

Leginon Protocol

cryo screening

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Glacios

What you're starting with

- You have performed the “Glacios Start Up Checklist” and have:
 - A cold, vacuum-stable microscope
 - The Turbo is on “Auto Off”
 - You know the grid you want to screen
 - WARP computer is logged in

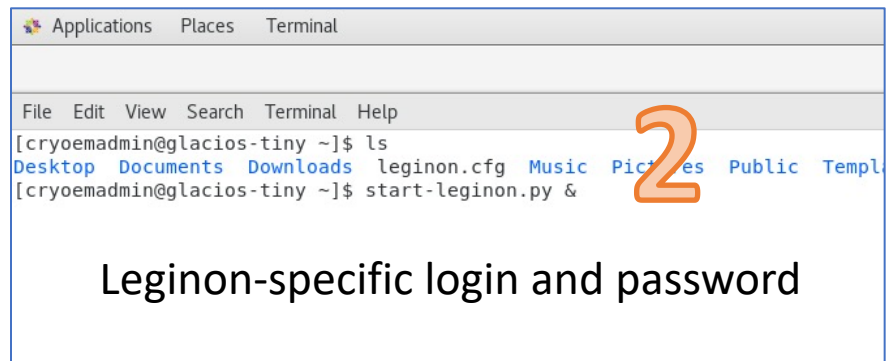
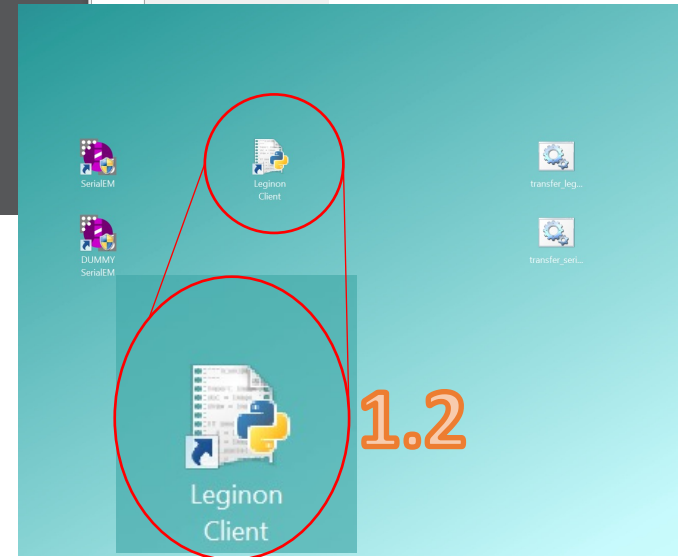
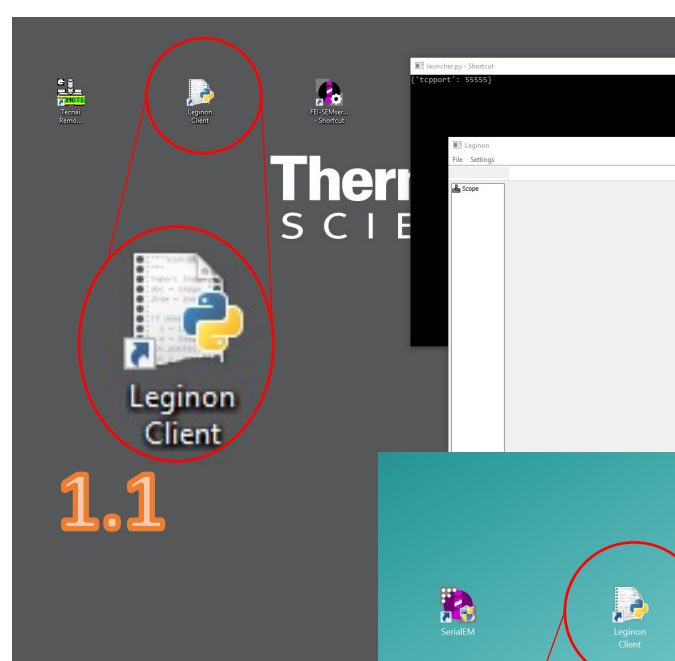
Start Leginon

1. Double click “Leginon Client” on the **microscope and K3 computers** to start

1. Two windows have to open on both computers before you start Leginon

2. Login to Leginon computer (glacios-tiny)

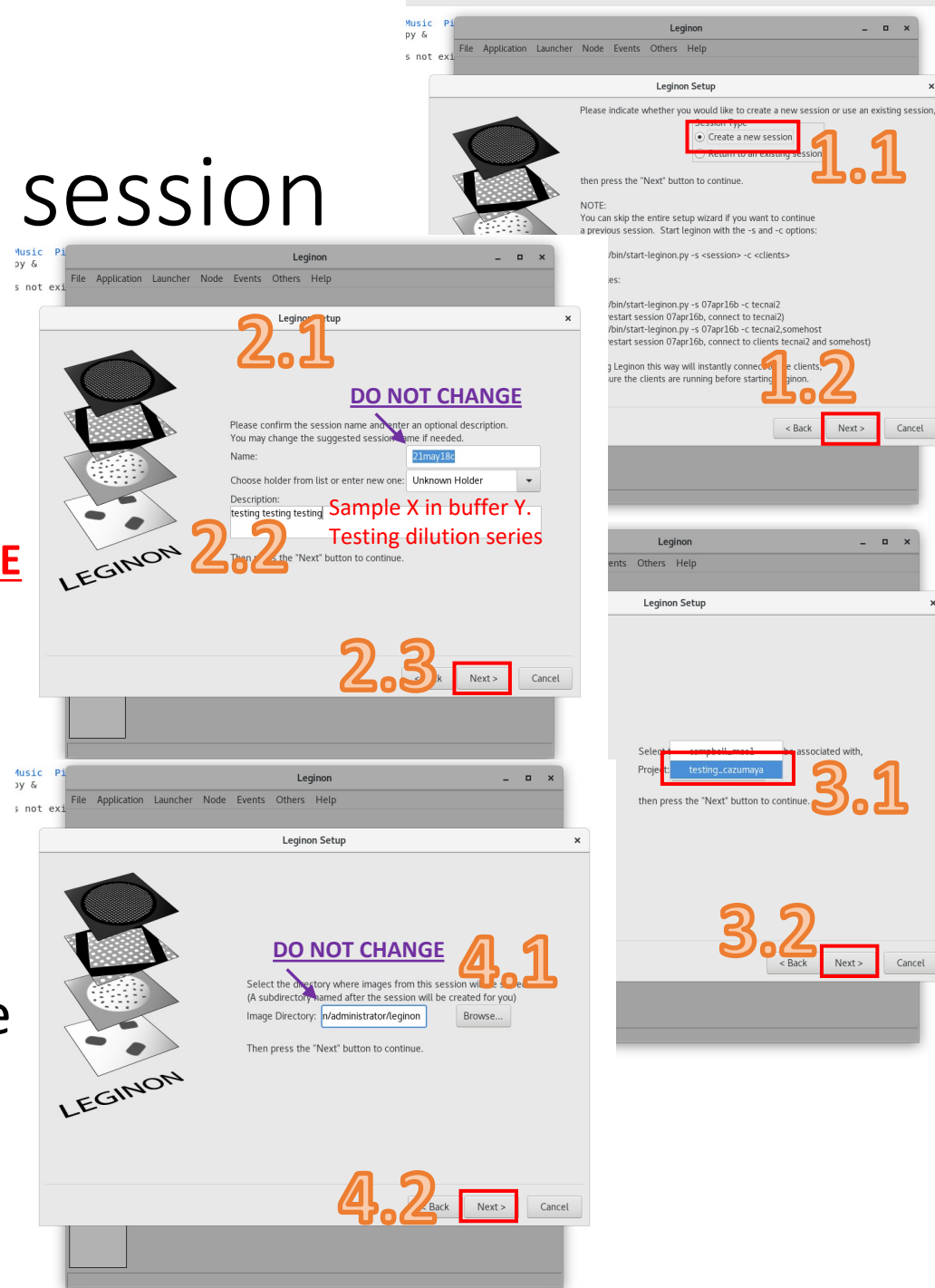
1. Open terminal (right click on background)
2. start-leginon.py



Leginon-specific login and password

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. Choose `glacios.fhcr.org` from dropdown
3. Click + !!
4. Choose `gatank3.fhcr.org` from dropdown
5. Click + !!
6. OK
7. Next

2. Define C2 aperture

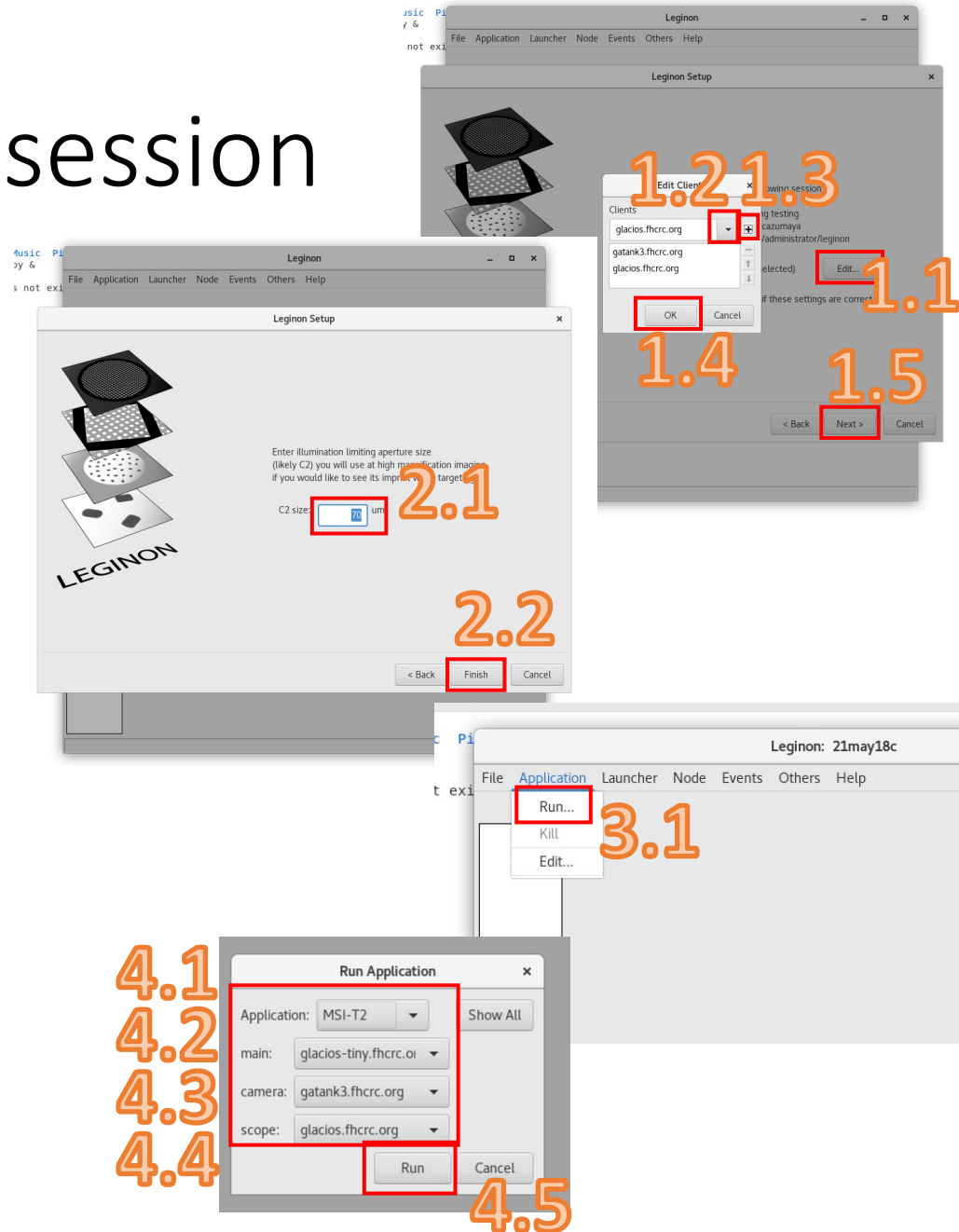
1. **50um**
2. Finish

3. Start session

1. Application -> Run

4. Choose application

1. Application: MSI-T2
2. Main: **glacios-tiny.fhcr.org**
3. Camera: **gatank3.fhcr.org**
4. Scope: **glacios.fhcr.org**
5. Run



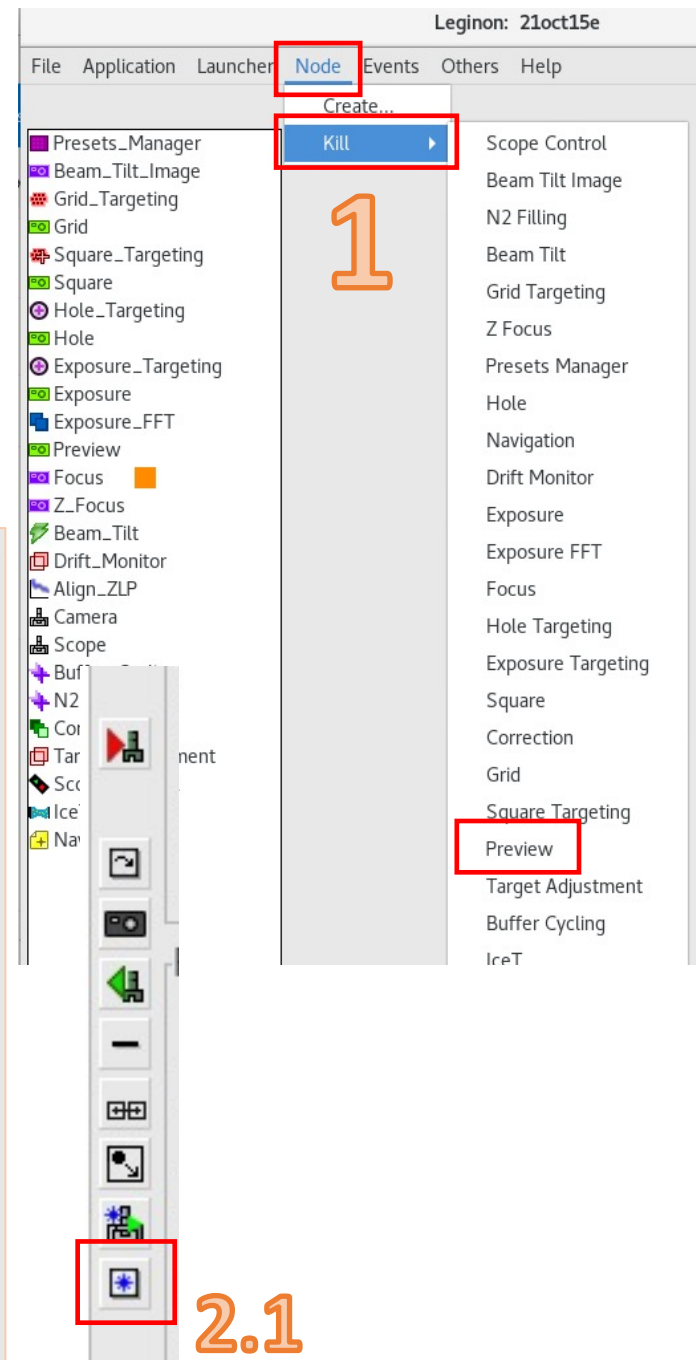
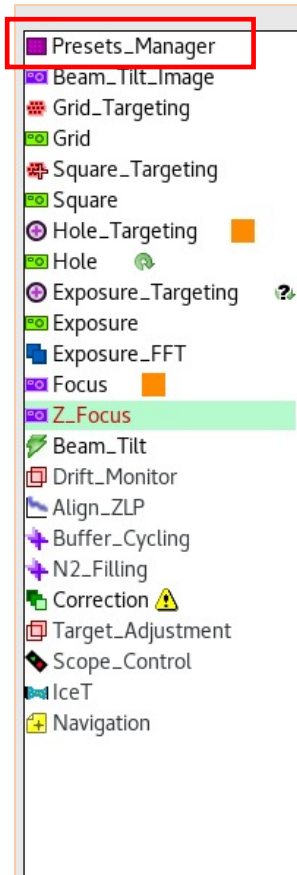
Setup session

1. Node -> Kill -> Preview

2. Import presets

1. Presets Manager -> Blue dot icon

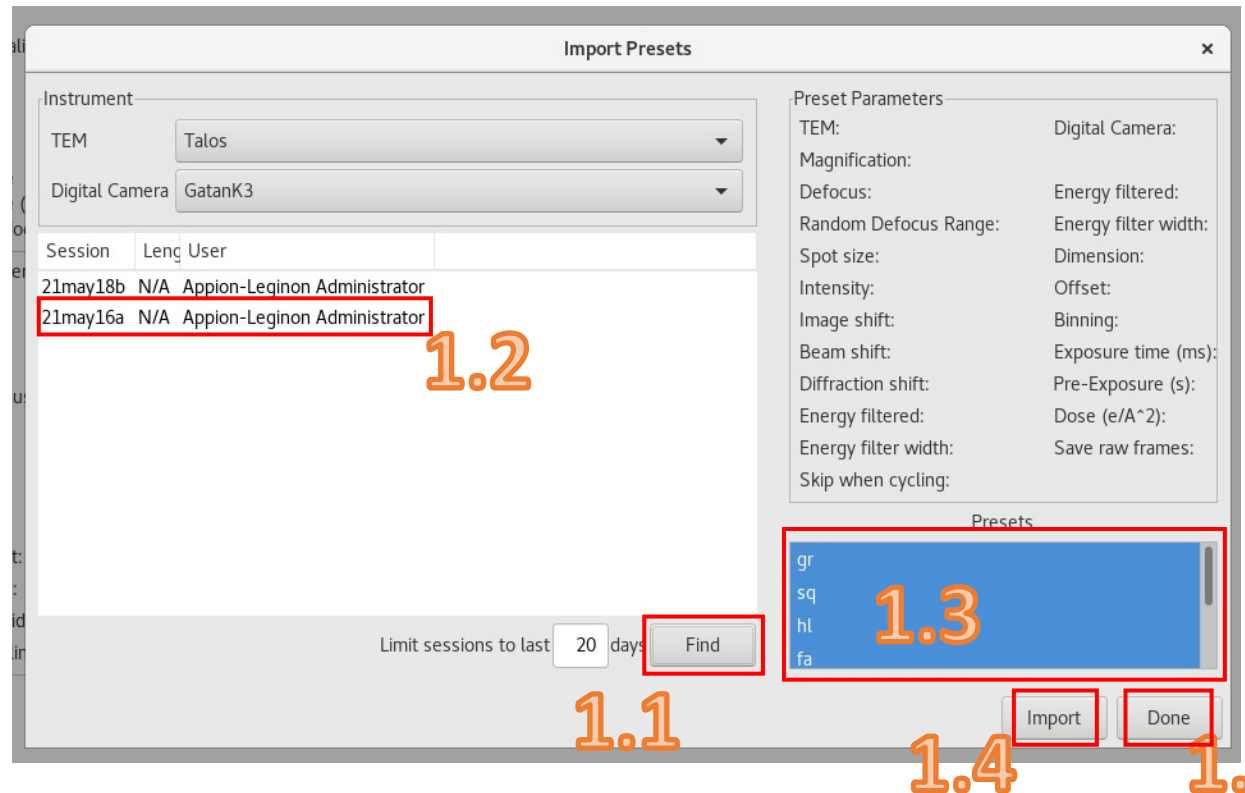
2



Setup session

1. Import presets

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



Load your grid

On the Autoloader tab of the microscope UI

1. Click the number grid you want to load
2. Click Load

1

2

Workset

Setup Autoloader Tune Search A ◀ ▶

Autoloader (User)

— Cassette

— Cartridge

12	
✓ 11	
10	
9	
8	
7	
6	
5	
✓ 4	
✓ 3	
✓ 2	
✓ 1	

Status

Temperature Control

— Status

All Nitrogen Temperature

— Dewar levels

Autoloader	47 %	4 h 30 min
Column	64 %	8 h 40 min

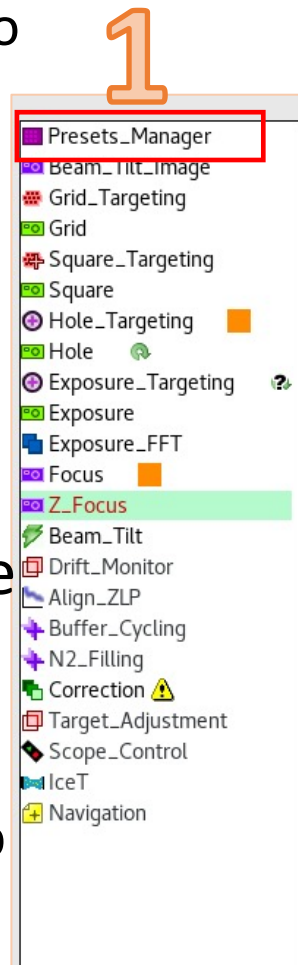
— Temperatures

Docker	89.7 K	-183.4 °C
Holder	79.8 K	-193.4 °C
Cassette gripper	85.2 K	-188.0 °C
Cartridge gripper	89.3 K	-183.8 °C
Autoloader Dewar	78.6 K	-194.5 °C
Column Dewar	78.7 K	-194.5 °C

Find a good square

1. Go to Presets Manager

1. Highlight gr and send to scope

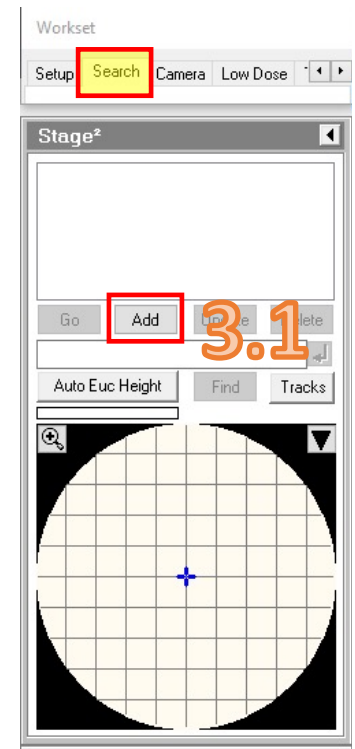


2. Insert screen on microscope (handpanel R1)



3. Use joystick to navigate around and choose a square

1. Mark squares of interest in "Search" tab on microscope TUI



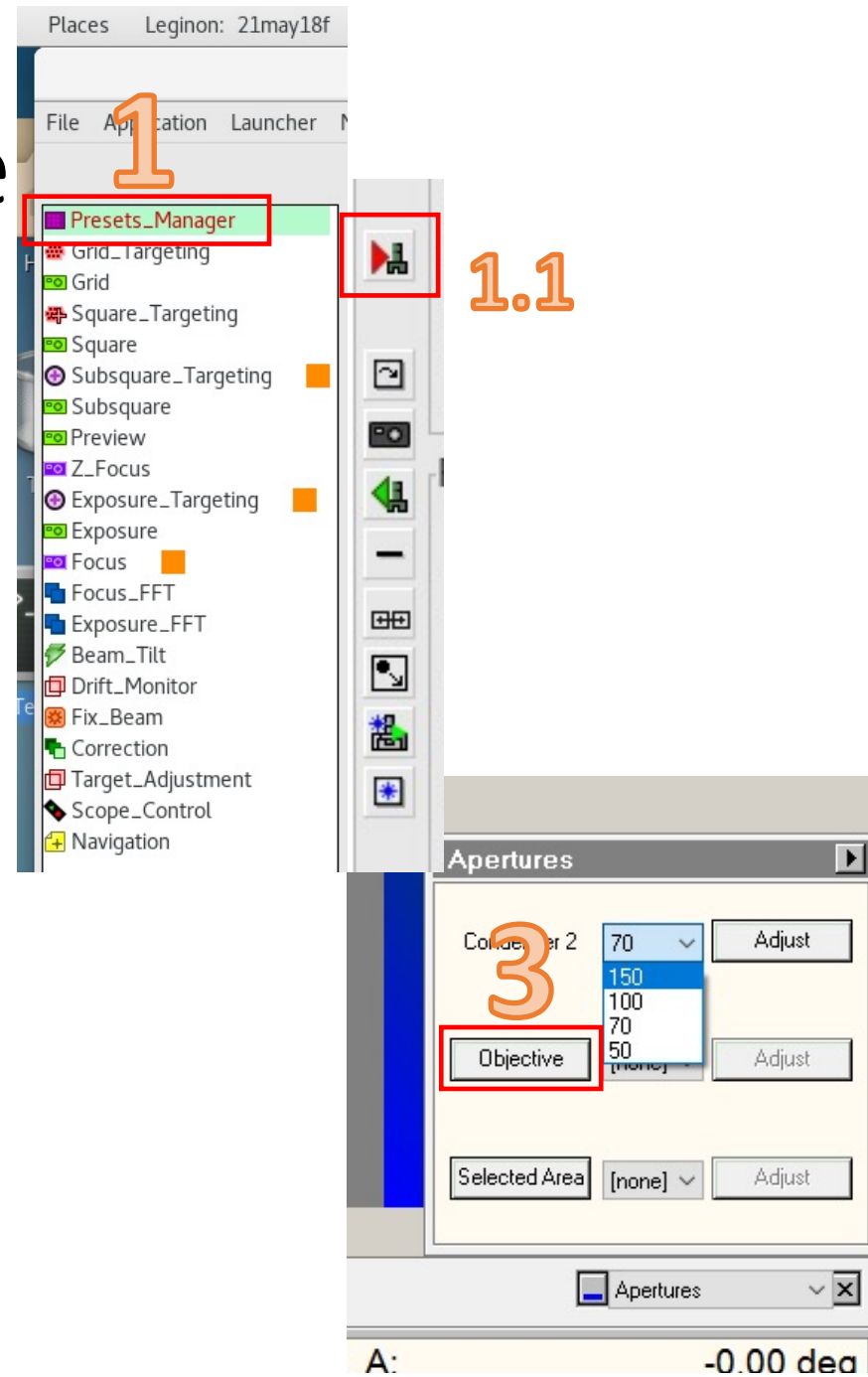
Zoom in on square

1. Go to Presets Manager

1. Highlight sq and send to scope

2. Use joystick to center square

3. Click "Objective" in "Apertures" to insert (will be yellow)



Simulate Square

1. Go to Square node

1. Click Simulate

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



1




1.1

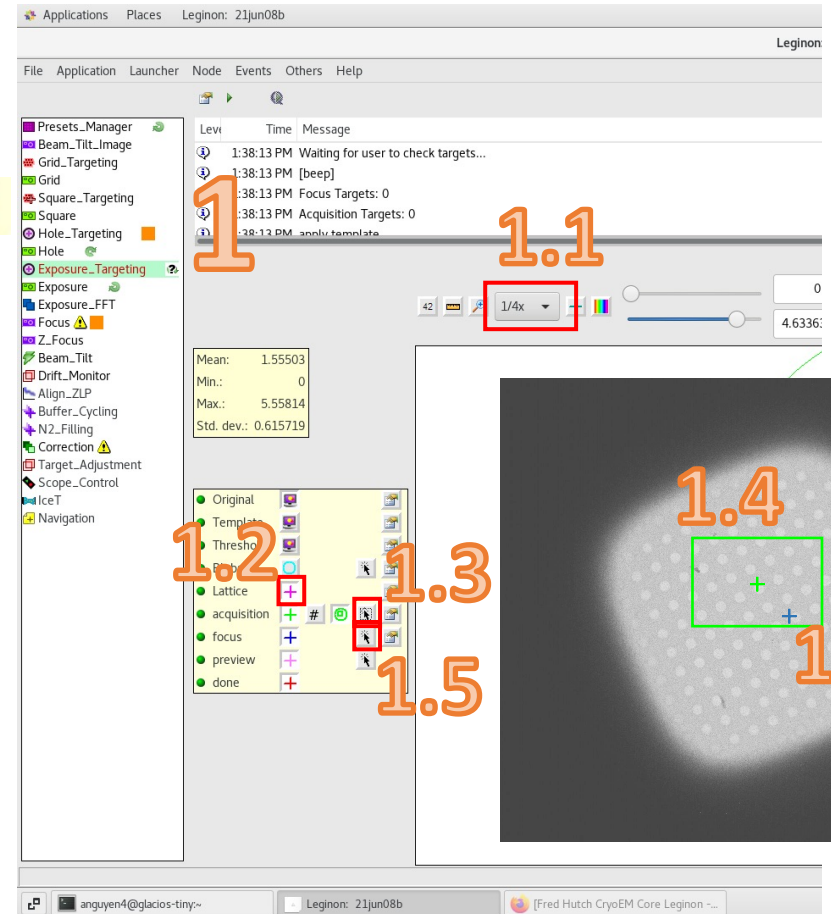
Level	Time	Message
?	12:48:12 PM	processing: waiting for 775t
?	12:48:12 PM	output: Image displayed
?	12:48:12 PM	output: Displaying image...
?	12:48:12 PM	output: Stats published...

Mean: 1.55467
Min.: 0
Max.: 5.88289
Std. dev.: 0.61092

Image

Choose hole and z-focus targets

1. Go to Hole Targeting
 1. Zoom out ($\sim 1/4x$)
 2. Turn off lattice points 
 3. Select acquisition 
 4. Left click to add targets where you want to image
 5. Select focus 
 6. Left click to add focus spot (make sure you're not near a grid bar)

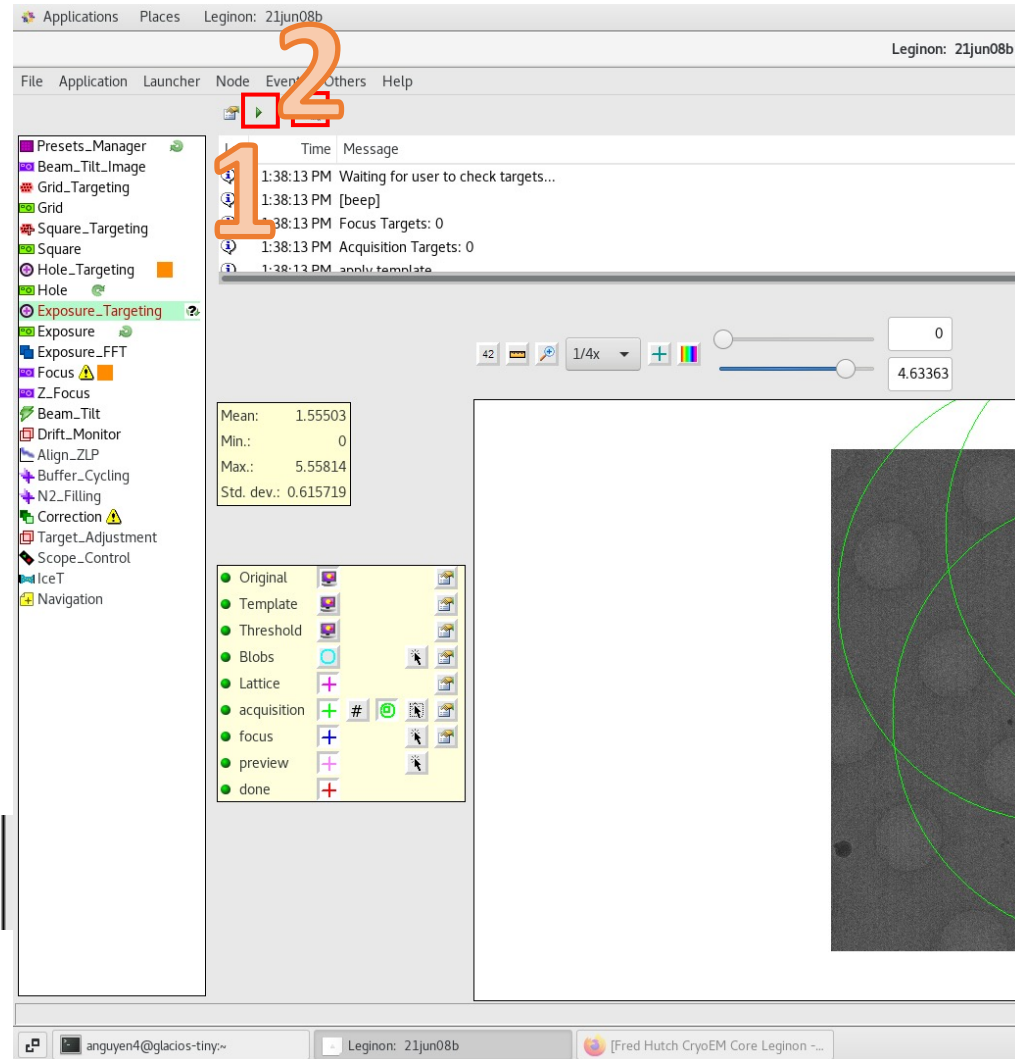


Submit hole target

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 “Hole”

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



Choose exposure and focus targets

1. Go to Exposure Targeting

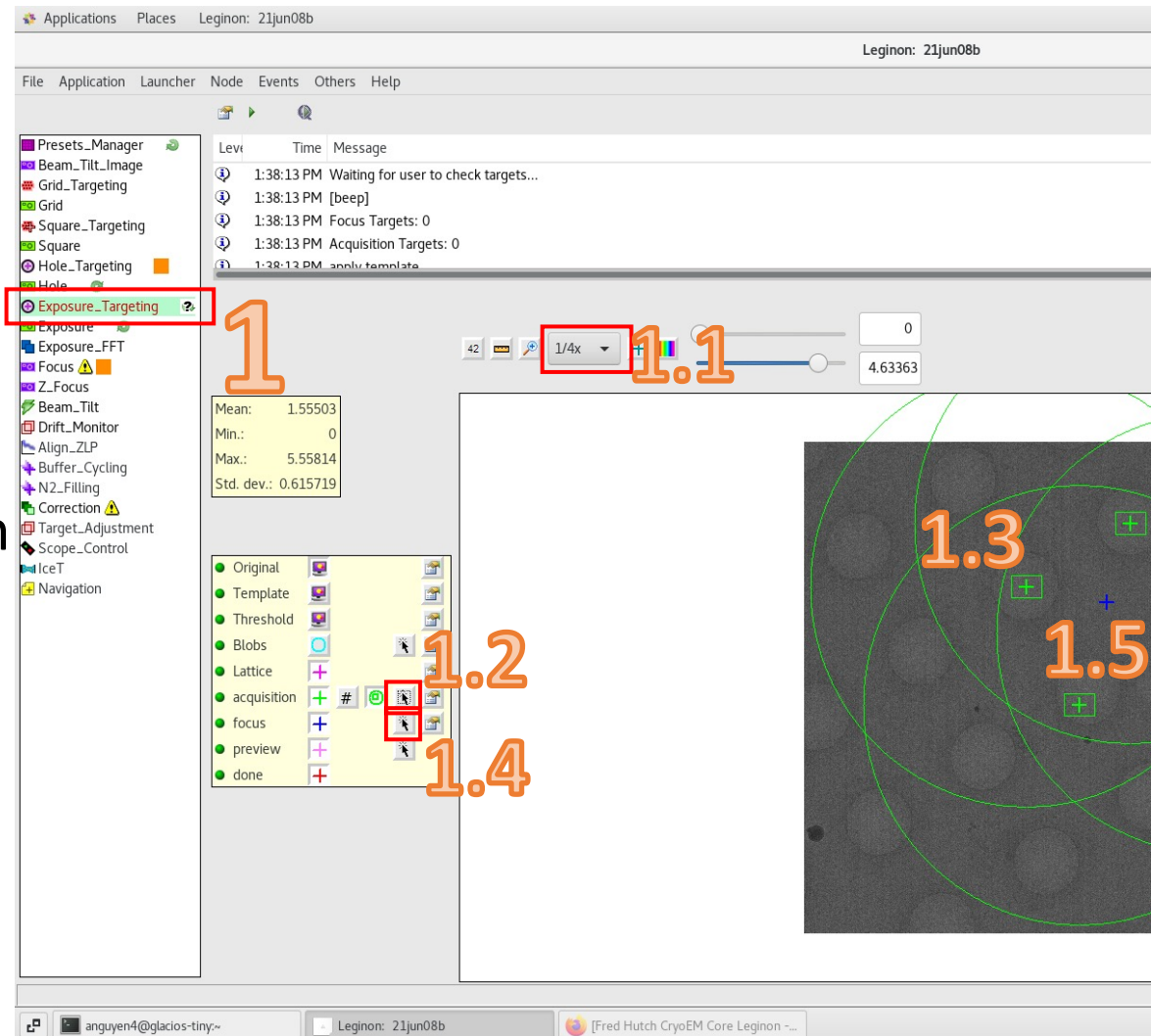
1. Zoom out (~1/4x)

2. Select acquisition 

3. Left click to add targets where you want to image – aim slightly up and to the left

4. Select focus 

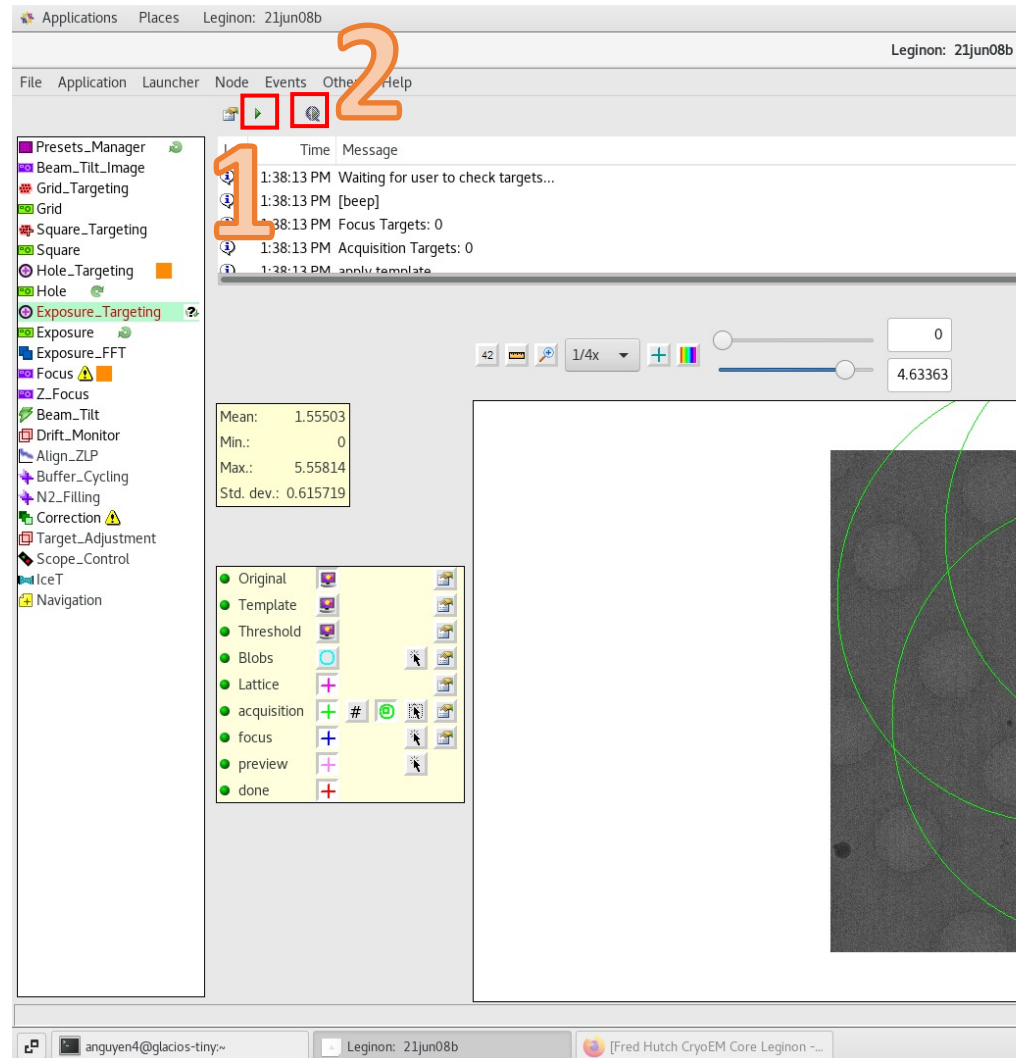
5. Left click to add focus spot (on the carbon between holes)



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

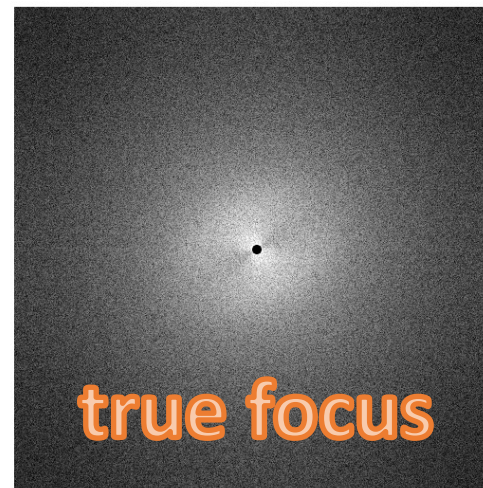
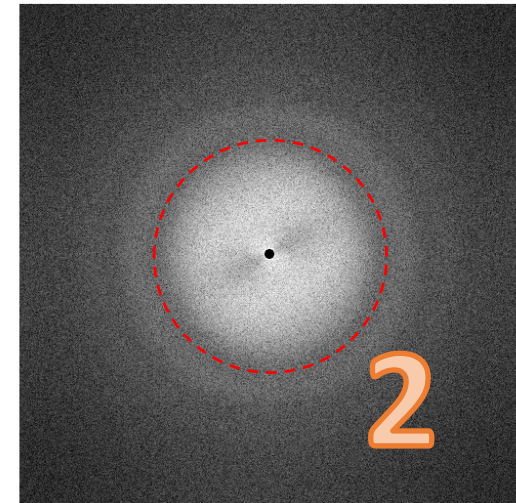
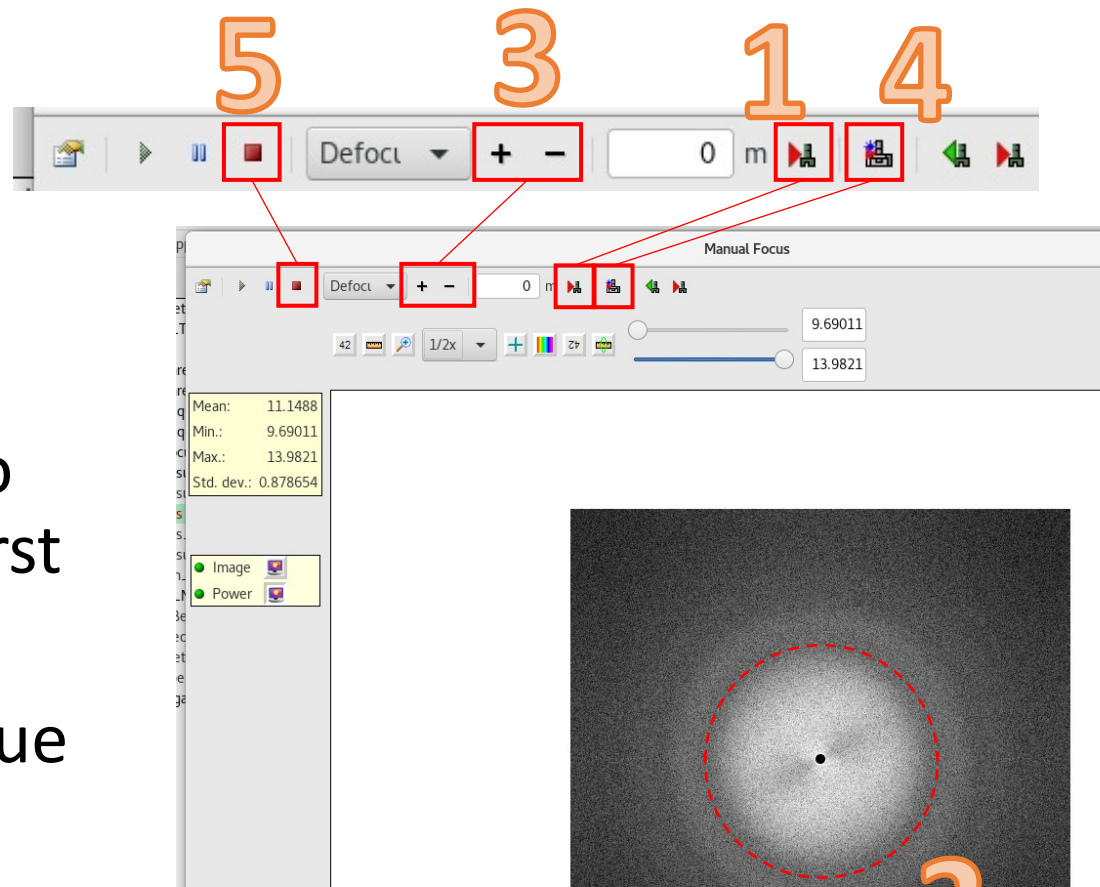
1

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

- 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**: 'Manual_after' is selected in the 'Focus sequence' list within the 'Focus Sequence (Focus)' dialog, highlighted with a red box.
- 1.3**: The 'Enabled' checkbox is checked in the 'Focus Sequence (Focus)' dialog, highlighted with a red box.
- 1.4**: The 'OK' button in the 'Focus Sequence (Focus)' dialog is highlighted with a red box.

Manual Focus

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



Monitor exposures

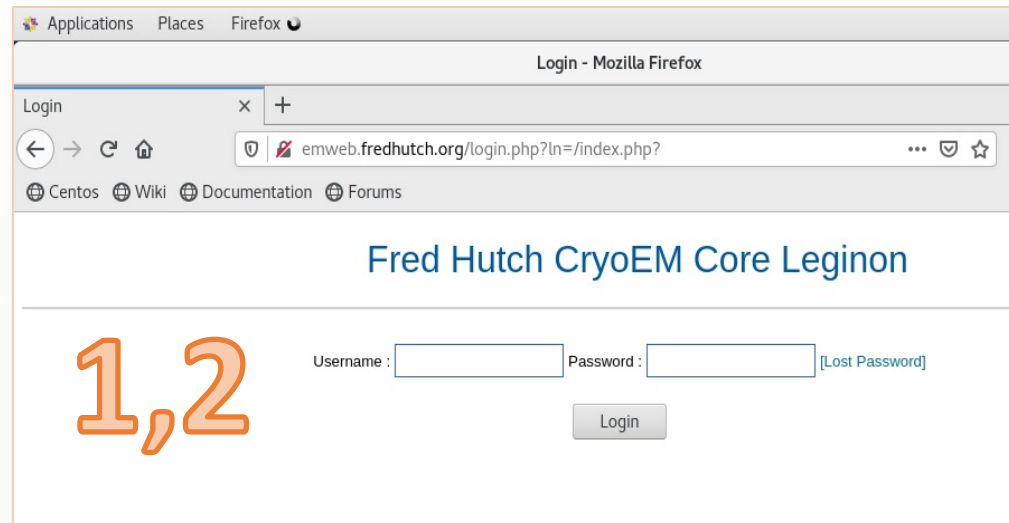
1. Open the internet on the Leginon computer or anywhere you are on VPN

1. **emweb.fredhutch.org**

2. Sign in with your leginon username and password

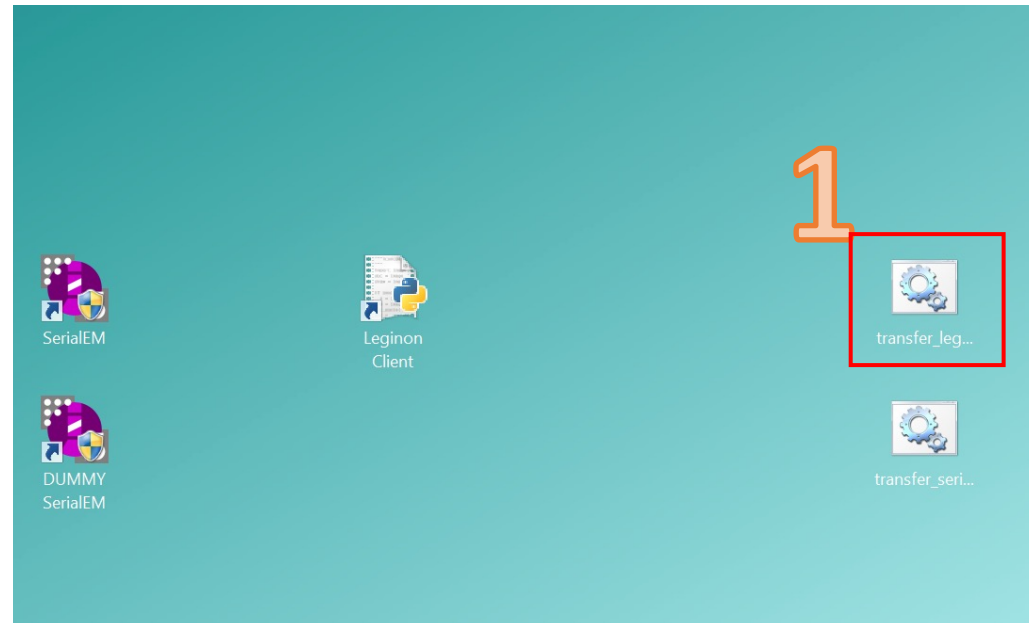
- View images in image viewer or 3-way image viewer
- Compare between sessions in 2-way image viewer
- More info on webserver on Teams channel

Best to observe exposures via
WARP



Start movie transfer

1. Double click on
“transfer_leginon.bat”
(K3 computer)



Open WARP on K3 remote desktop



DURCHLICHTELEKTRONENMIKROSKOPIEBILDDATENENTZERRUNGSWERKZEUG 1.0.9

SAVE SETTINGS LOAD SETTINGS

Overview

Fourier Space Real Space

EXPORT MICROGRAPH LIST ADJUST PARTICLE DEFOCUS EXPORT PARTICLES IMPORT PARTICLE COORDINATES MATCH TEMPLATE EXPORT BOXNET EXAMPLES

Processing Status

Astigmatism (use up to 3.0 σ)

Defocus (use 0.10–3.50 μm) — average |CTF|:

0 1000 1/1.1 Å

Estimated resolution (use better than 4.6 Å)

1.03 0.51 0.00

Average motion per frame in first 1/3 (use up to 2.0 Å)

1.03 0.51 0.00

(use up to 10 %)

1.03 0.51 0.00

Input

Input: M:\cryoem\cazumaya\frames\21may14\rawdata\ — *.tif
Pixel X/Y: 0.5610/0.5610 Å, 0.0 °
Bin: 1.00x (1.1220 Å/px)
Exposure: 0.51 e/Å²/frame (group)

Preprocessing

Correct gain using: M:\cryoem\GainRefs\K3GainRefx1m3_gatanCD...
 Correct defects using: Select defect map...
 Flip X axis Flip Y axis Transpose

CTF
Window: 512 px Range: 30.0–2.5 Å Use Movie Sum
Voltage: 200 kV C_s: 2.70 mm Phase Shift
Amplitude: 0.07 Defocus: 0.0–5.0 μm

Motion
Consider 44.9–9.0 Å, weight with B = -500 Å²

Models

Defocus: 5 x 5 x 1

Motion: 7 x 5 x 99

Pick Particles
Use Select BoxNet model...
Expect 200 Å cryo particles; use scores above 0.95
Maintain a minimum distance of 0 Å from

Extract 128 px boxes, 1.1220 Å/px, invert, normalize
 Maintain a separate list of the latest 10000 particles

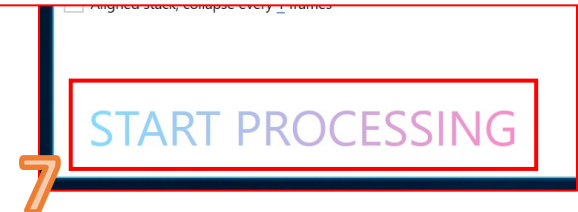
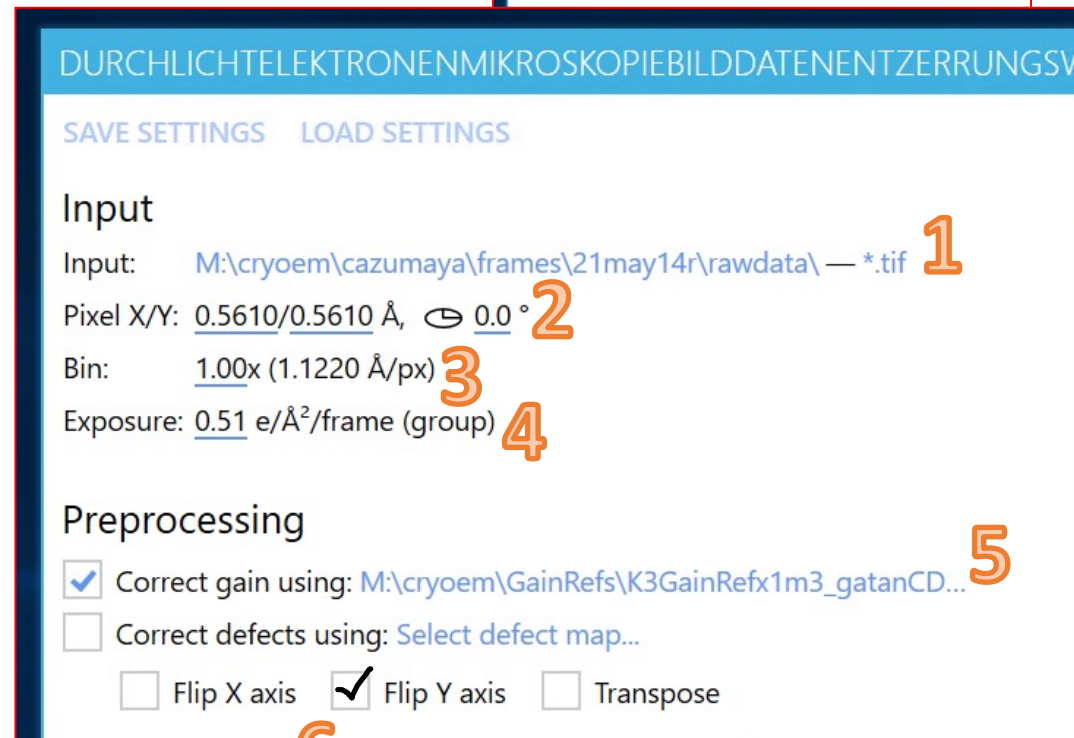
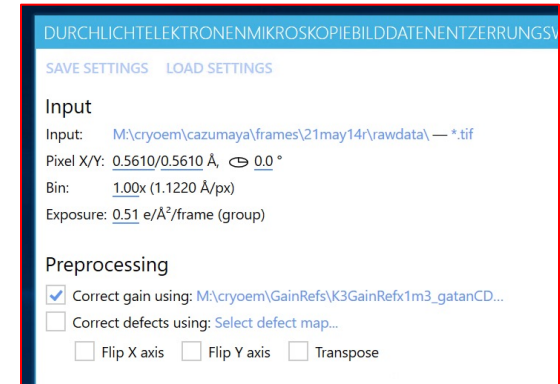
Output

Skip first 0, last 0 frames,
 Average
 Aligned stack, collapse every 1 frames

START PROCESSING

Setup WARP

1. **Input:** Browse for frames/rawdata folder
2. **Pixel size:** probably 0.561
3. **Bin:** 1.00x
4. **Exposure:** probably 0.51
5. **Correct gain using:**
Browse for gain image:
/cryoem/GainRefs/most recent
6. **Flip Y axis for gain**
7. **Start Processing**



Setup collection on this grid
(SerialEM Collection protocol)

OR

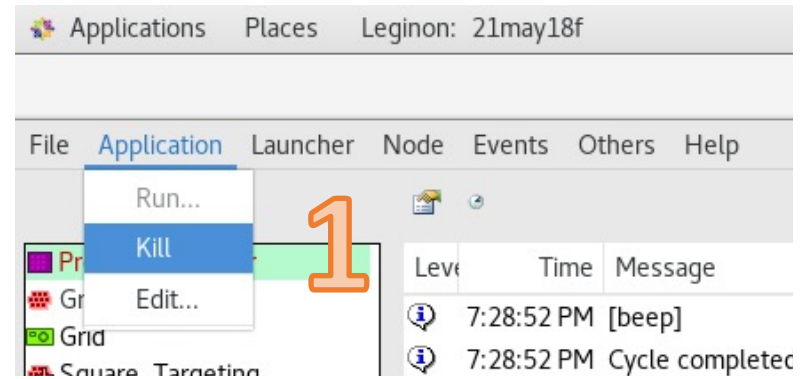
Screen more or another grid
(repeat from slide 10 or 8)

OR

Shutdown
(next slides).

Shutdown Leginon

1. Application -> Kill
2. File -> Exit
3. Logout of computer
 1. Power logo ->
Username -> Sign out
4. Close client on microscope and K3 computers



Do shutdown without
collection Glacios check list!

End iLab time and sign out!