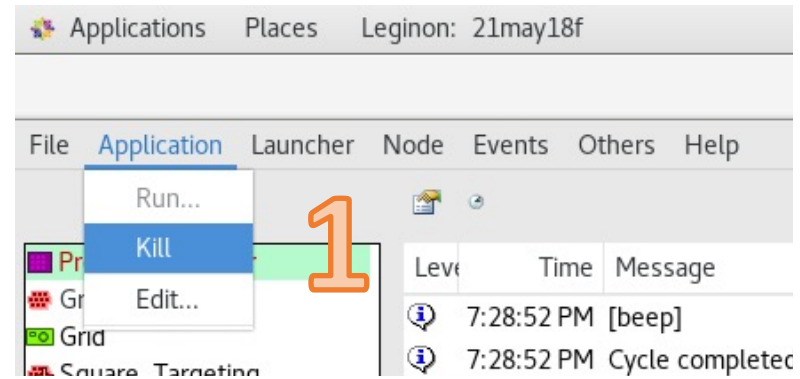
Screen more or another grid  
(repeat from slide 10)

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!