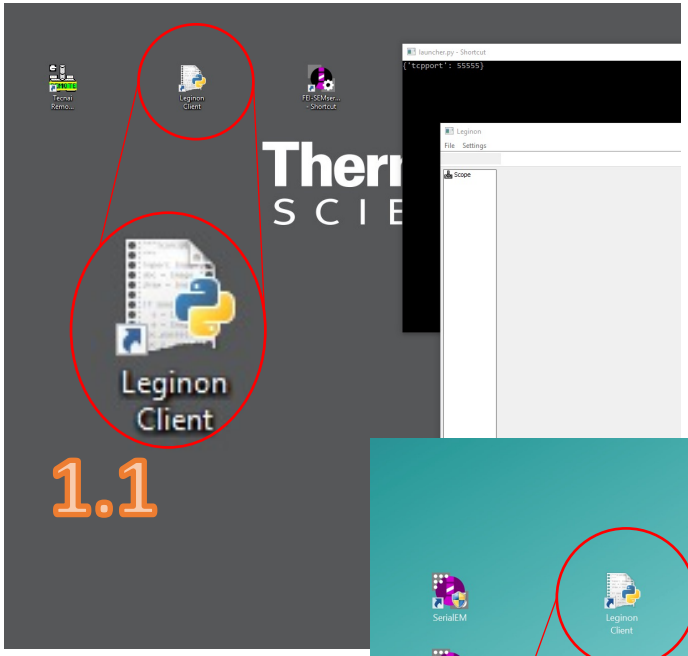
Leginon Protocol cryo screening Fred Hutch Glacios

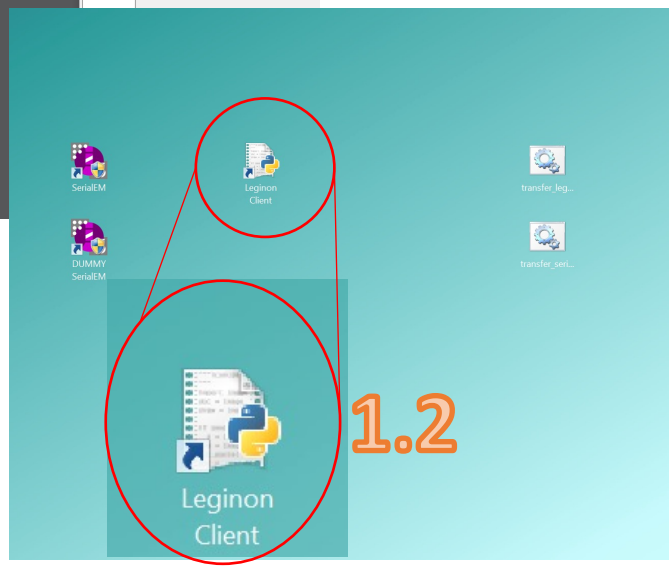
What you're starting with

- 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

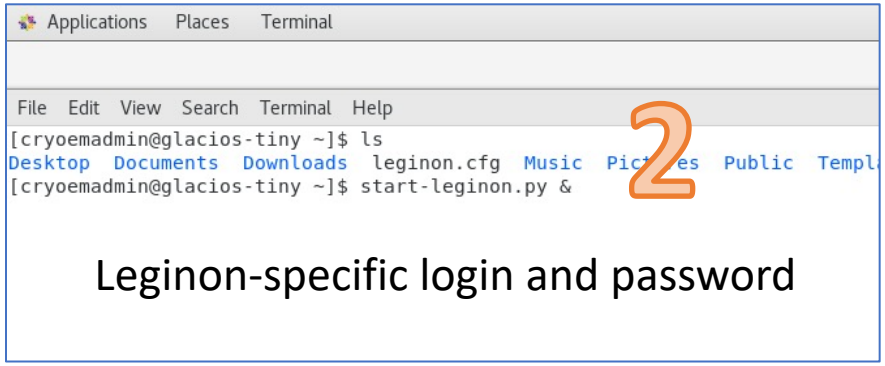
Start Leginon

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



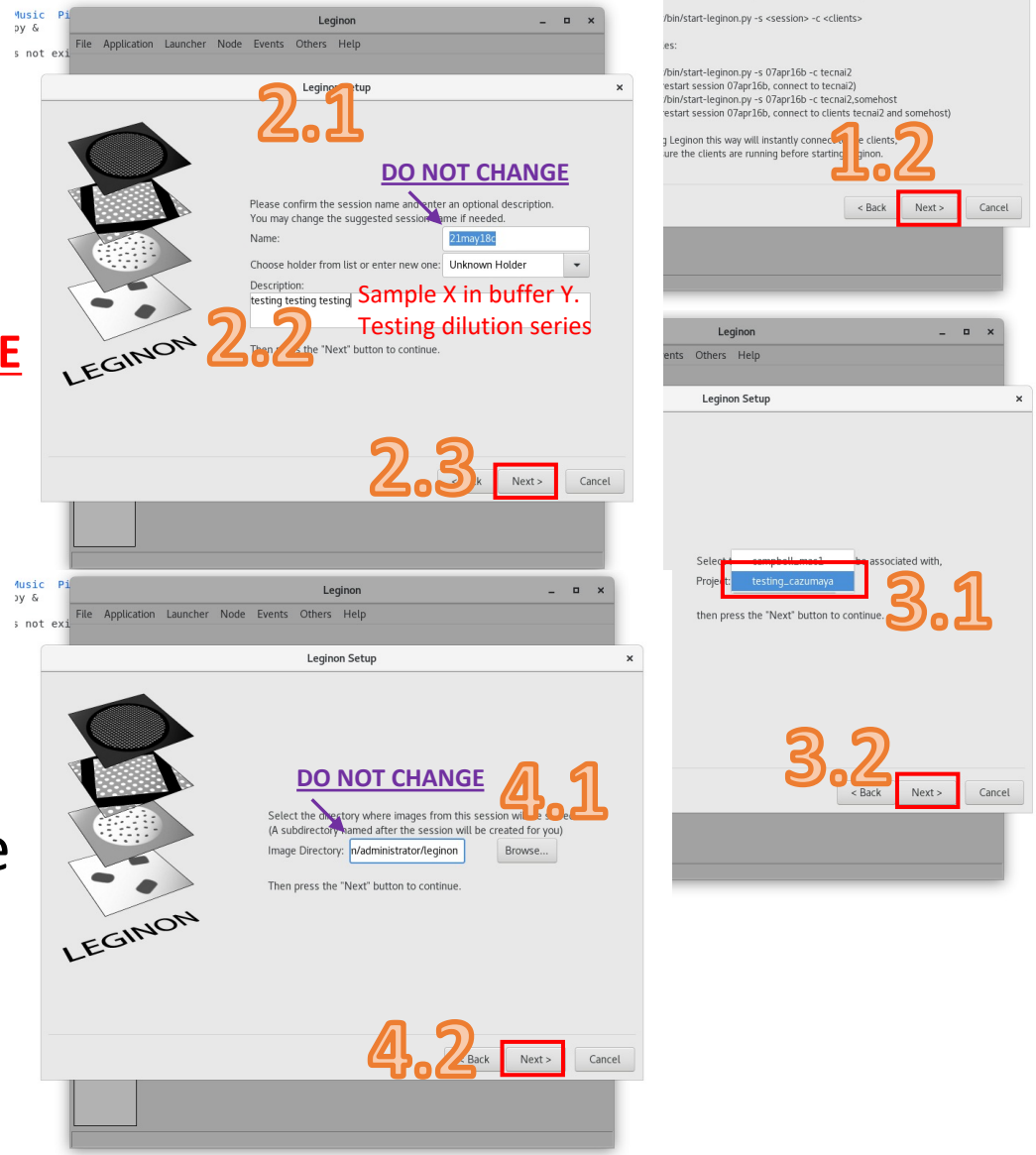


2. Login to Leginon computer (glacios-tiny)
    1. Open terminal (right click on background)
    2. start-leginon.py



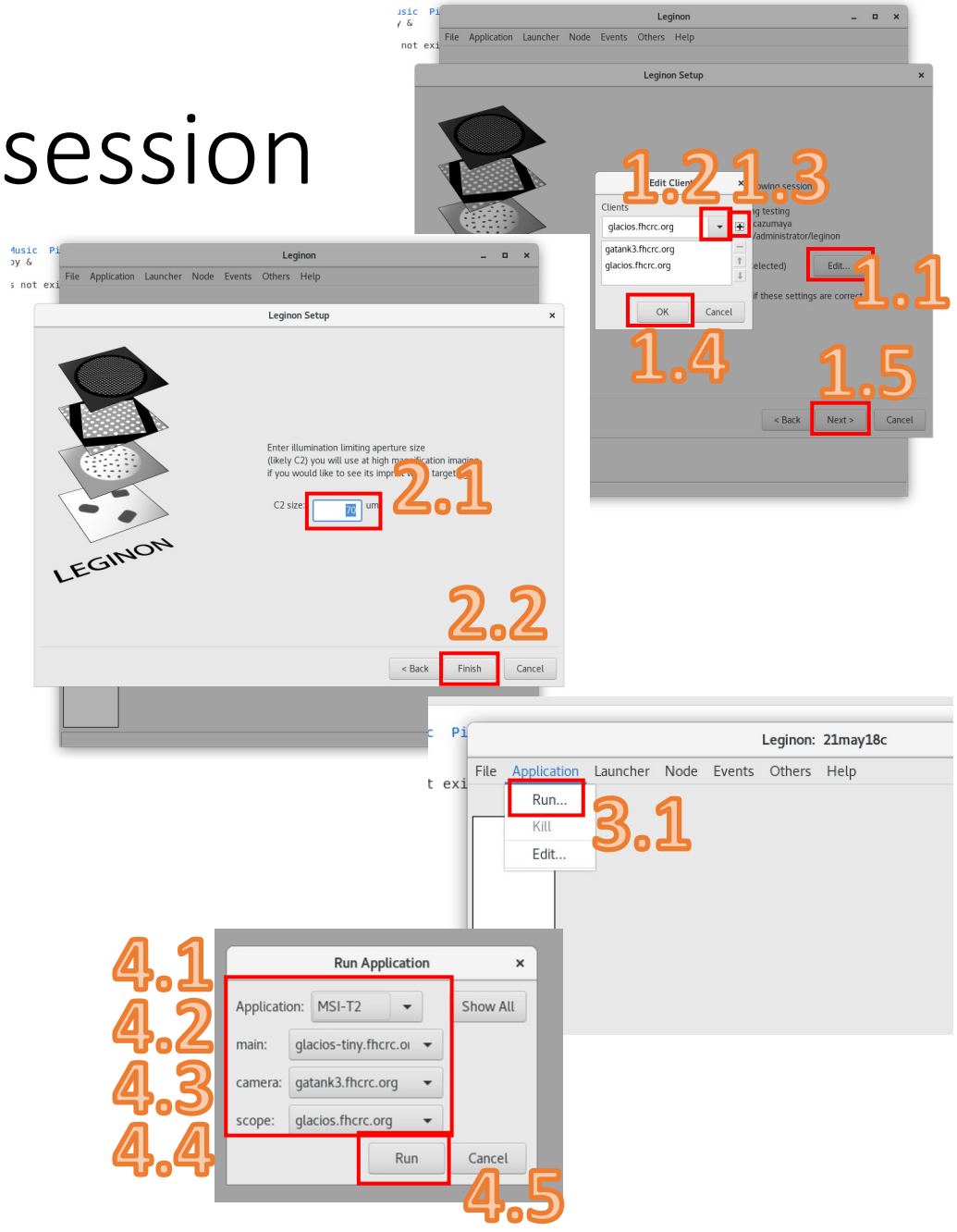
Create Leginon session

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

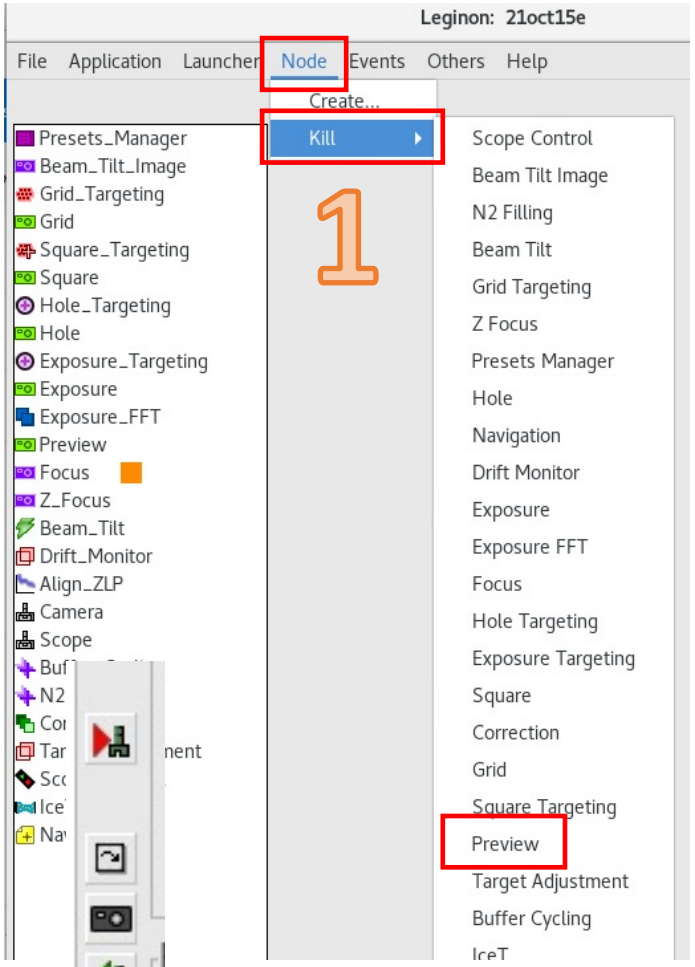
1. Add clients
   1. Edit
   2. Choose glacios.fhcrc.org from dropdown
   3. Click + !!
   4. Choose gatank3.fhcrc.org from dropdown
   5. Click + !!
   6. OK
   7. Next
2. Define C2 aperture
   1. **50um**
   2. Finish
3. Start session
   1. Application -> Run
4. Choose application
   1. Application: MSI-T2
   2. Main: **glacios-tiny.fhcrc.org**
   3. Camera:**gatank3.fhcrc.org**
   4. Scope: **glacios.fhcrc.org**
   5. Run

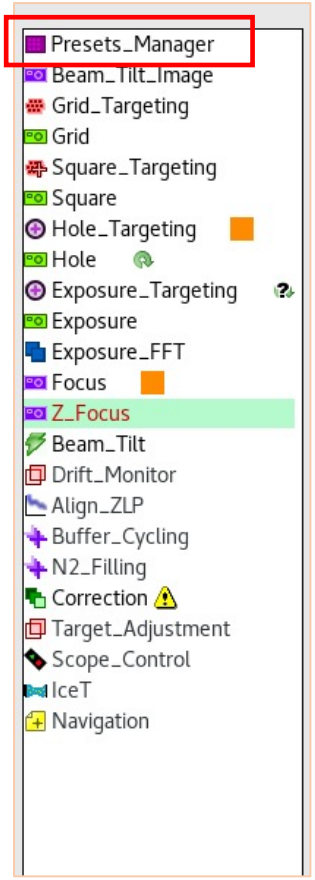


Setup session

1. Node -> Kill -> Preview

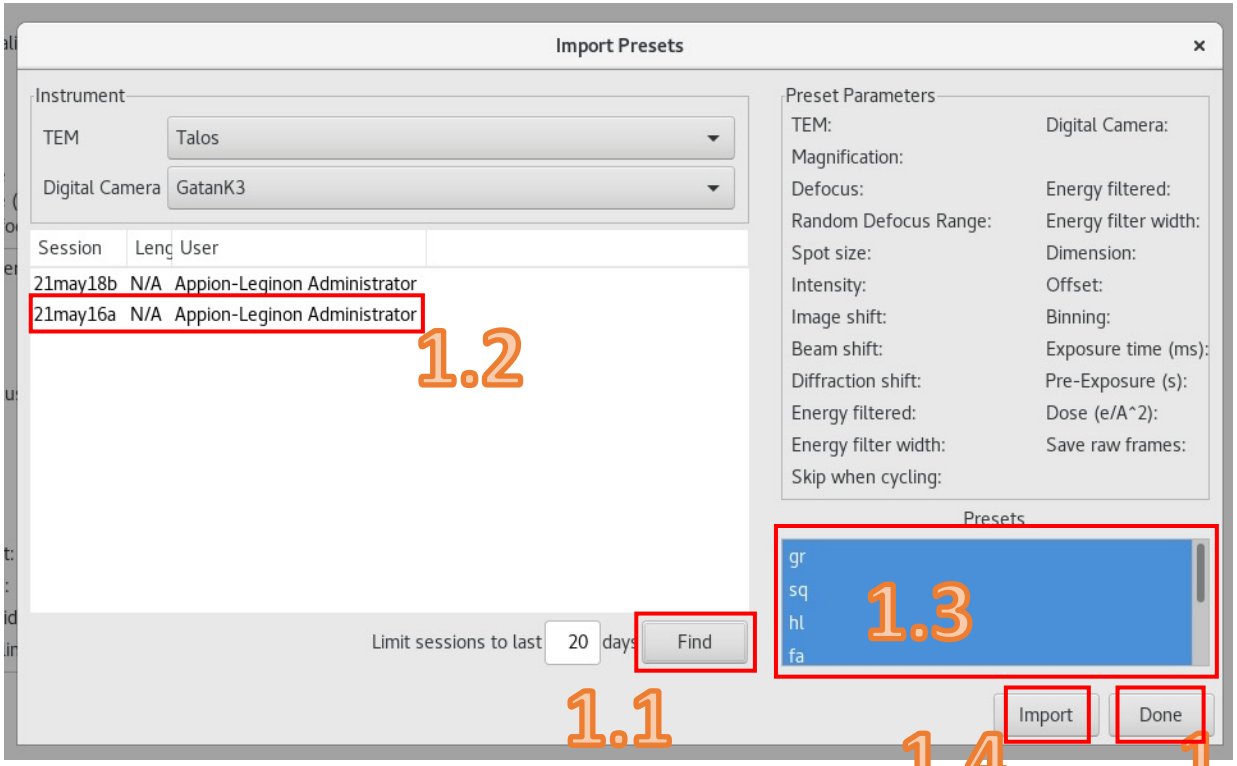
2. Import presets
    1. Presets Manager -> Blue dot icon





Setup session

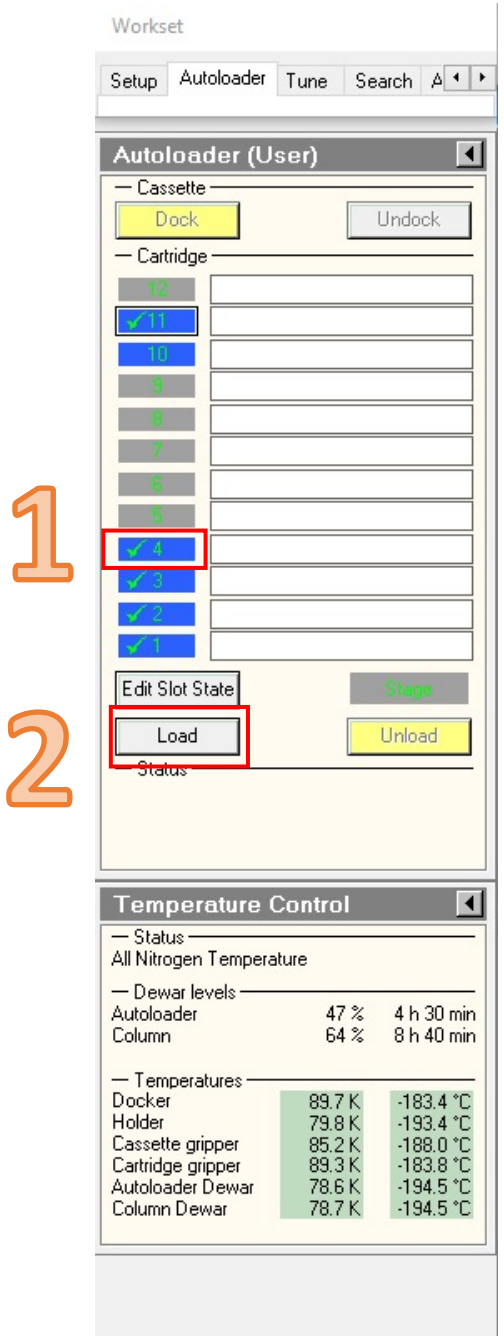
1. Import presets
    1. Find
    2. Choose person you trust uses same settings
    3. Highlight all presets
    4. Import
    5. Done



Load your grid

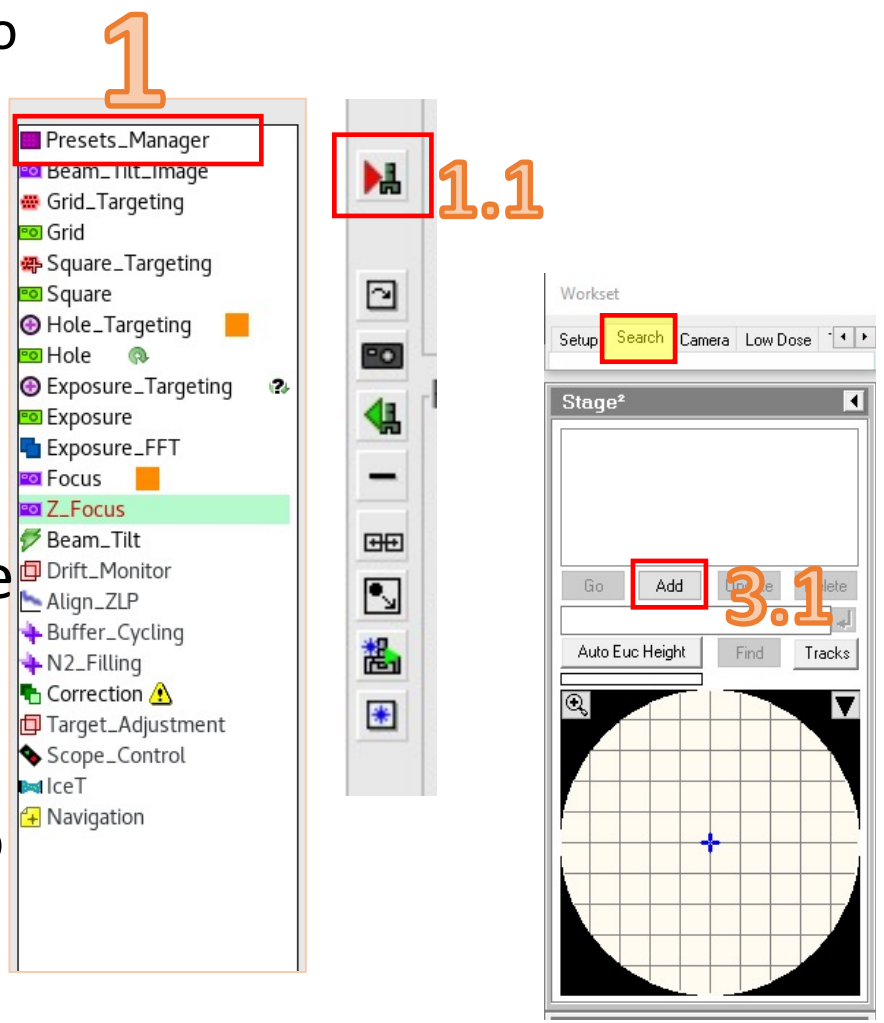
On the Autoloader tab of the microscope UI

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



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

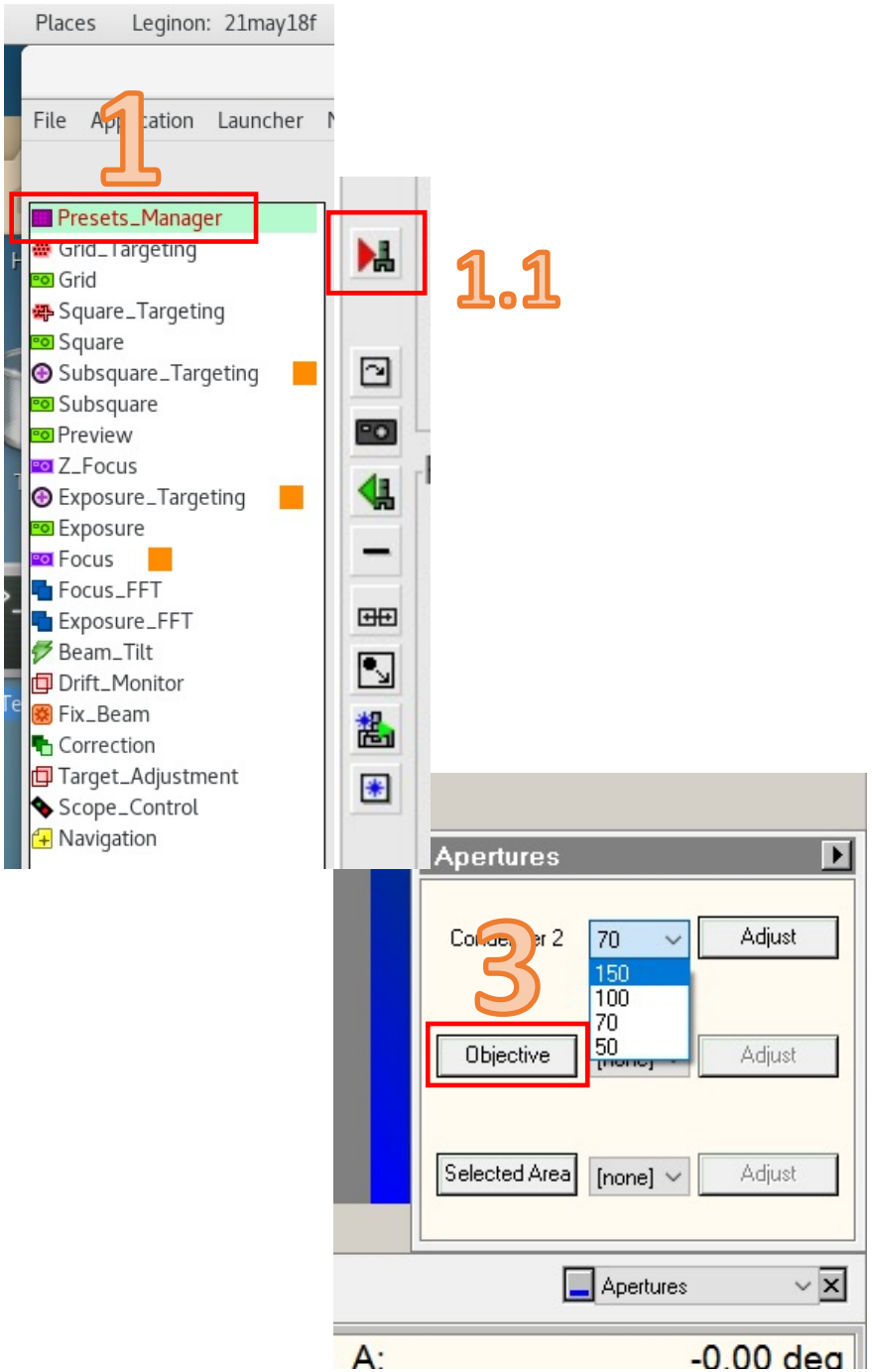


Zoom in on square

1. Go to Presets Manager
    1. Highlight sq and send to scope

2. Use joystick to center square

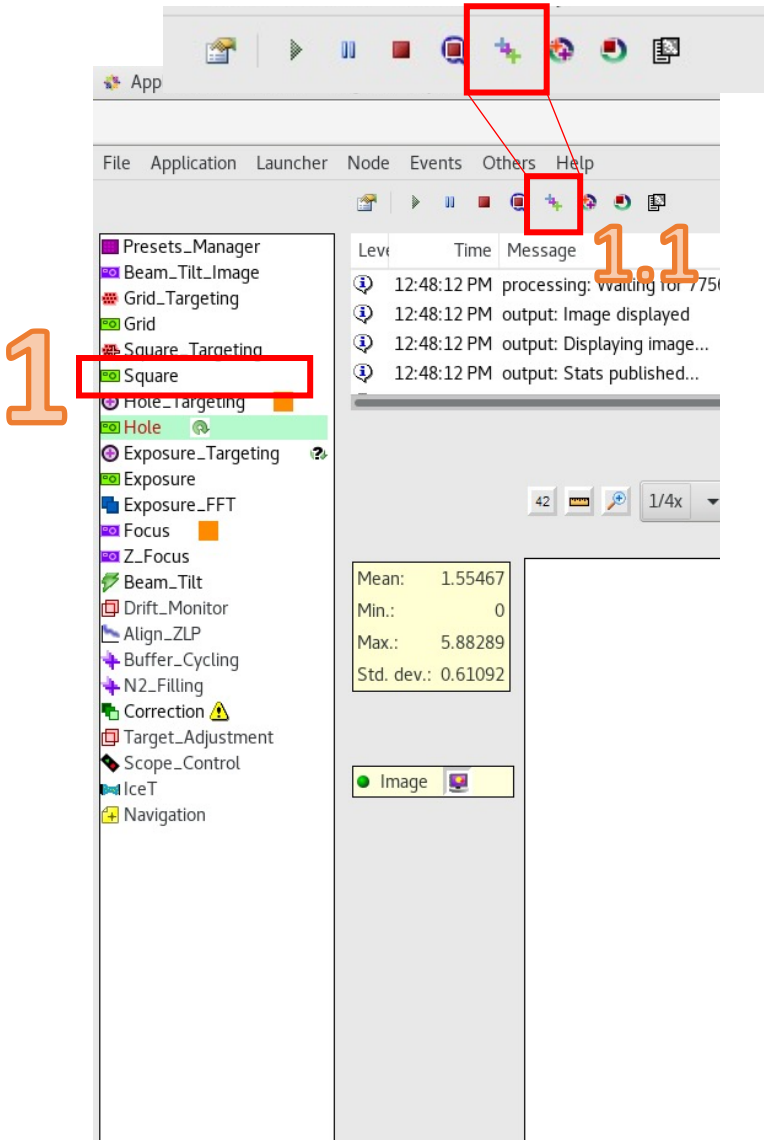
3. Click “Objective” in ”Apertures” to insert (will be yellow)



Simulate Square

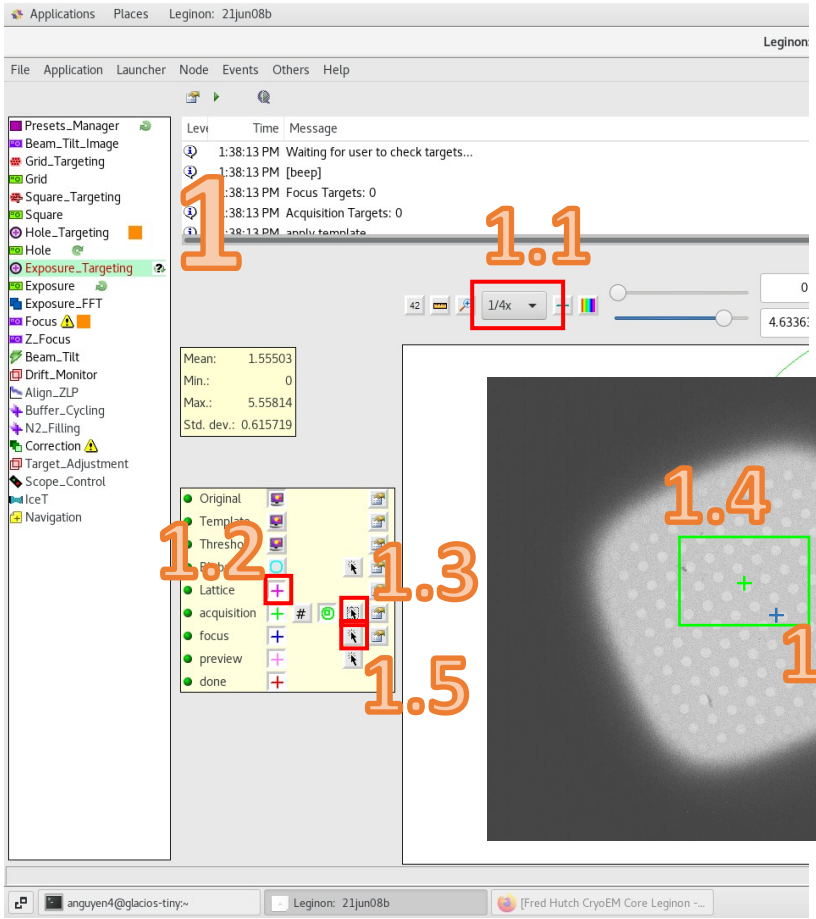
1. Go to Square node
    1. Click Simulate

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



Choose hole and z-focus targets

1. Go to Hole Targeting
    1. Zoom out (~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)



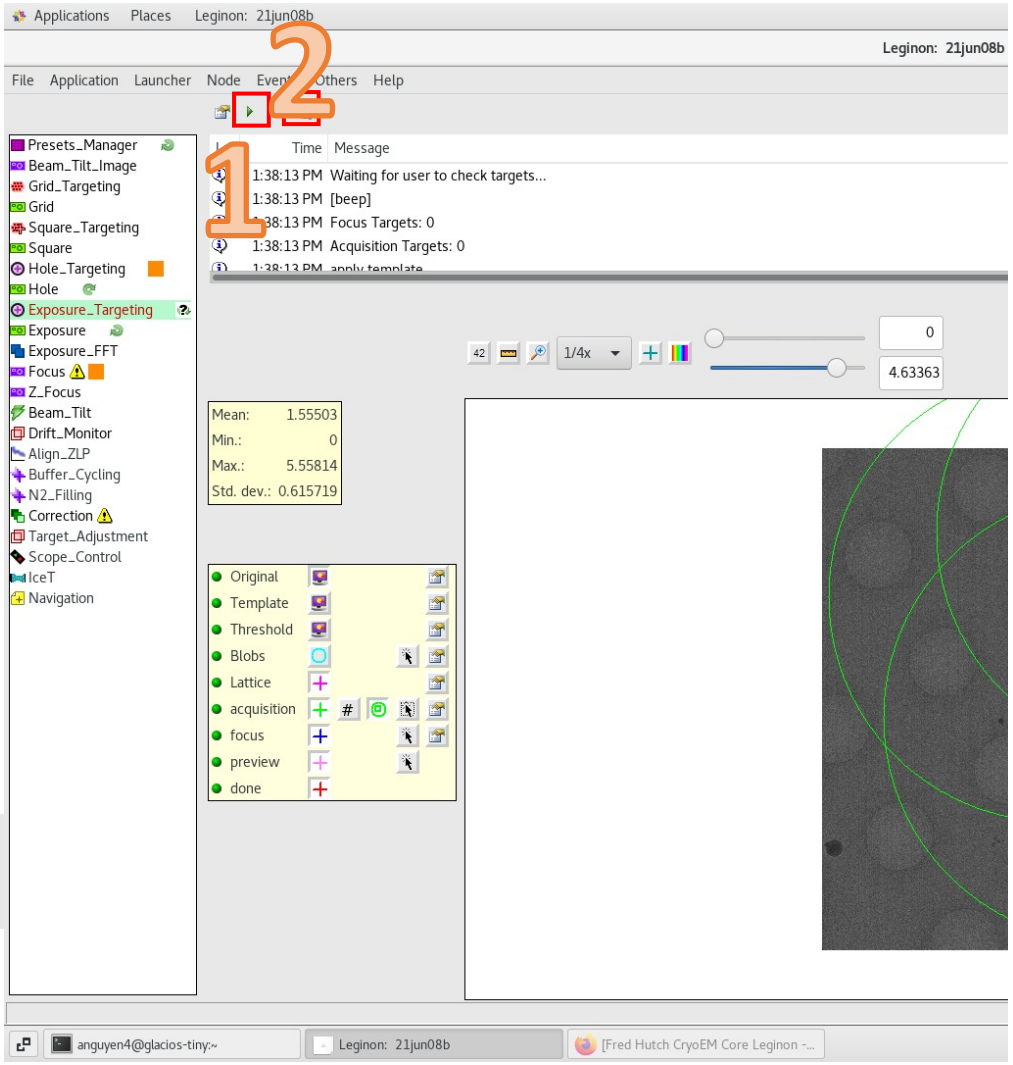
Submit hole target

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

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

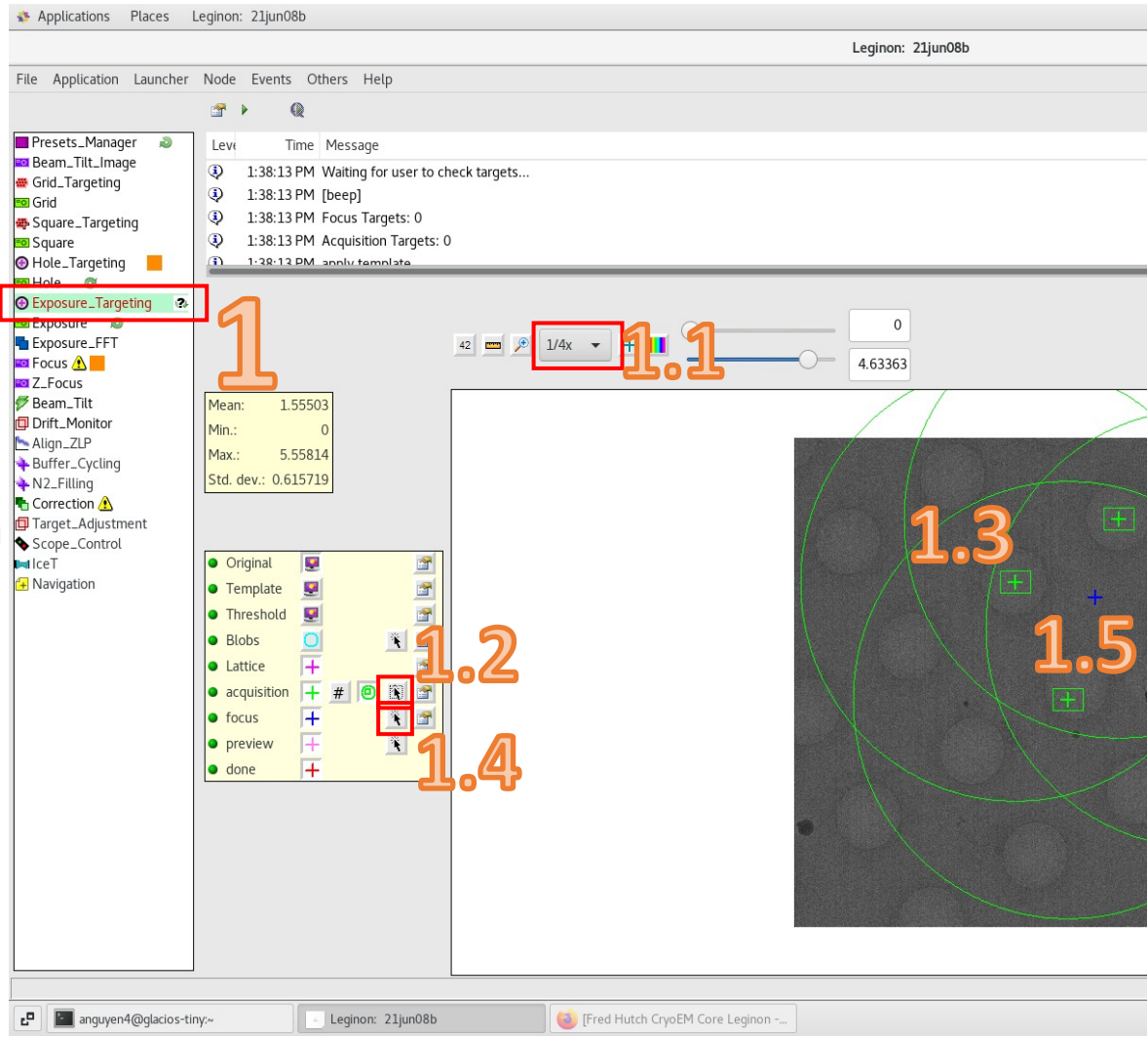
Focus sequence will start to run through “Target Adjustment”, “Z-focus”, and “Hole”

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)

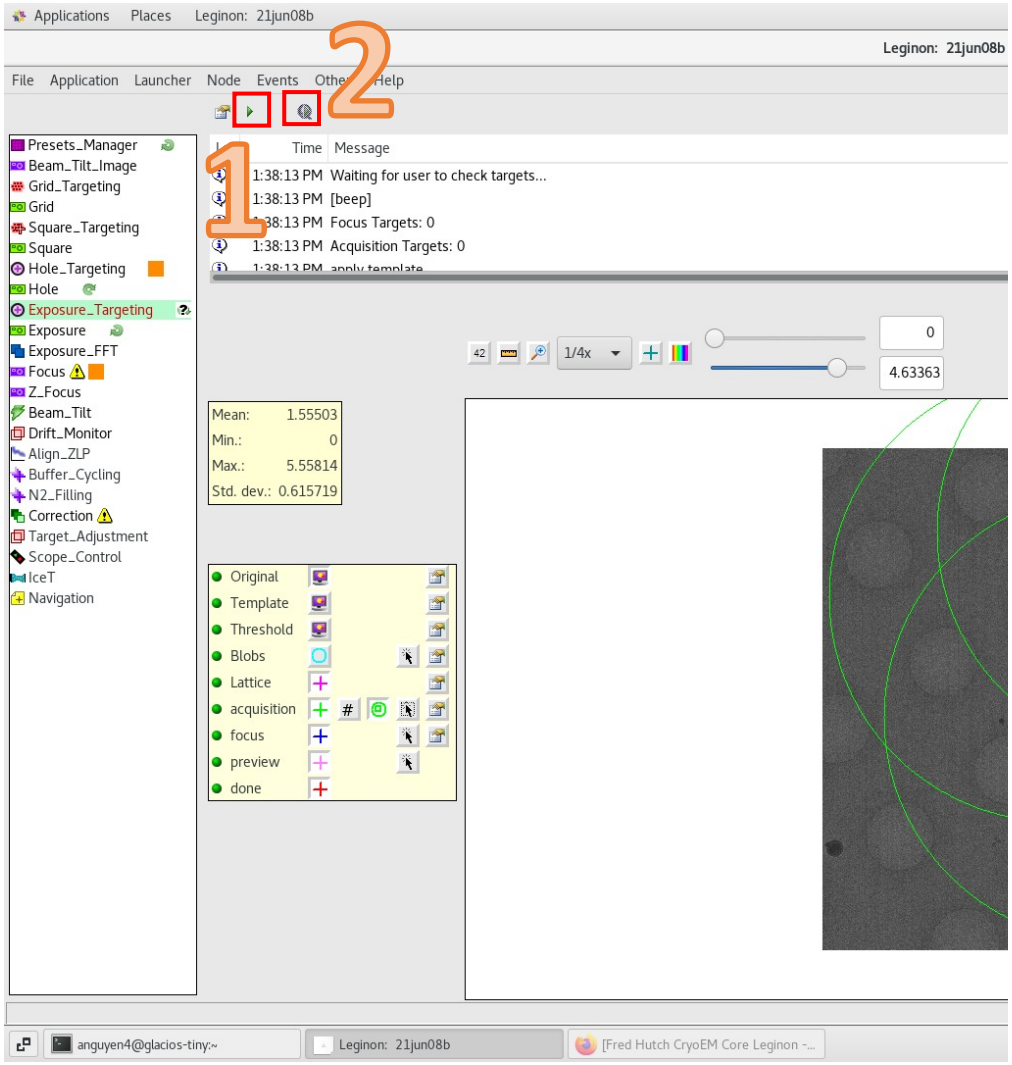


Submit exposure targets

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

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

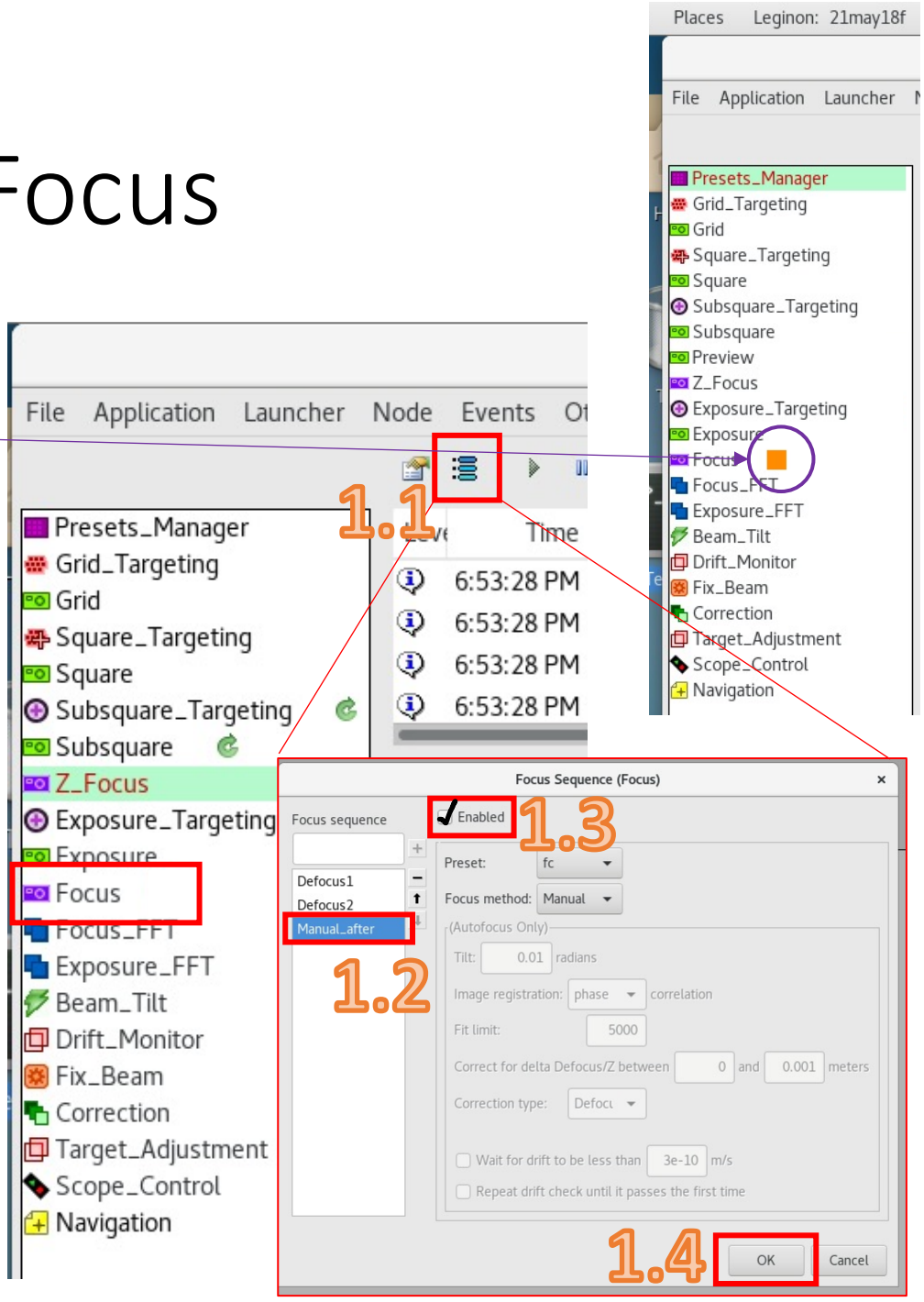
Focus sequence will start to run through “Target Adjustment”, “Drift Monitor”, and ”Focus”



Enable Manual Focus

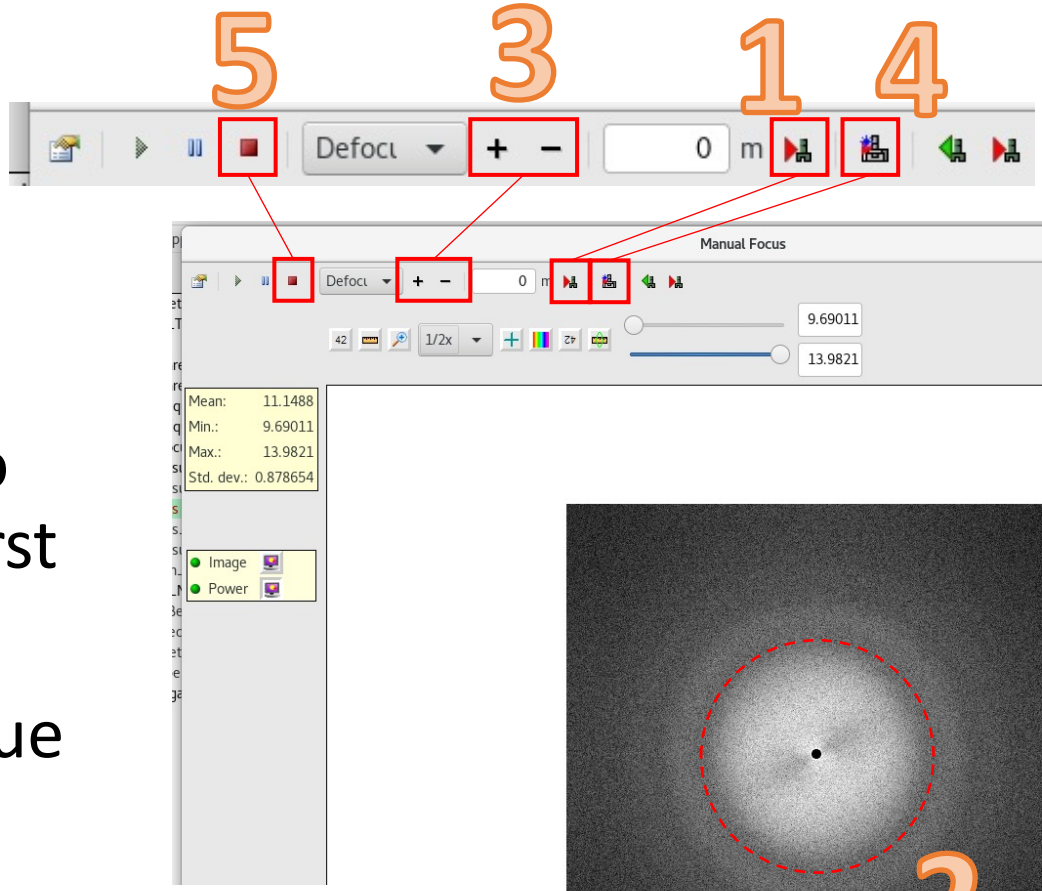
Orange square means it is enabled.

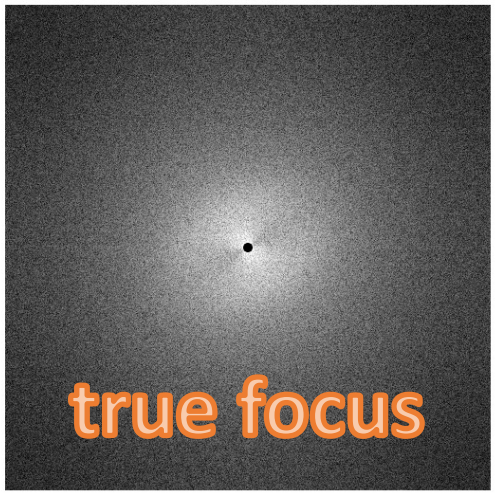
1. If not, Go to Focus
    1. Click blue bullet list
    2. Select Manual_after
    3. Check Enabled
    4. Ok



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

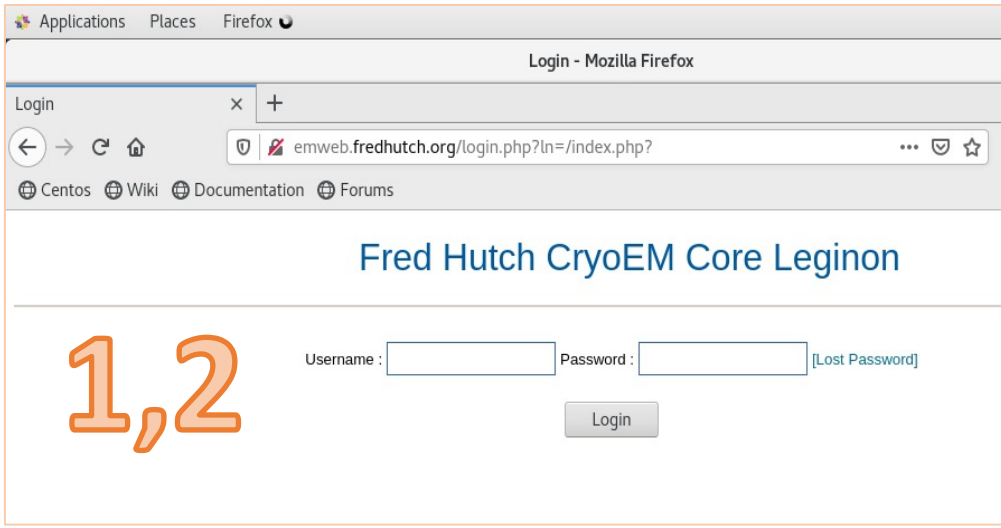




Monitor exposures

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



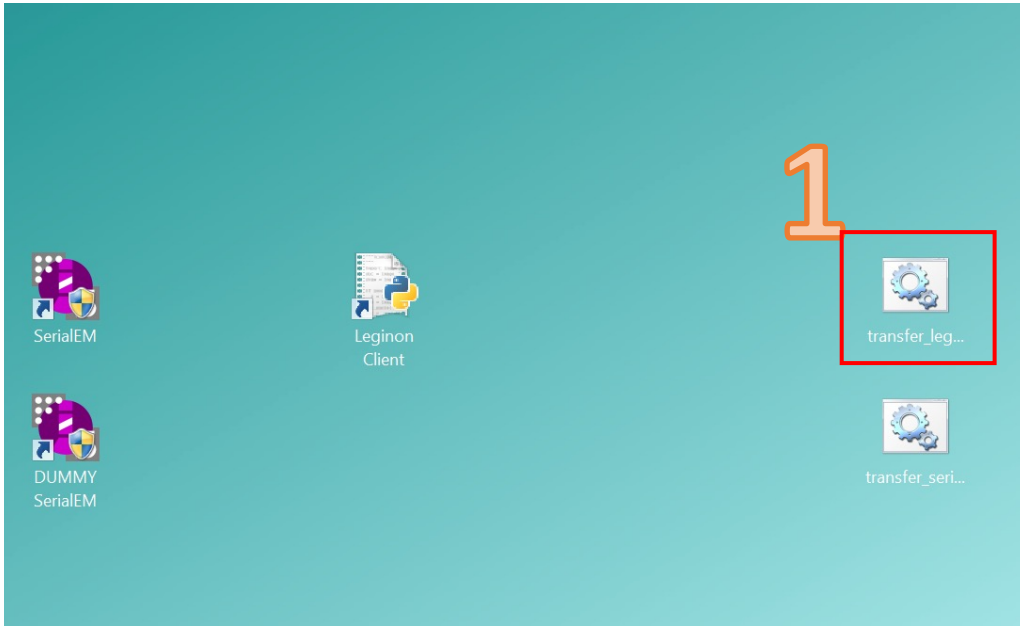
- View images in image viewer or 3-way image viewer
- Compare between sessions in 2-way image viewer
- More info on webserver on Teams channel

Best to observe exposures via WARP

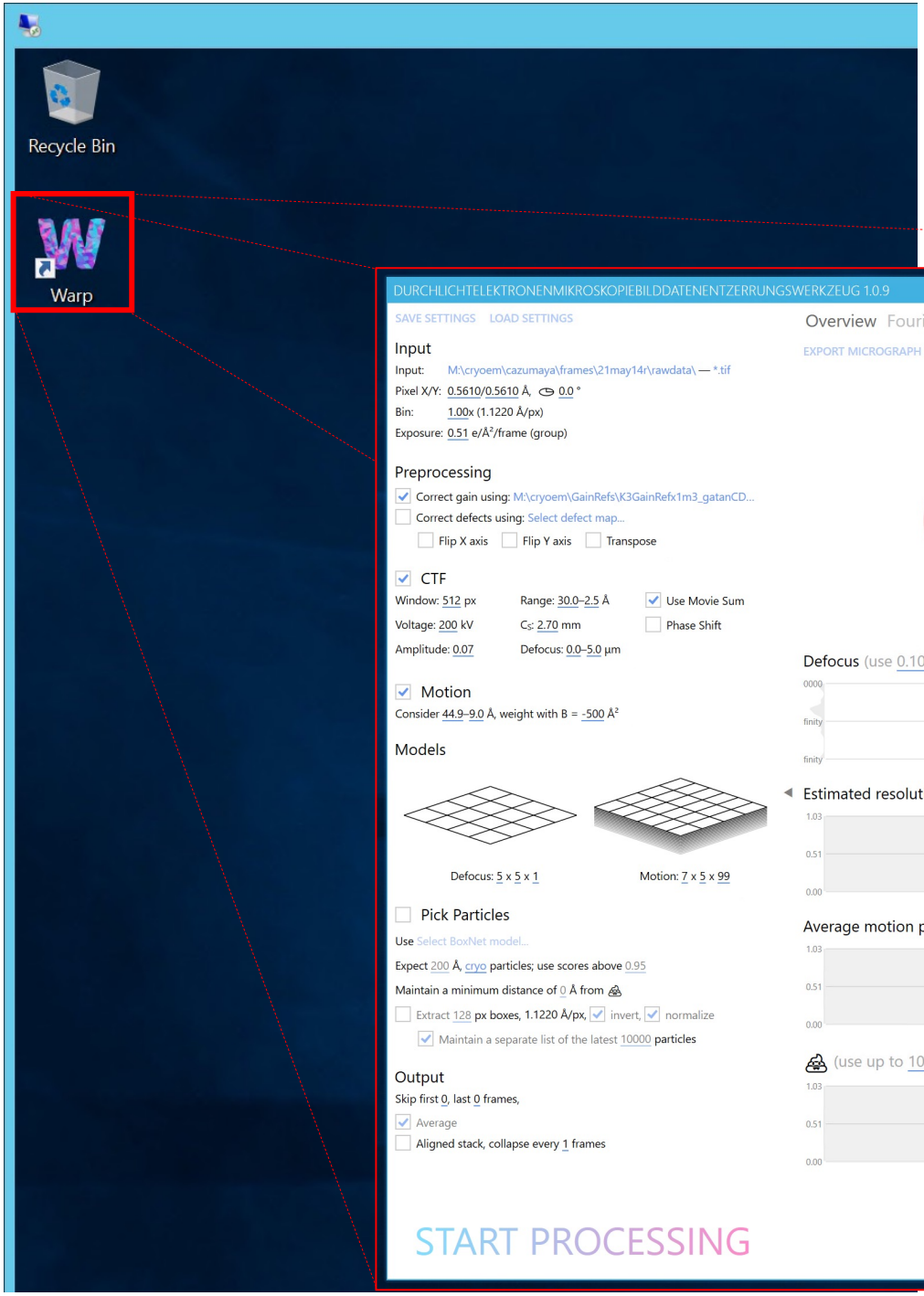
Start movie transfer

1. Double click on “transfer_leginon.bat”

   (K3 computer)

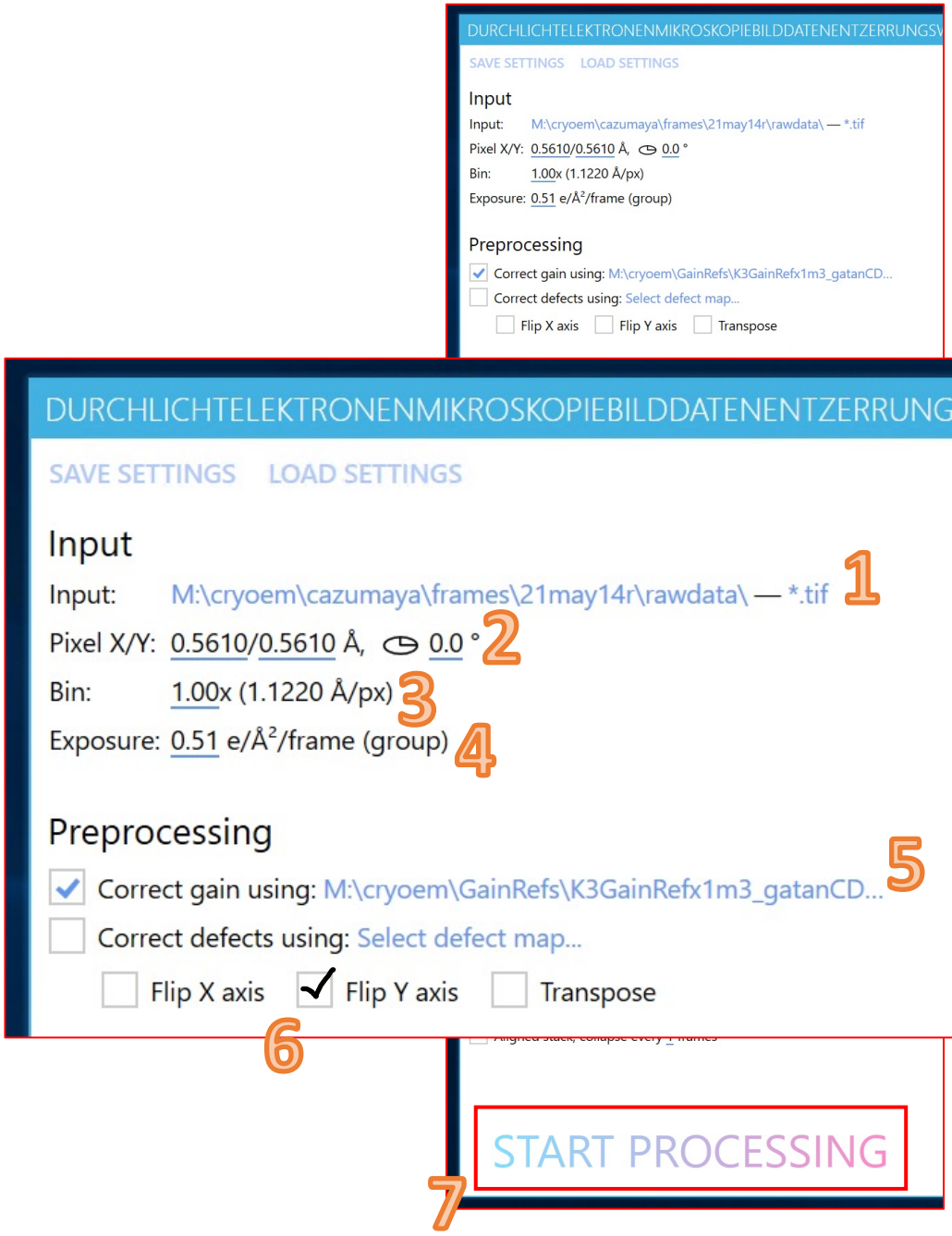


Open WARP on K3 remote desktop



Setup WARP

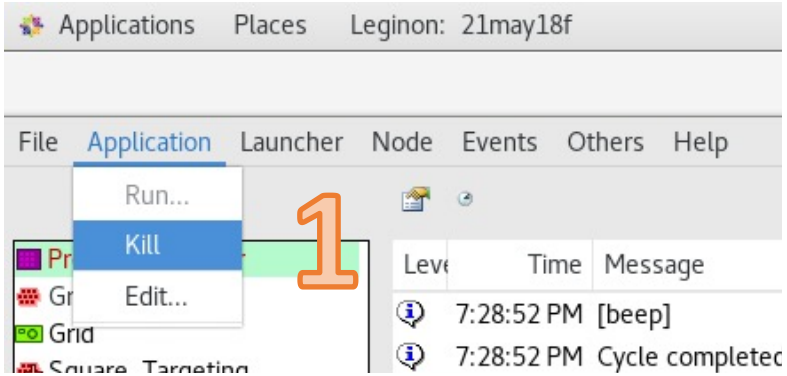
1. **Input:** Browse for frames/rawdata folder
2. **Pixel size:** probably 0.561
3. **Bin:** 1.00x
4. **Exposure:** probably 0.51
5. **Correct gain using:**
   Browse for gain image:
   /cryoem/GainRefs/most recent
6. **Flip Y axis for gain**
7. **Start Processing**

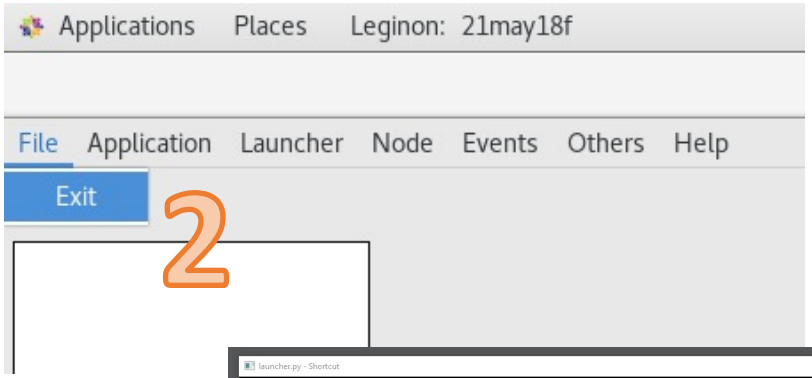


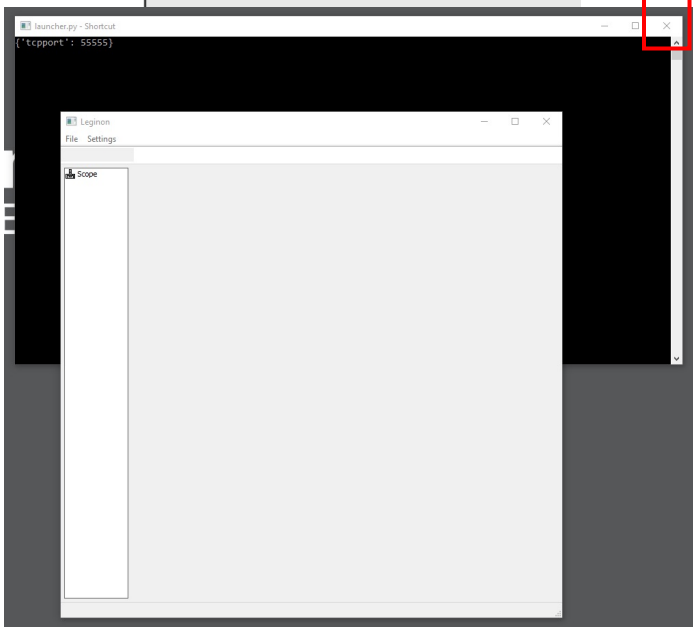
Setup collection on this grid
(SerialEM Collection protocol)
OR
Screen more or another grid
(repeat from slide 10 or 8)
OR
Shutdown
(next slides).

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!