

**Leginon Protocol
collection
negative stain
Fred Hutch
Talos L120C**

What you're starting with

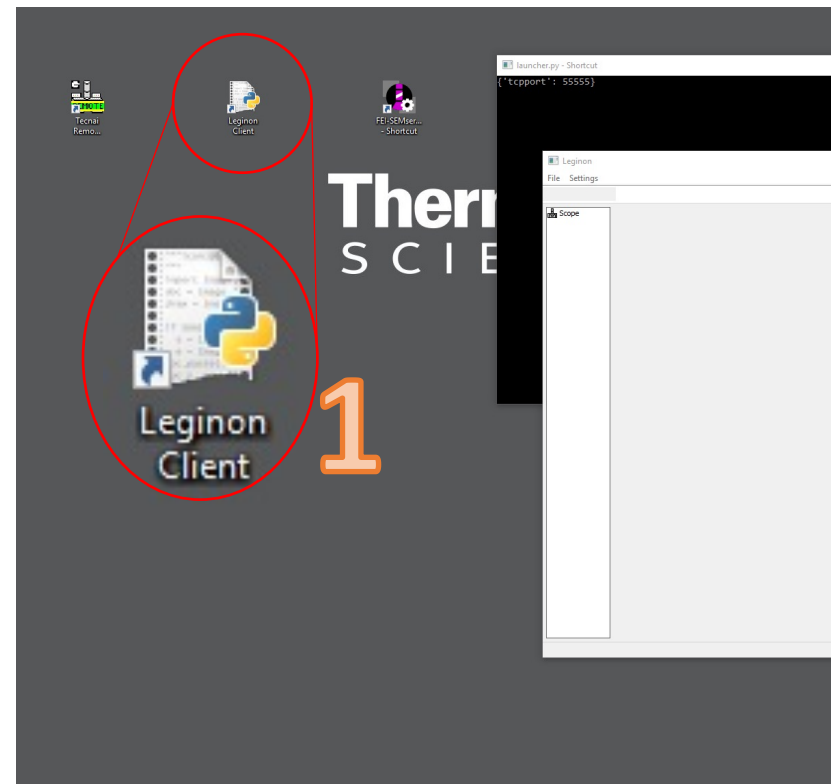
- You have performed the “Talos Start Up Checklist” and have:
 - A cold, vacuum-stable microscope
 - A beam
- You have screened your grid to confirm:
 - Good sample concentration
 - What good staining looks like at low/high magnification
 - Roughly how many images you would like to collect

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



Leginon-specific login and password

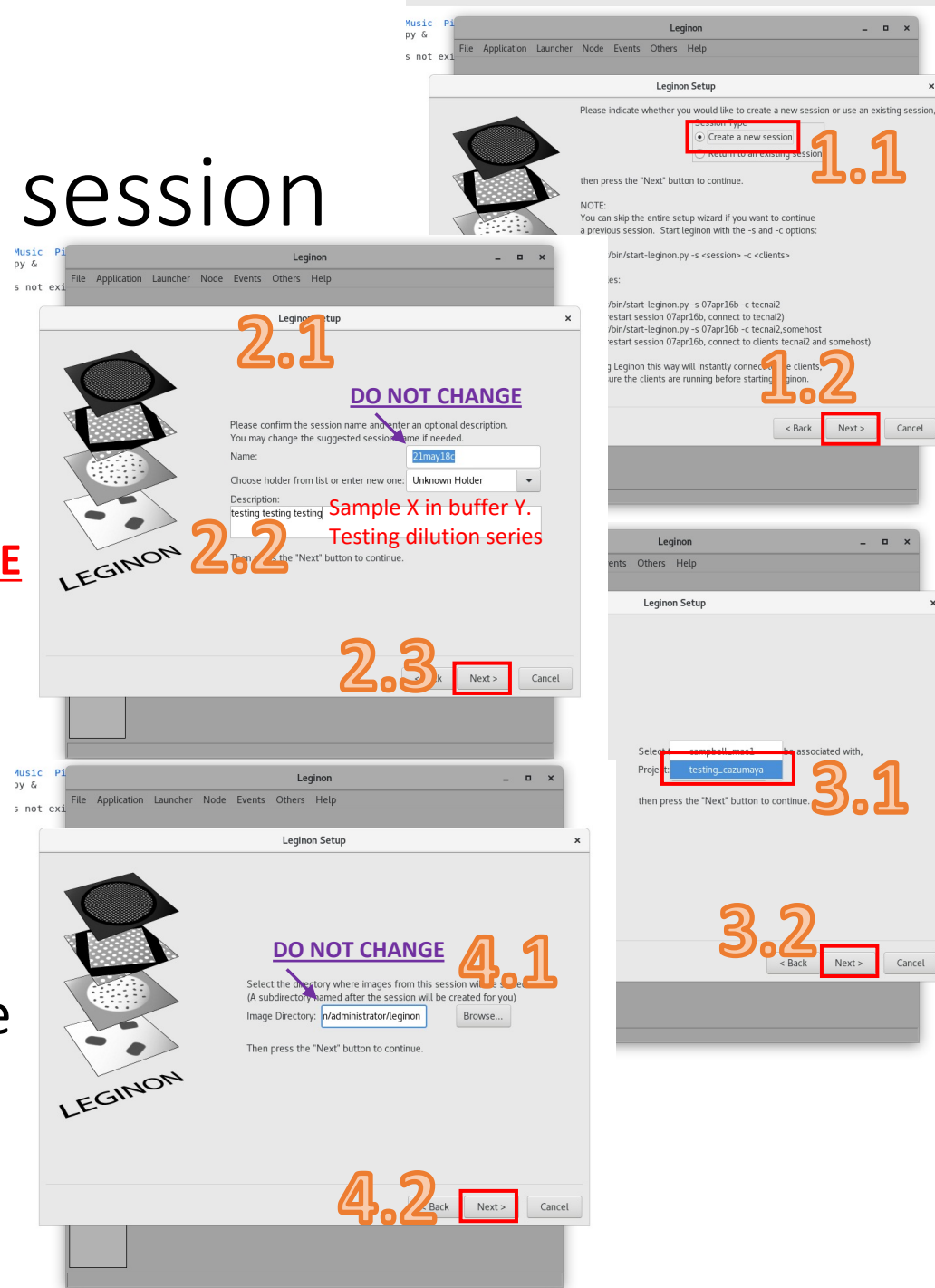
2. Login to Leginon computer
 1. Open terminal (right click on background)
 2. start-leginon.py

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

2

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

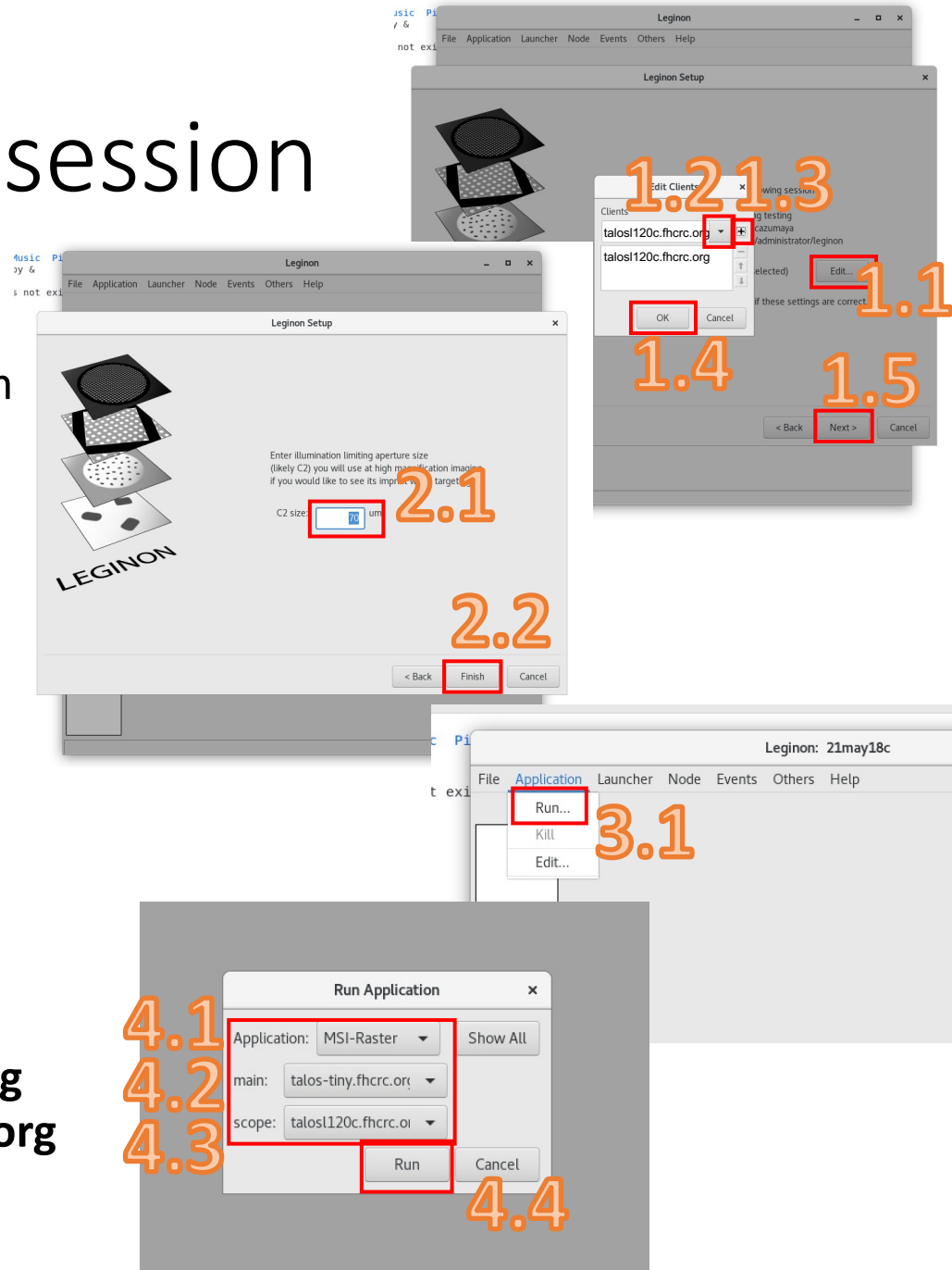
1. 70um
2. Finish

3. Start session

1. Application -> Run

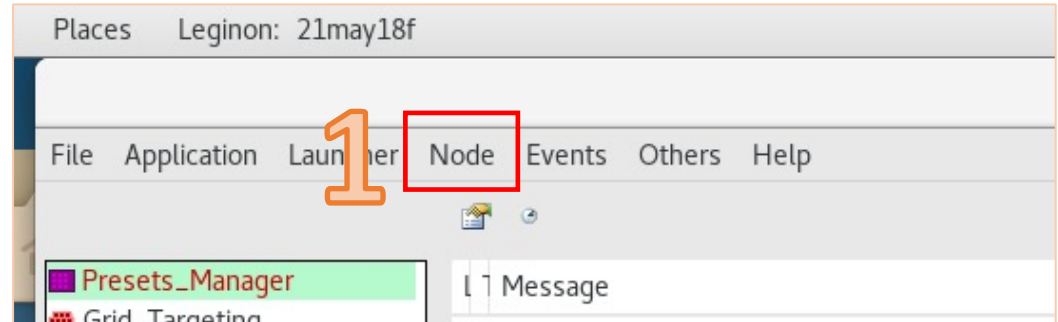
4. Choose application

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



Setup session

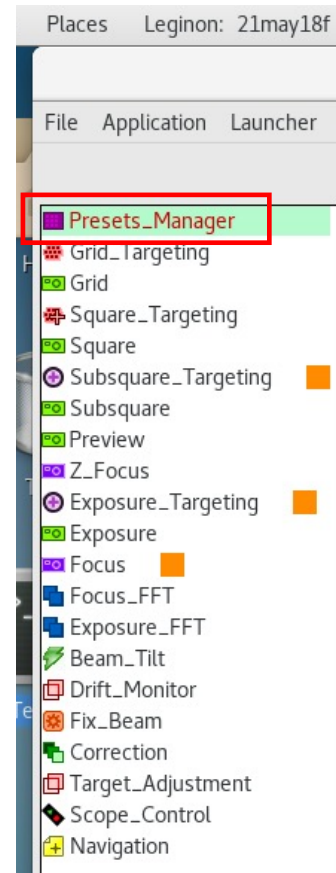
1. Node -> Kill -> Preview



2. Import presets

1. Presets Manager -> Blue dot icon

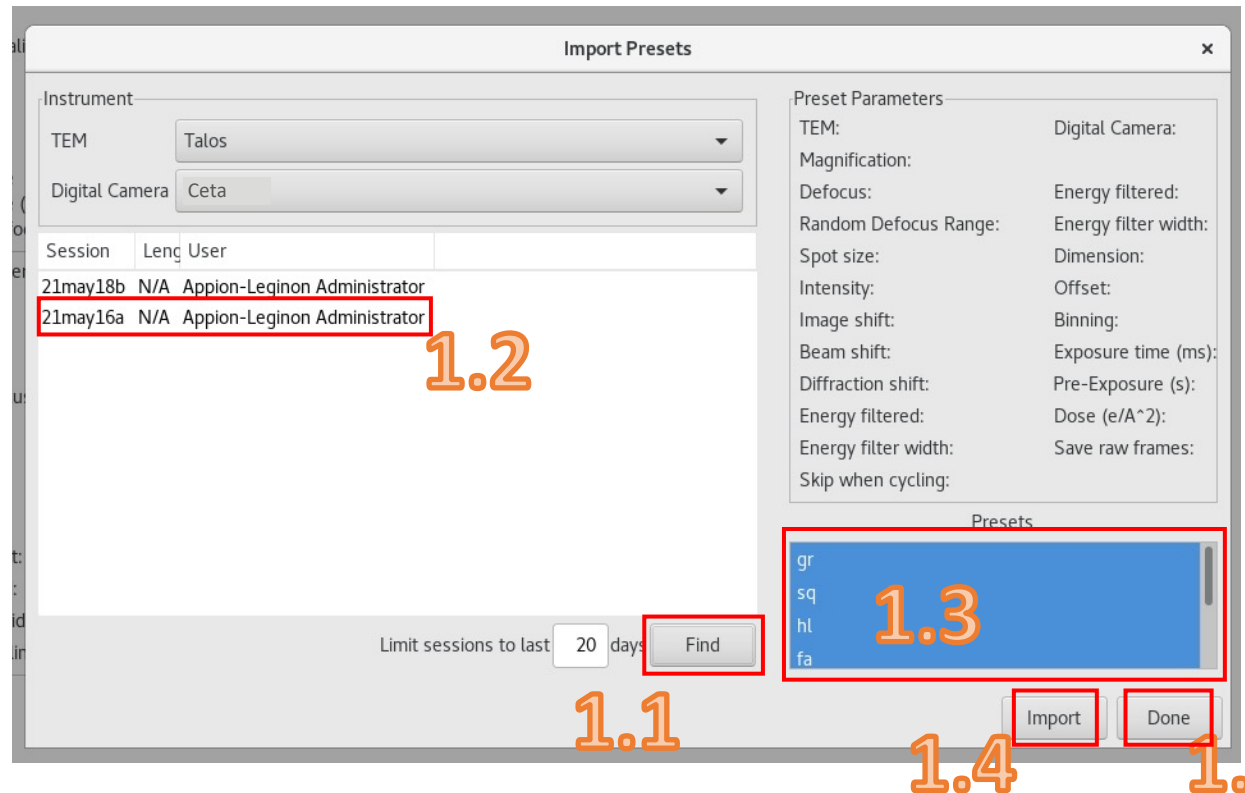
2



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):

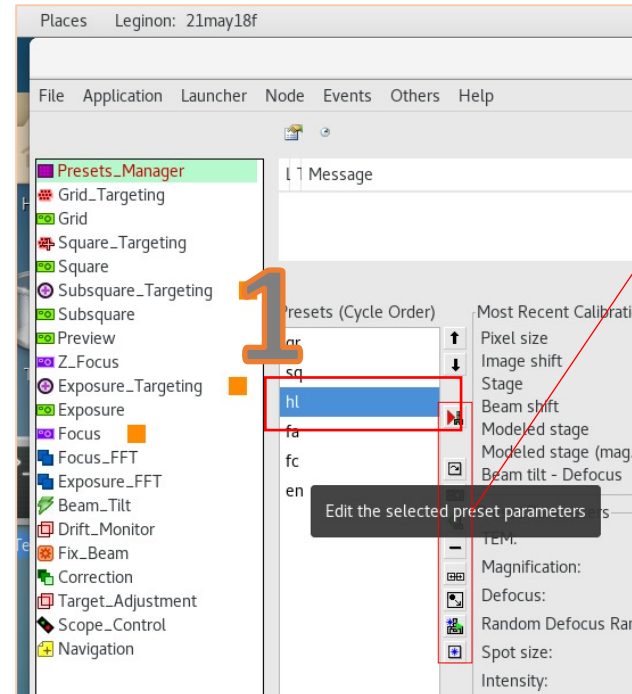
Dose (e/A²):

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

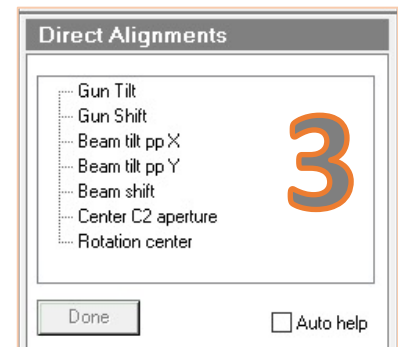
To change your presets

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



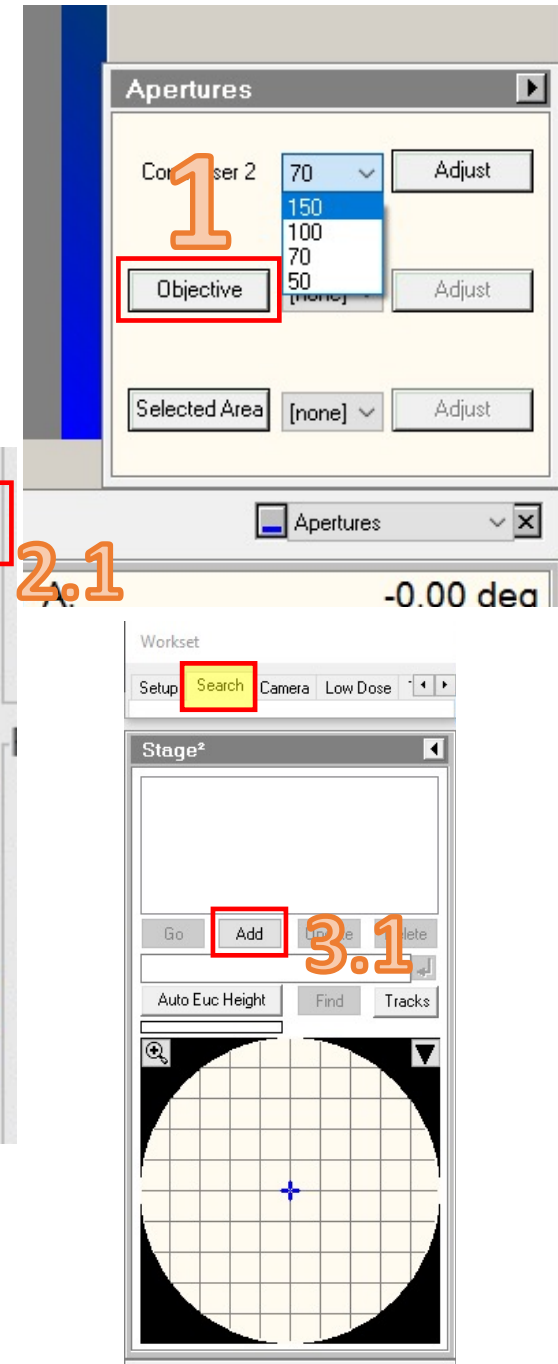
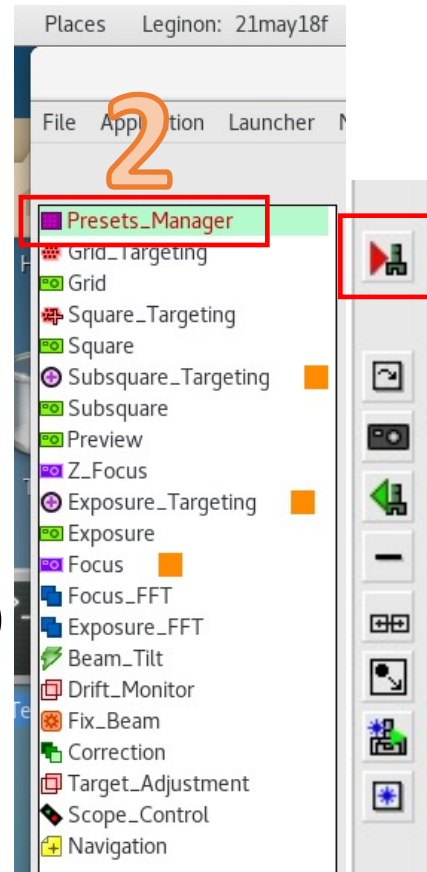
You should be near eucentric height when you adjust

- 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



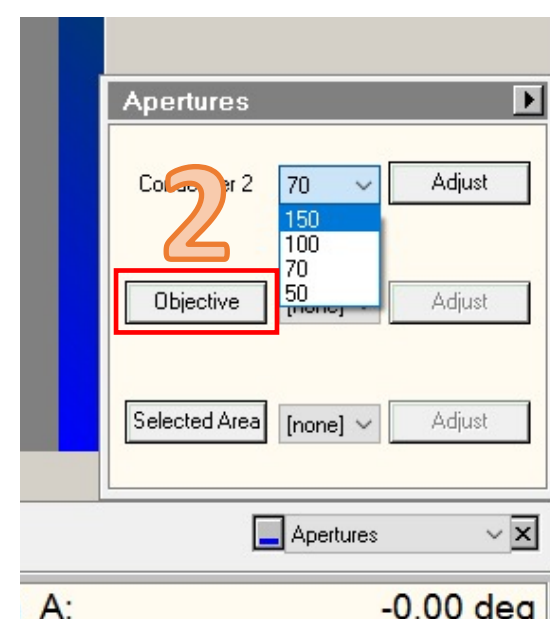
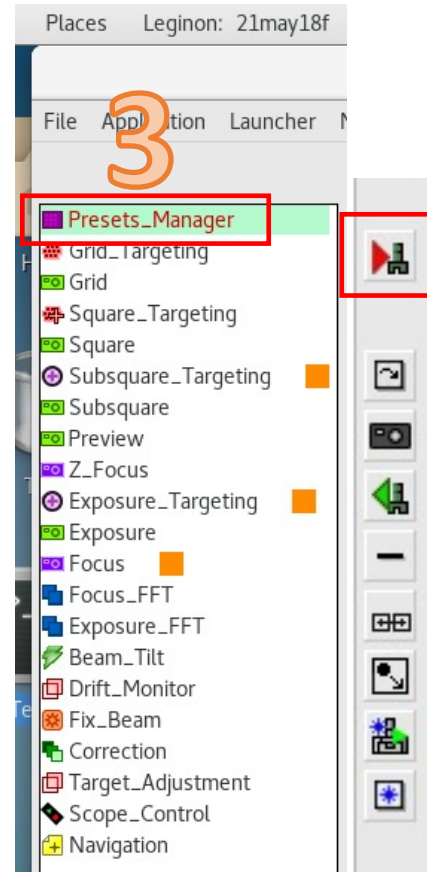
Find good square(s)

1. Click "Objective" on microscope "Apertures" to remove (will turn grey)
2. Go to Presets Manager
 1. Highlight grid 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

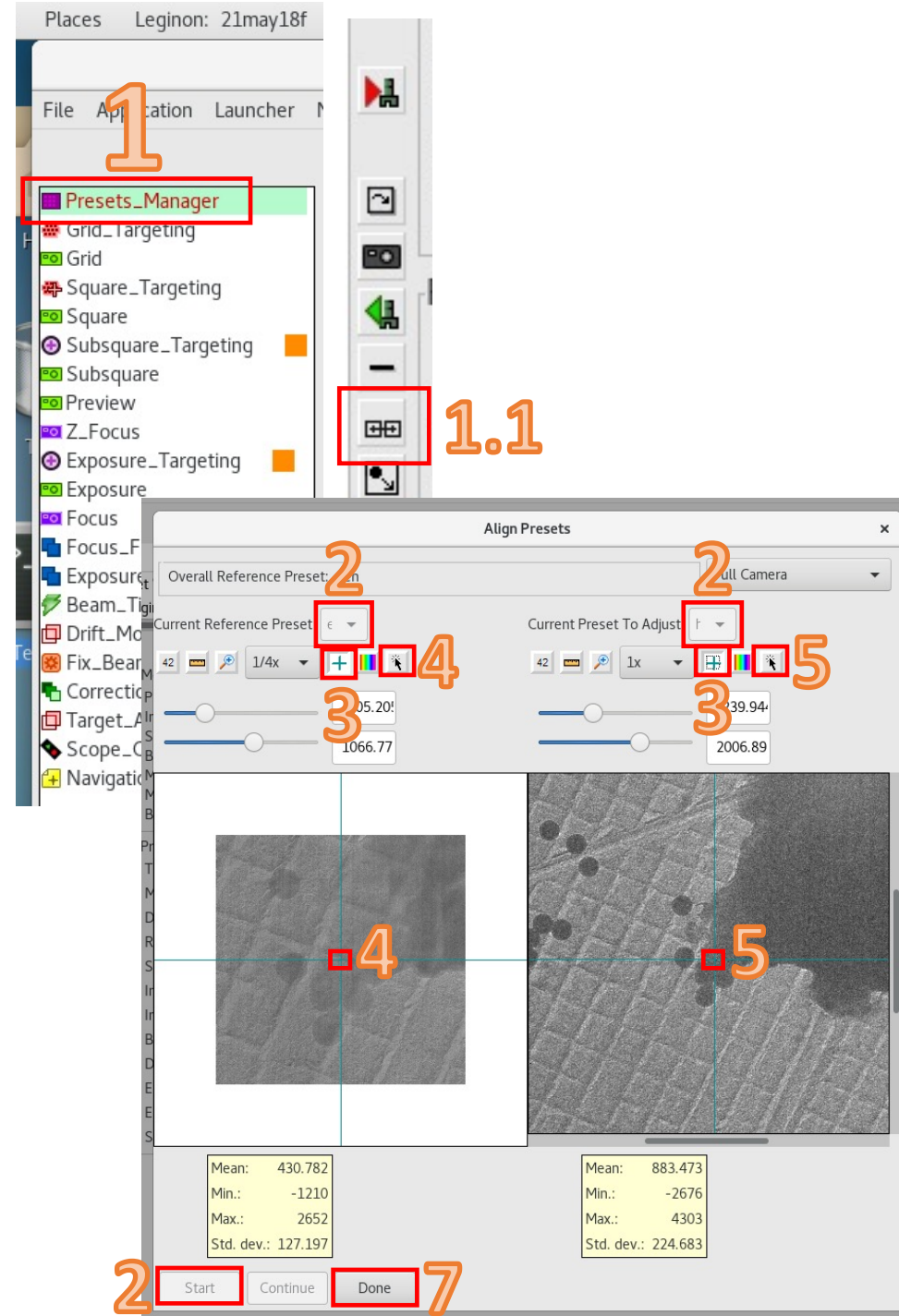


3, 4, 5


A: -0.00 dea

Align en and hl

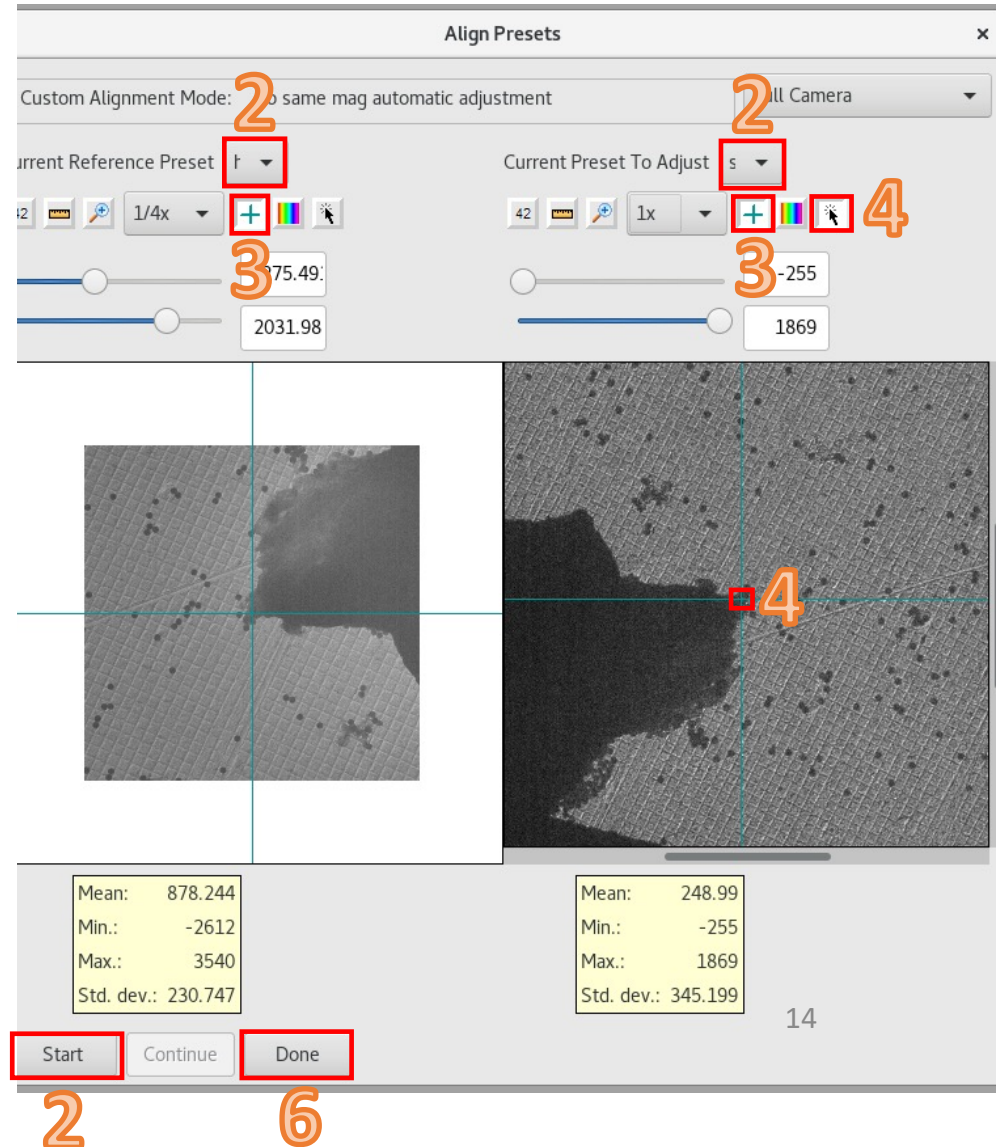
1. In Presets Manager:
 1. Click 'Align Presets'
2. Select **en** on left and **hl** on right and click Start
 1. Wait for images to populate
3. Turn on crosshair for both views
4. Turn on clicker and center feature in **en** by clicking on image
 1. Wait for scope to react (it's moving the stage)
5. 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

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

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



The screenshot shows the 'Align Presets' window with the following elements:

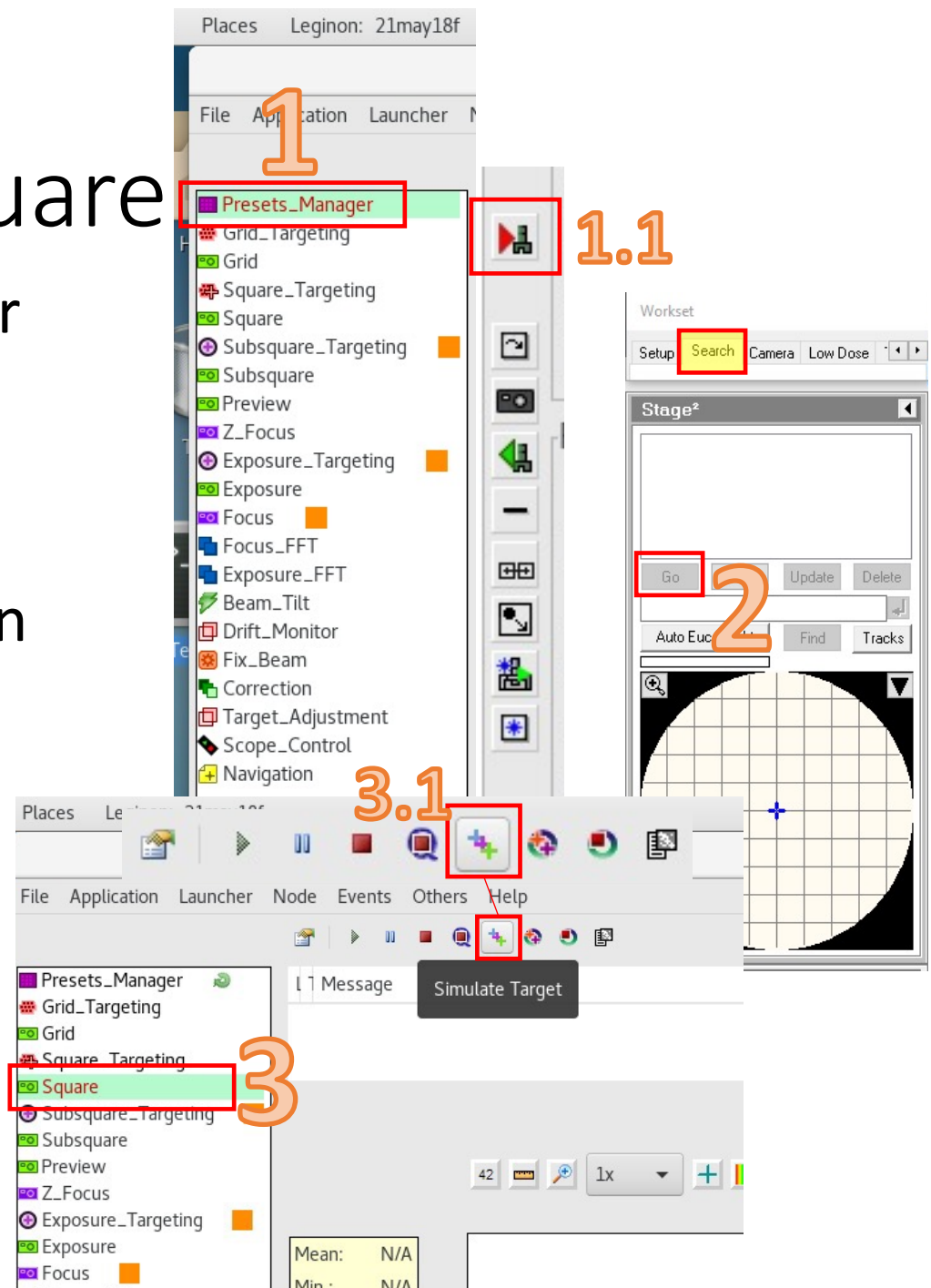
- Custom Alignment Mode:** 'to same mag automatic adjustment' (labeled 2)
- Current Reference Preset:** 'hl' (labeled 2)
- Current Preset To Adjust:** 'sq' (labeled 2)
- Buttons:** '+', '-', and 'Clicker' icons (labeled 4)
- Sliders:** Two sliders for alignment, with values 75.49 and 2031.98 on the left, and -255 and 1869 on the right (labeled 3)
- Images:** Two side-by-side microscope images with crosshairs. The right image has a red square on a feature (labeled 4).
- Statistics:** Two boxes showing mean, min, max, and std. dev. for each image.

Mean:	878.244
Min.:	-2612
Max.:	3540
Std. dev.:	230.747

Mean:	248.99
Min.:	-255
Max.:	1869
Std. dev.:	345.199
- Buttons:** 'Start', 'Continue', and 'Done' buttons (labeled 2 and 6)



Go to good square

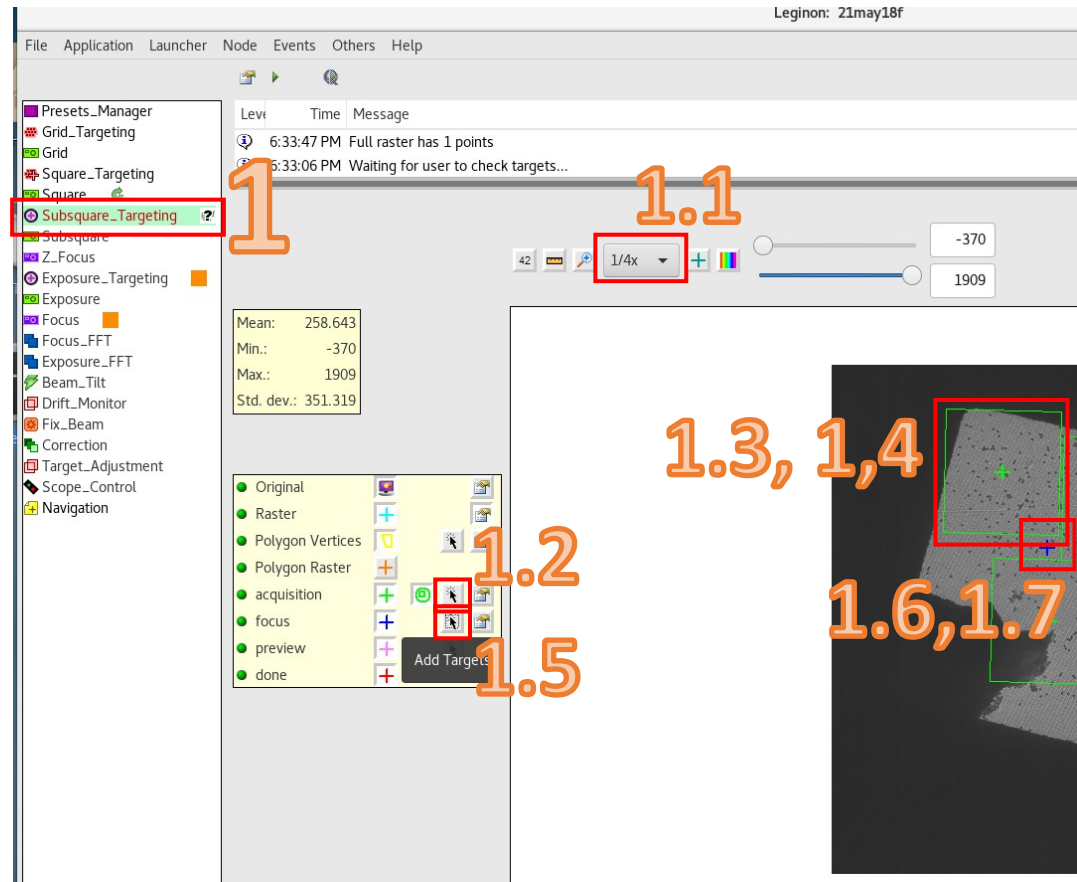
1. Go to Presets Manager
 1. Highlight sq and send to scope
2. "Go" to good square from saved positions in Search tab
3. Go to Square node
 1. "Simulate Target"
4. Repeat 2&3 for all good squares for collection



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

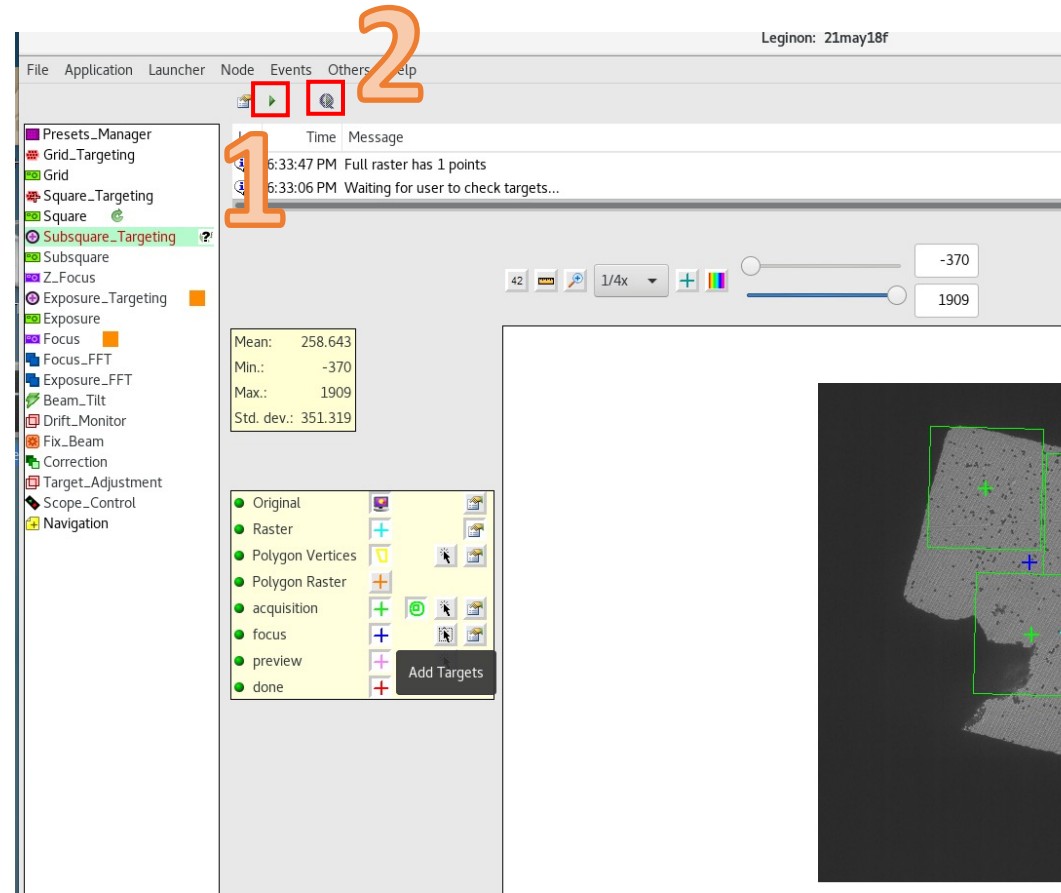


Green boxes will be approximate images
Dark blue + is Z-focus spot (put in middle)

Submit subsquare targets

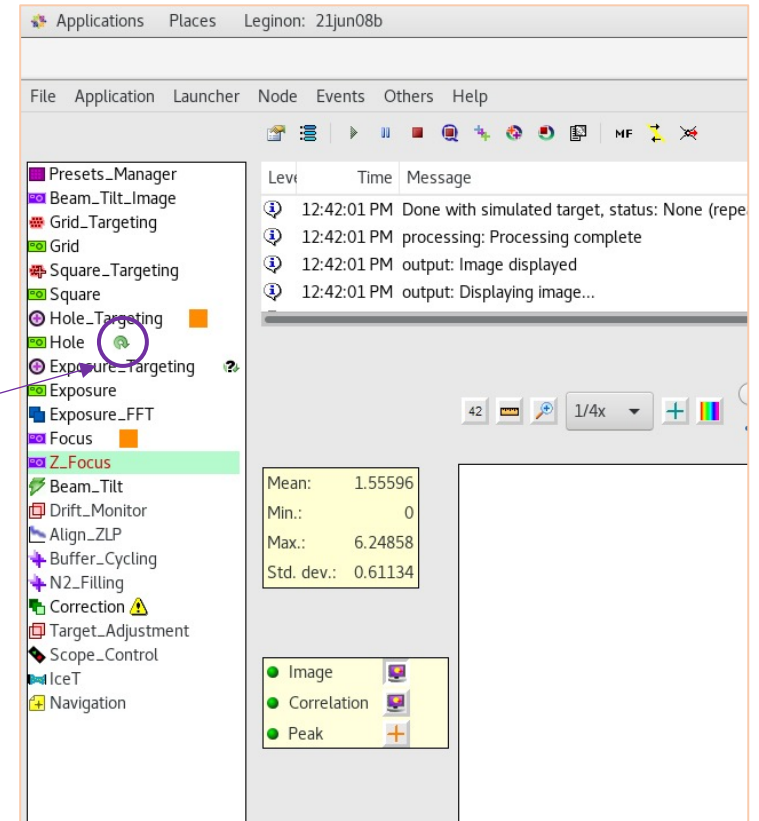
1. Click “play” button to add targets to put targets in queue
2. Click “Qplay” button to submit the queue for collection

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





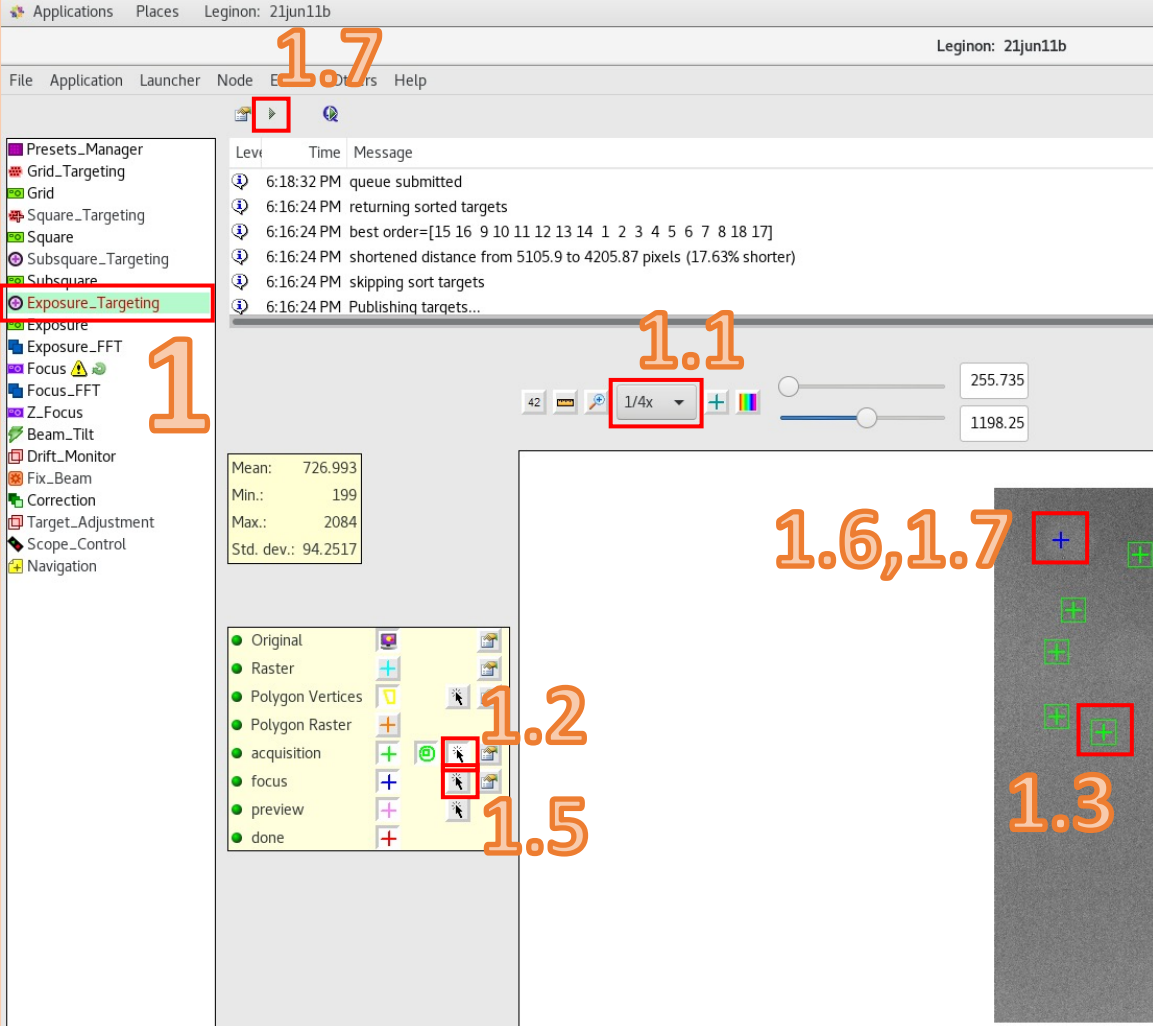
Do not submit exposure queue before subsquare queue finishes

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



The screenshot shows a software interface with the following components and annotations:

- 1.7**: Points to the "Play" button in the toolbar.
- 1**: Points to the "Exposure_Targeting" option in the left-hand menu.
- 1.1**: Points to the zoom level dropdown menu showing "1/4x".
- 1.2**: Points to the "acquisition" layer in the layer list.
- 1.5**: Points to the "focus" layer in the layer list.
- 1.6, 1.7**: Points to the main image area where targets are visible.
- 1.3**: Points to a specific target in the main image area.

The interface also displays a log window with the following messages:

Level	Time	Message
Info	6:18:32 PM	queue submitted
Info	6:16:24 PM	returning sorted targets
Info	6:16:24 PM	best order=[15 16 9 10 11 12 13 14 1 2 3 4 5 6 7 8 18 17]
Info	6:16:24 PM	shortened distance from 5105.9 to 4205.87 pixels (17.63% shorter)
Info	6:16:24 PM	skipping sort targets
Info	6:16:24 PM	Publishing targets...

Statistics window:

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

Layer list:

- Original
- Raster
- Polygon Vertices
- Polygon Raster
- acquisition
- focus
- preview
- done

Alter your raster spacing (usually just one time)

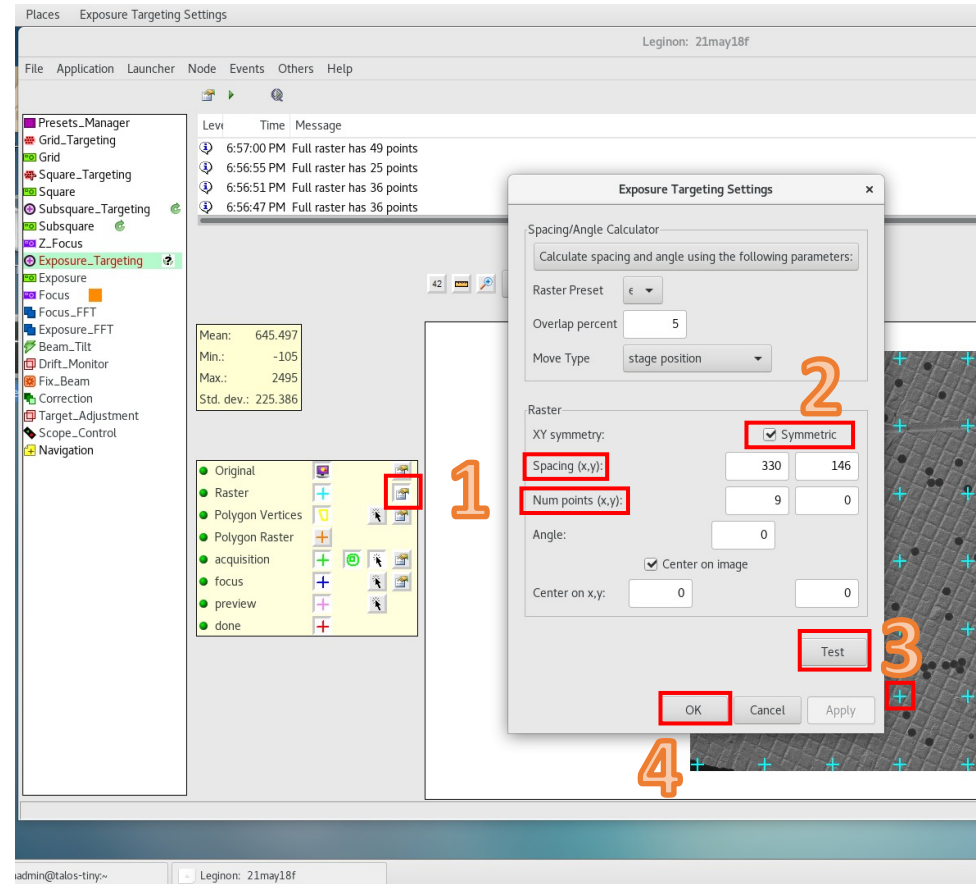
1. Click pointer finger by 'raster' to adjust spacing and number of points to overlap the amount you want (usually none)

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

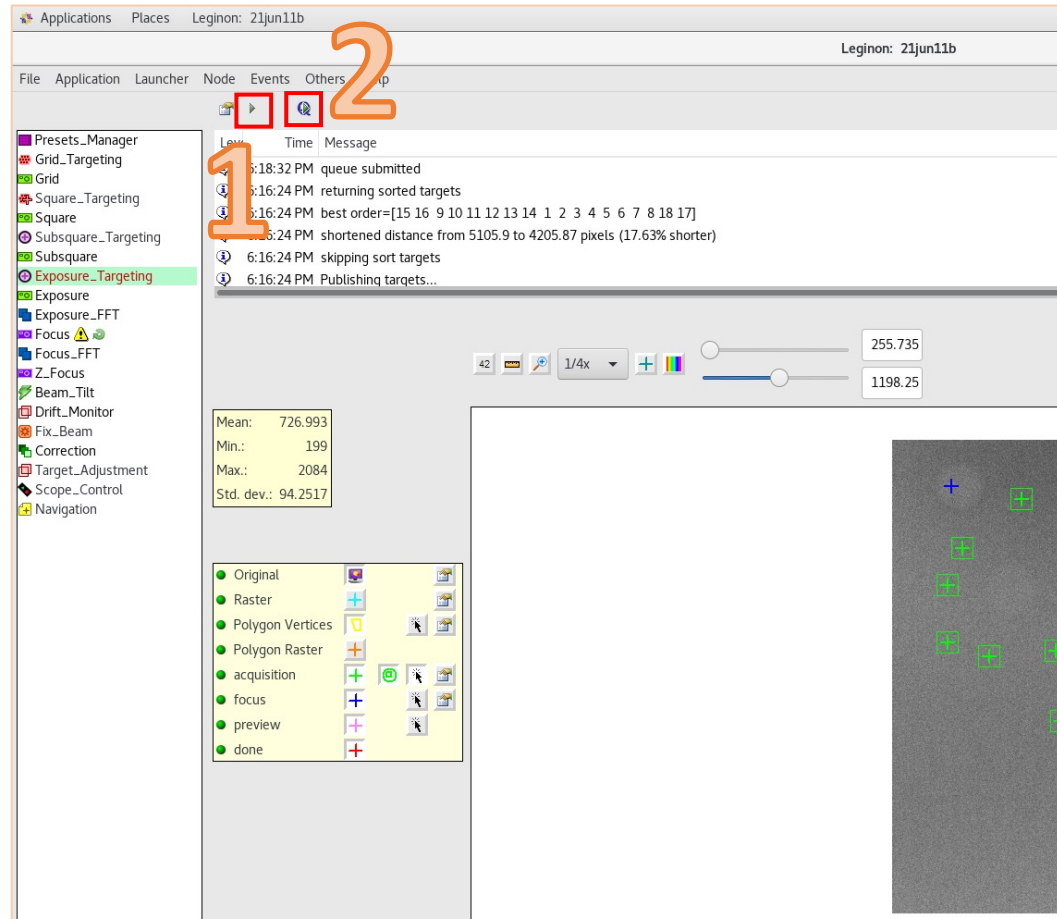


Submit exposure targets

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

1

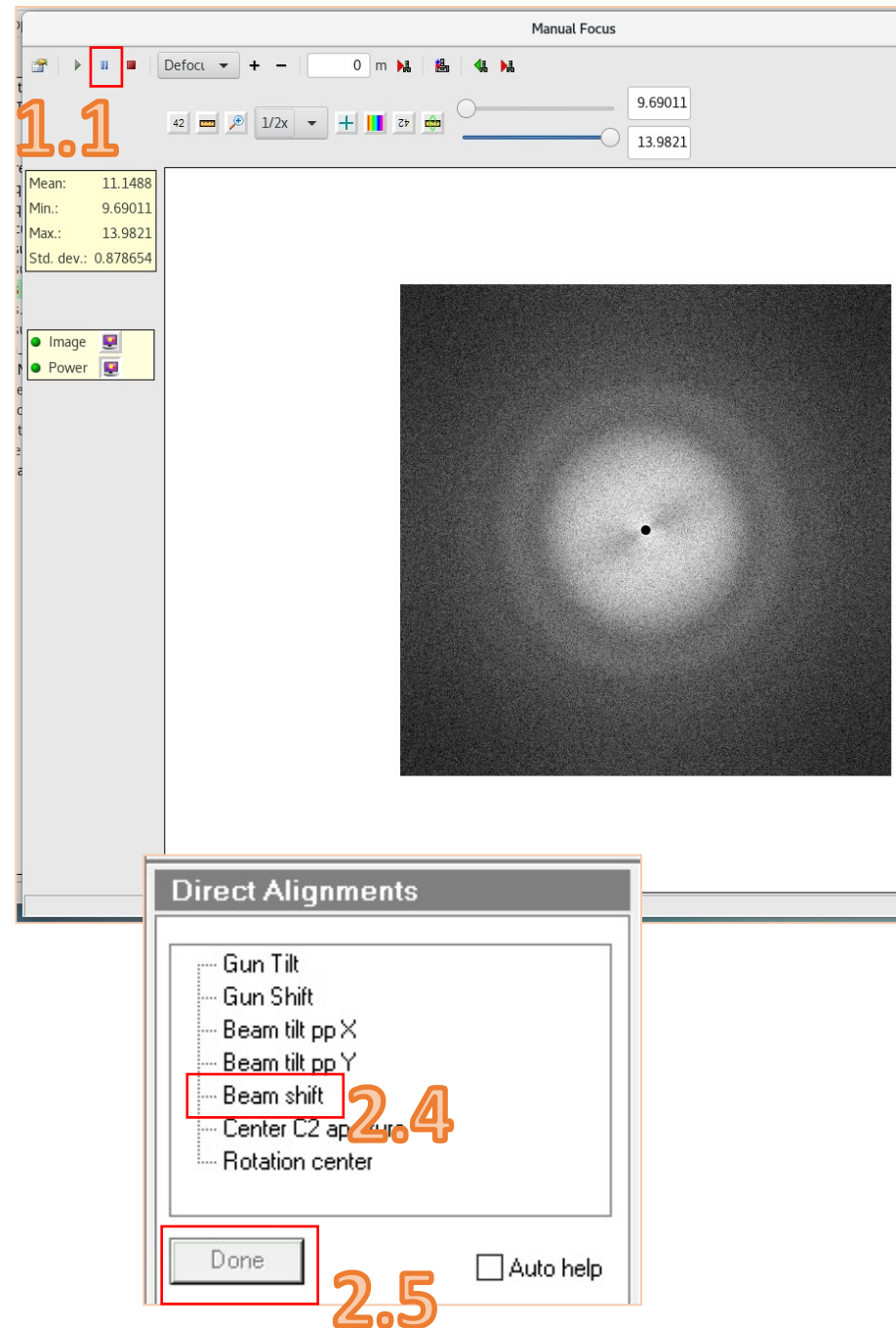
The screenshot shows the software interface with the following elements highlighted and labeled:

- 1**: The 'Focus' item in the left sidebar is highlighted with a red box.
- 1.1**: A blue bullet list icon in the top toolbar is highlighted with a red box.
- 1.2**: The 'Manual_after' option in the 'Focus sequence' list is highlighted with a blue box.
- 1.3**: The 'Enabled' checkbox is checked and highlighted with a red box.
- 1.4**: The 'OK' button is highlighted with a red box.

An orange square next to the 'Focus' item in the sidebar is circled in purple, indicating it is enabled.

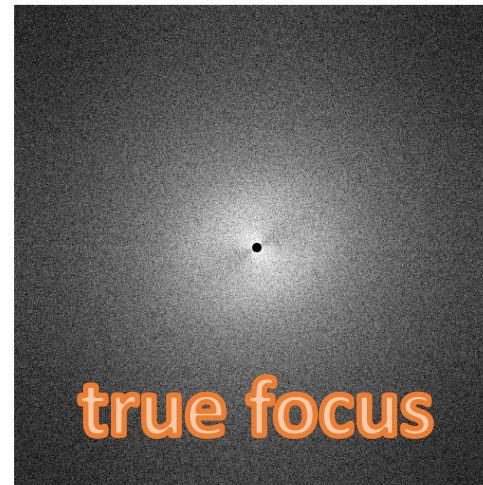
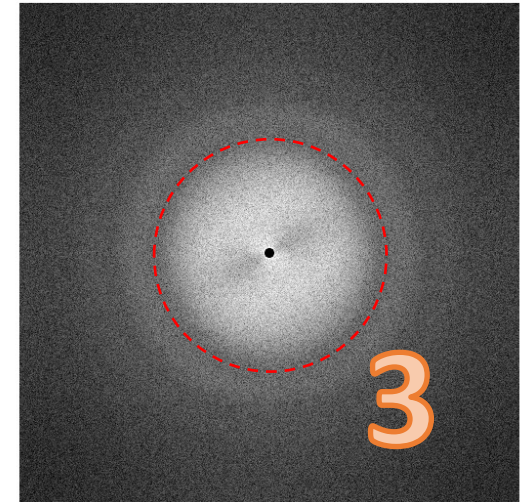
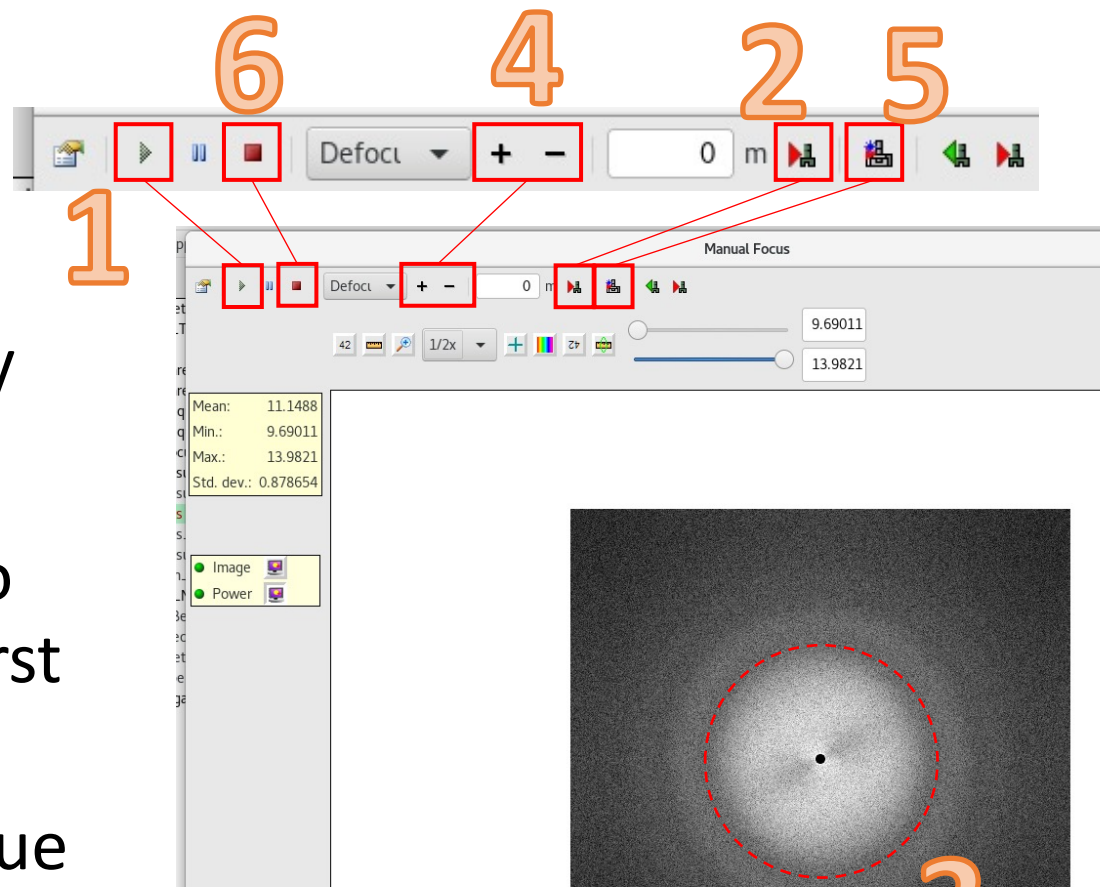
Manual Focus

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

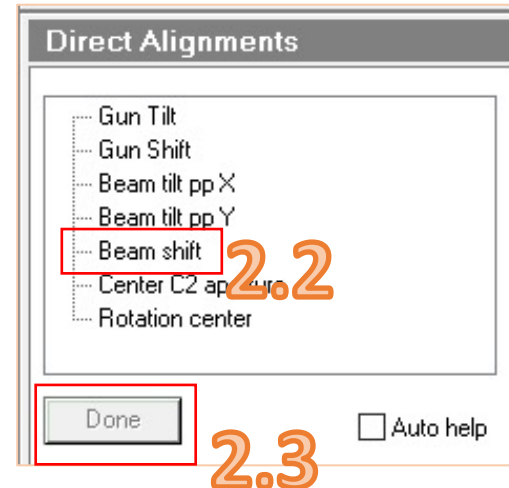
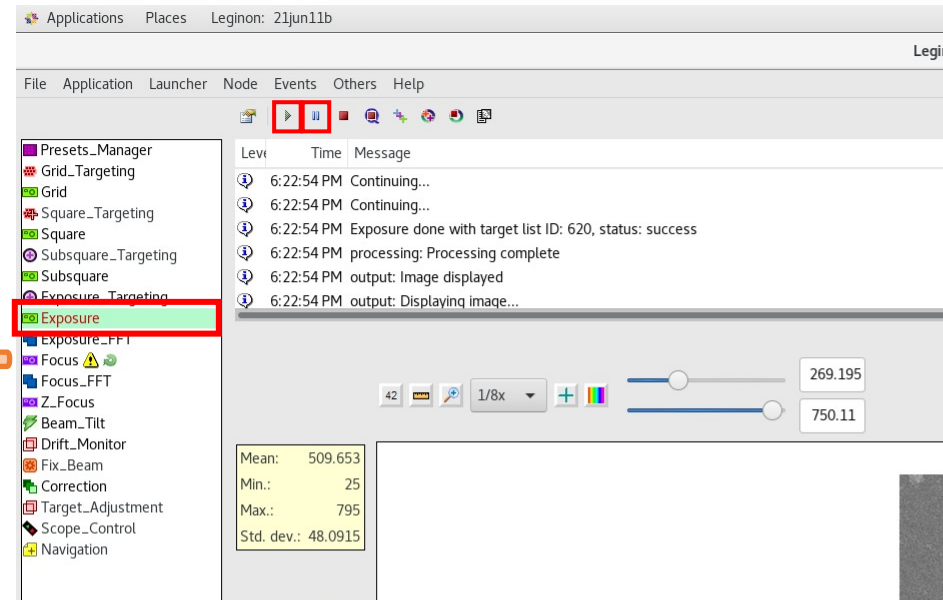


Monitor exposures



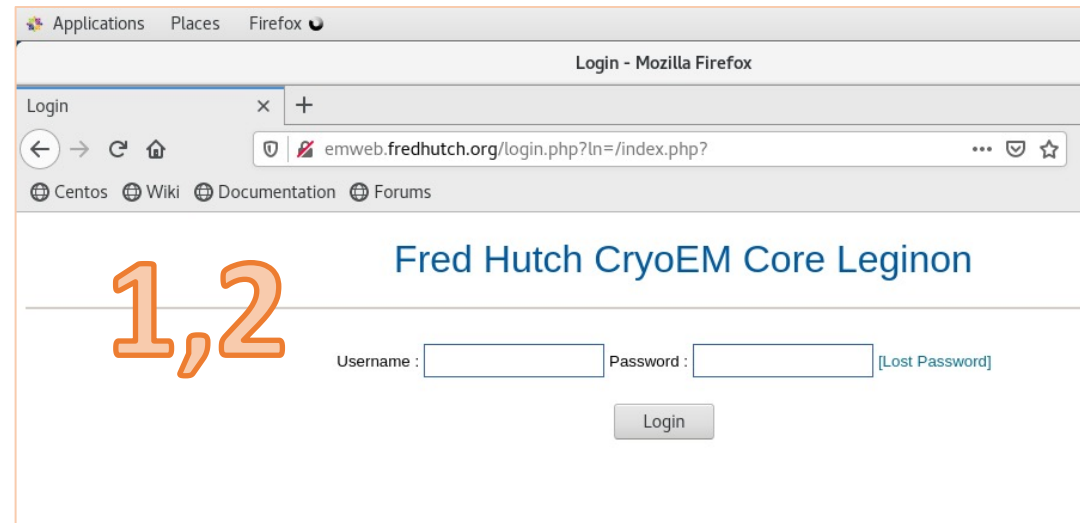
1. Go to Exposure
 1. If you can see the edge of the beam: Pause
2. On Microscope computer
 1. Insert screen (handpanel R1)
 2. Click “Beam shift” in “Direct Alignments” and center beam with multifunction X and Y knobs
 3. Done
3. Back in Exposure: Play

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
 - Use LOI to determine how much time is left in your collection
 - More info on webserver on Teams channel



estimated queue processing time

Subsquare Targeting (29 targets)
unprocessed queue = 0
avg time so far = 32 s
remaining time = 0 min 0 s

Exposure Targeting (252 targets)
unprocessed queue = 0
avg time so far = 32 s
remaining time = 0 min 0 s

estimated non-queue processing time

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

The screenshot shows the software interface with the following elements:

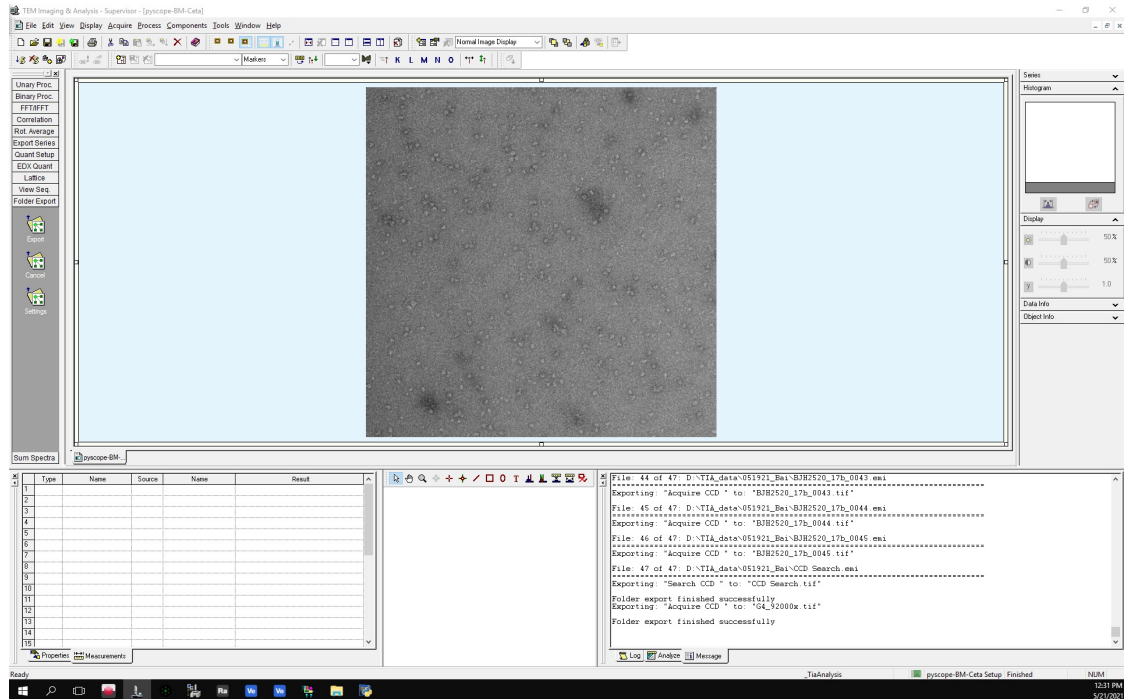
- Menu Bar:** File, Application, Launcher, Node, Events, O
- Component List (Left):** Presets_Manager, Grid_Targeting, Grid, Square_Targeting, Square, Subsquares_Targeting, Subsquares, Z_Focus, Exposure_Targeting, Exposure, Focus, Focus_FFT, Exposure_FFT, Beam_Tilt, Drift_Monitor, Fix_Beam, Correction, Target_Adjustment, Scope_Control, Navigation.
- Focus Sequence (Focus) Dialog:**
 - Focus sequence:** [Empty field]
 - Defocus1:** [Empty field]
 - Defocus2:** [Empty field]
 - Manual_after:** [Selected]
 - Enabled:** (1.3)
 - Preset:** fc
 - Focus method:** Manual
 - (Autofocus Only):**
 - Tilt:** 0.01 radians
 - Image registration:** phase correlation
 - Fit limit:** 5000
 - Correct for delta Defocus/Z between:** 0 and 0.001 meters
 - Correction type:** Defocus
 - Wait for drift to be less than 3e-10 m/s
 - Repeat drift check until it passes the first time
- Buttons:** OK (1.4), Cancel

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

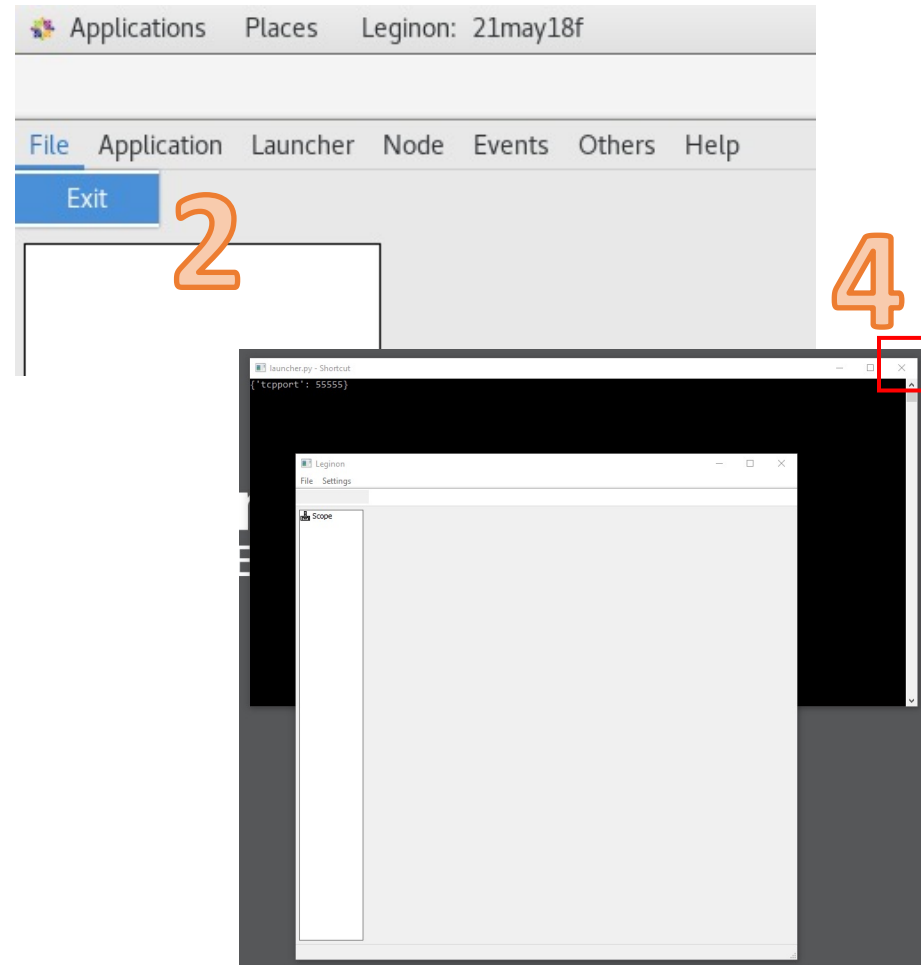
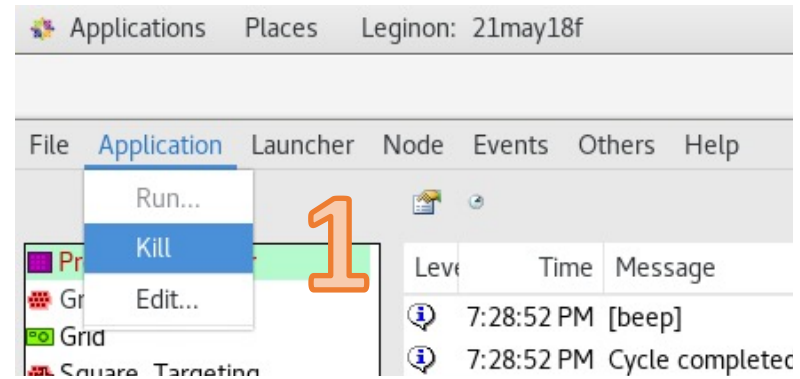
When you leave the room:

- Leave TIA window up when you leave the room and Legikon 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!