

Only recommended  
for gold/gold grids

**SerialEM**

**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 is ready to launch

# Load your first 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

Dock Undock

— Cartridge

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

Edit Slot State Steps

Load Unload

Status

### Temperature Control

— Status

All Nitrogen Temperature

— Dewar levels

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

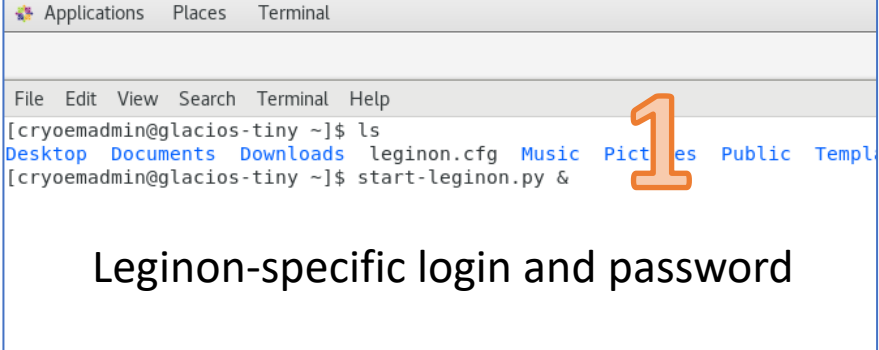
— Temperatures

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

# Make fake Leginon session

## 1. Login to Leginon computer

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



A terminal window titled "Applications Places Terminal" with a menu bar containing "File Edit View Search Terminal Help". The terminal shows the user "cryoemadmin@glacios-tiny" in the home directory. The command "ls" is entered, showing a directory listing with files "leginon.cfg", "Music", "Pictures", "Public", and "Templates". The command "start-leginon.py &" is then entered. A large orange number "1" is overlaid on the terminal output. Below the terminal window, the text "Leginon-specific login and password" is displayed.

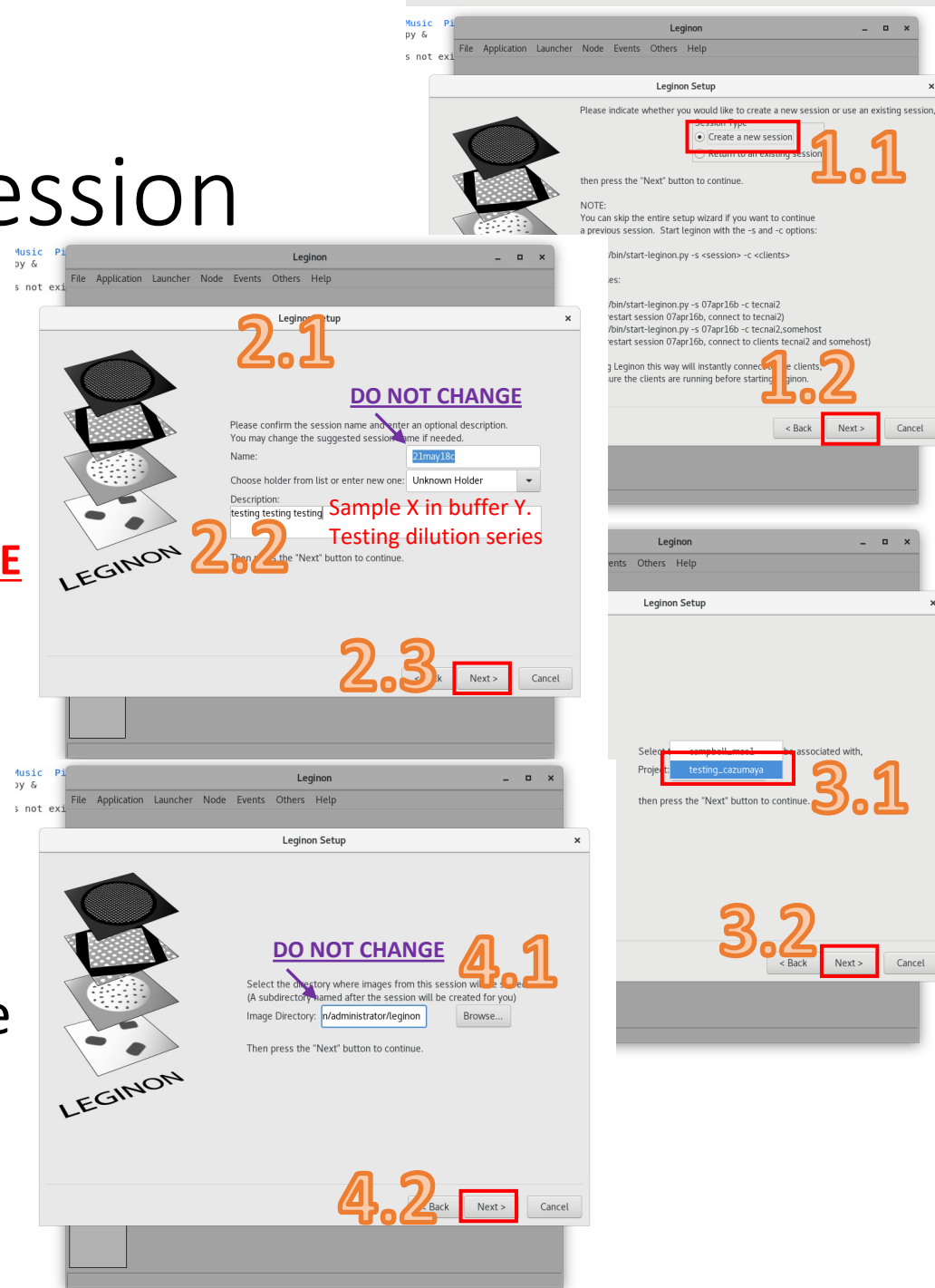
```
[cryoemadmin@glacios-tiny ~]$ ls
Desktop Documents Downloads leginon.cfg Music Pictures Public Templates
[cryoemadmin@glacios-tiny ~]$ start-leginon.py &
```

Leginon-specific login and password

Just once per session

# Fake Leginson 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



# Fake Leginson 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

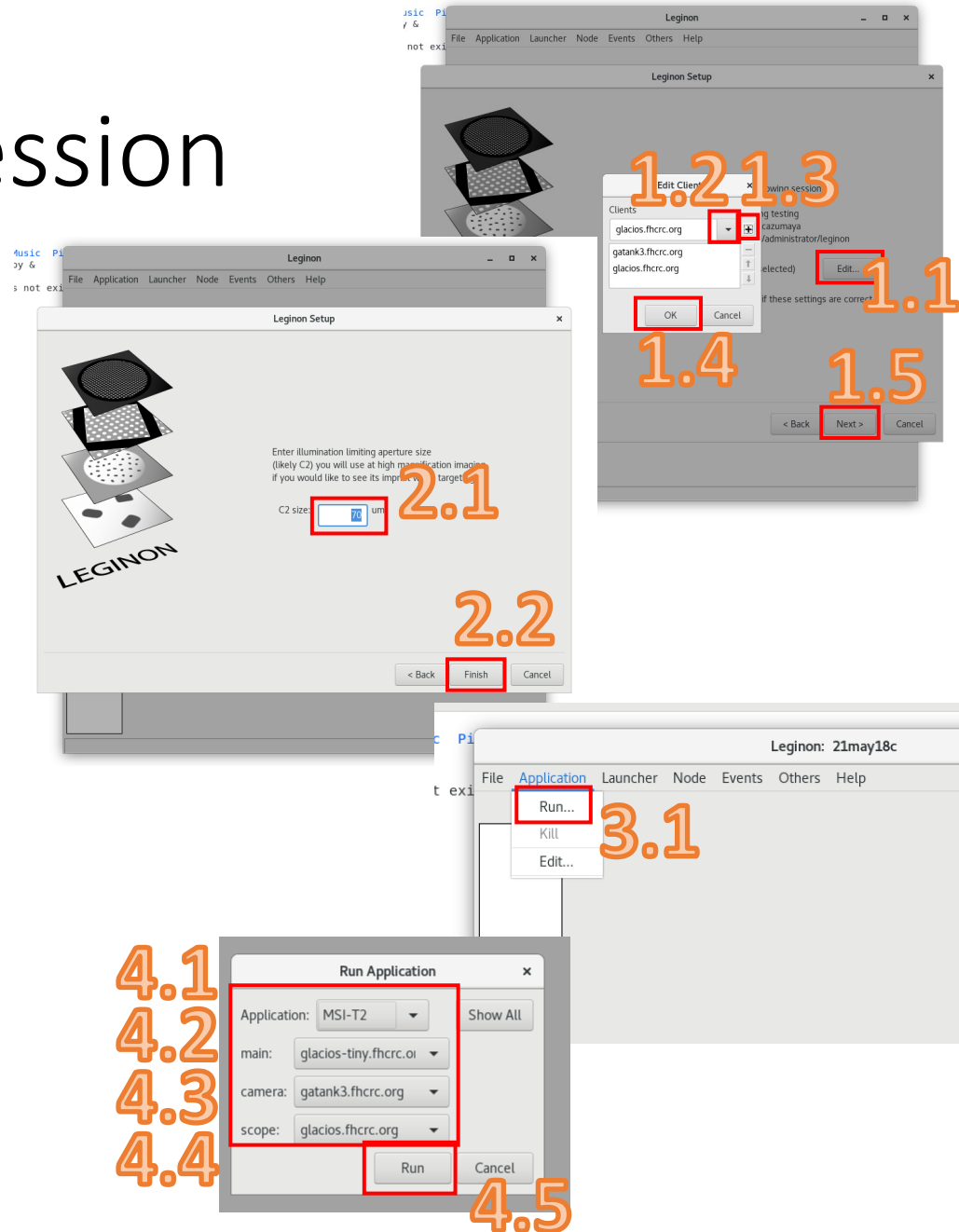
1. 50um
2. Finish

## 3. Start session

1. Application -> Run

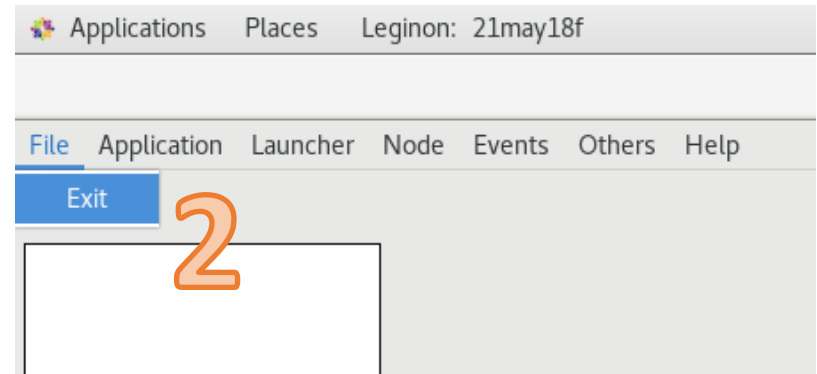
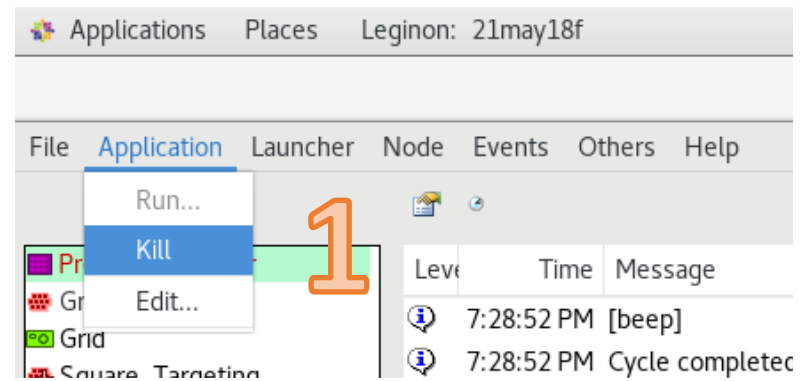
## 4. Choose application

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



# Close Leginon

1. Application -> Kill
2. File -> Exit
3. Logout of computer
  1. Power logo ->  
Username -> Sign out



# Create folder for movies

1. Go to File Explorer (on K3 computer)
2. Go to M:/cryoem/**username**/frames/
3. Right click -> new folder (**name of your fake login session**)
4. Go to fake session
5. Right click -> new folder (**rawdata**)



# Prep SerialEM

1. Open SerialEM
2. Load Settings file
  1. Settings -> SerialEMsetting\_10cds\_36kx\_nP.txt
3. Load Navigator
  1. Navigator -> Read & Open -> nav.nav (in your today folder)
4. When grid is loaded and ready!!
  1. Open column valves
  2. Put in 100um objective aperture

1

2

3

4.1

4.2

Component	Value	Log
Accelerator	1	Log
Column	1	Log
Autoloader	17	Log
Detection Unit	21	Log
Buffer tank	57	Log
Backing line	60	Log

Aperture	Value	Adjust
Condenser 2	70	Adjust
Objective	100	Adjust
Selected Area	[none]	Adjust

Are your  
column  
valves open?

# Go to square of interest

1. Double click on atlas in Navigator window
2. Left click in the middle of a square of interest
3. Click "Add Marker"
4. Click "Go to Marker"
5. Check "Low Dose" in LowDose
6. Go to "View" in Low Dose
7. Insert screen and scoot yourself into the square with joystick

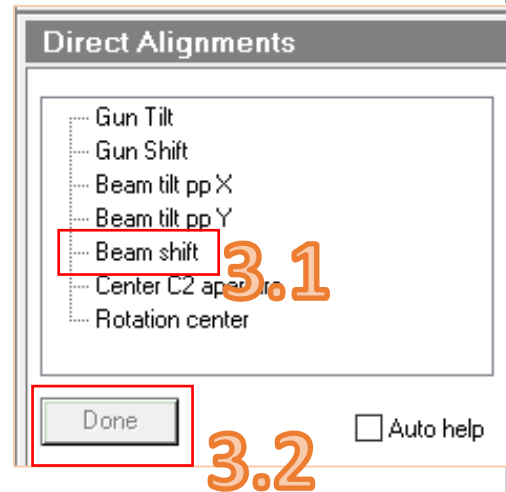
The screenshot shows the Navigator software interface. The main window displays a list of markers with columns for Label, Color, X, Y, Z, Type, Reg., Acq., and Note. The first marker is highlighted in blue. A 'Low Dose Control' dialog box is open in the foreground, showing various settings and buttons. Red boxes and orange numbers 1-6 highlight specific UI elements:

- 1: The 'Add Marker' button in the left sidebar.
- 2: The first row of the marker list.
- 3: The 'Go To Marker' button in the left sidebar.
- 4: The 'Go to' button in the 'Low Dose Control' dialog.
- 5: The 'View' button in the 'Go to' section of the 'Low Dose Control' dialog.
- 6: The 'View' button in the 'Go to' section of the 'Low Dose Control' dialog.

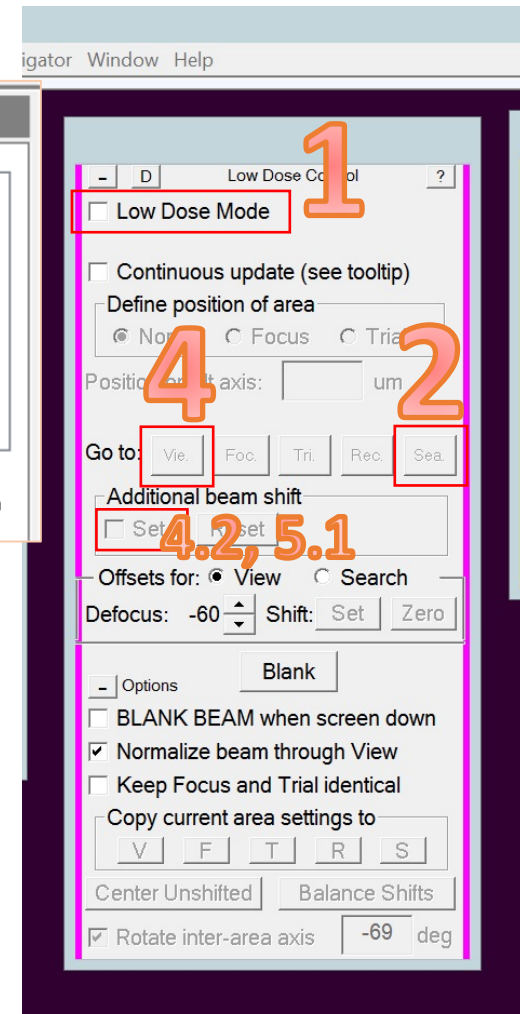
Label	Color	X	Y	Z	Type	Reg.	Acq.	Note
1	Blu	-38.9	0.7	-19.6	Map	1		Sec 0 - LMM.st
2	Blu	-38.9	0.7	-0.0	Map	1		Sec 1 - LMM.st
3	Blu	-38.9	0.7	-0.0	Map	1		Sec 2 - LMM.st
4	Blu	-38.9	0.7	-0.0	Map	1		Sec 3 - LMM.st
5	Red	-681.7	-581.5	-0.0	Pt	1		
6	Red	-817.3	-353.7	-0.0	Pt	1		
7	Red	-740.7	-640.5	-21.6	Pt	1		
8	Red	-796.5	-699.7	-26.7	Pt	1		
9	Red	-738.3	-753.6	-26.8	Pt	1		
10	Red	-559.1	-574.7	-15.6	Pt	1		
11	Red	-743.5	-523.8	-17.7	Pt	1		
12	Red	-619.4	-519.3	-15.4	Pt	1		
13	Red	-684.1	-462.8	-15.1	Pt	1		
14	Red	-622.3	-401.2	-12.3	Pt	1		
15	Red	-689.8	-347.8	-13.1	Pt	1		
16	Red	-748.6	-292.7	-14.5	Pt	1		
17	Red	-807.3	-352.3	-18.3	Pt	1		
18	Red	-868.0	-297.9	-19.9	Pt	1		
19	Red	-813.3	-236.4	-16.7	Pt	1		
20	Red	-754.1	-178.2	-14.1	Pt	1		
21	Red	-564.7	-458.3	-12.6	Pt	1		
22	Red	-555.3	-925.4	-31.0	Pt	1		
23	Red	-404.7	-888.8	-27.7	Pt	1		

# Find your beam in Low Dose

1. Go to: Rec in **LowDose**
2. Insert screen and
  1. Center 'beam shift' in Direct Alignments
  2. Click Done
3. Go to: View in **LowDose**
  1. Make sure that defocus on microscope is -80, if not change defocus to -80 with hand panel focus knob
  2. If beam is not centered:
    1. Check Set
    2. Center beam with track ball
    3. Uncheck Set
4. Go to: Focus in **LowDose**
  1. If beam is not centered:
    1. Check Set
    2. Center beam with track ball
    3. Uncheck Set

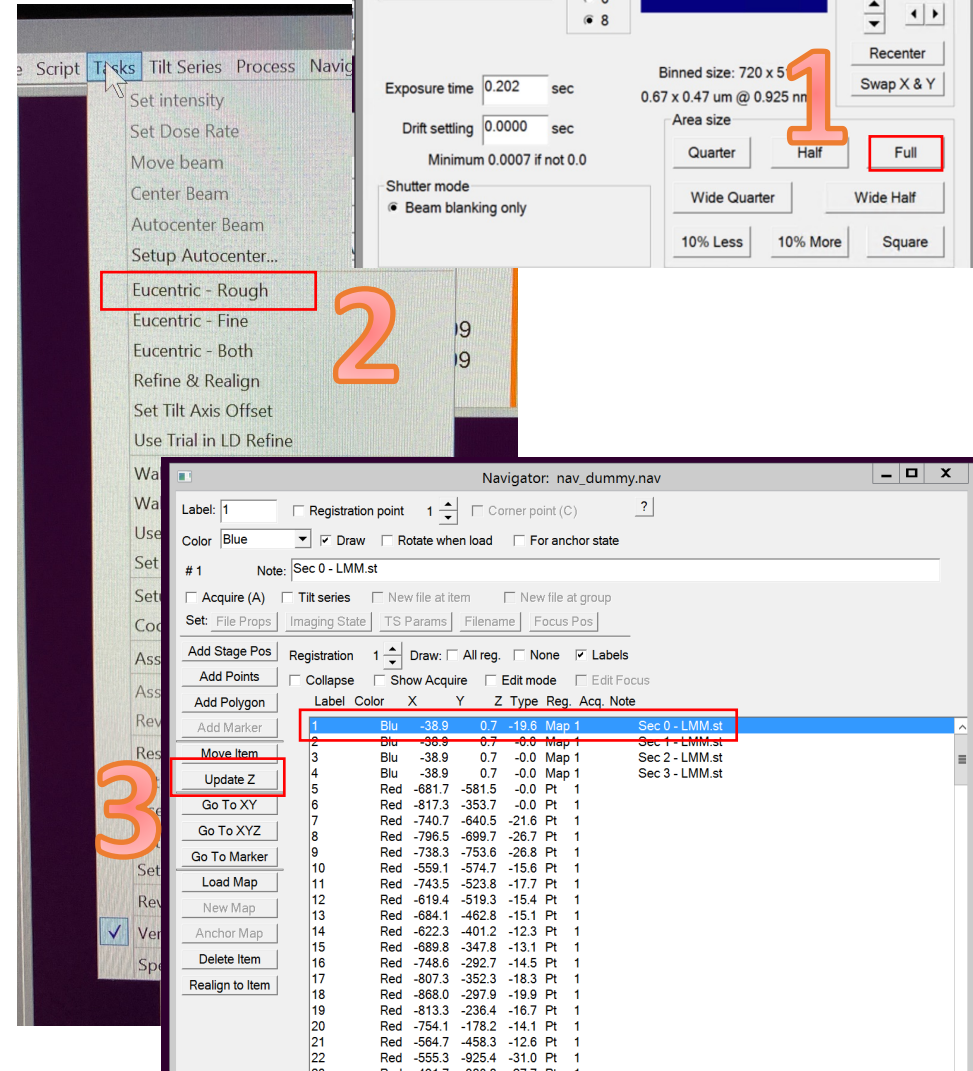


Once per session,  
**probably not**  
necessary if you  
screened in Legikon  
first.



# Find eucentric height

1. In **Camera** “Setup” make sure View has area size = Full
2. Tasks → Eucentric-Rough
3. Select atlas in Navigator window and click “Update Z”
4. Take a “View” image in **Camera**



# Center on a hole

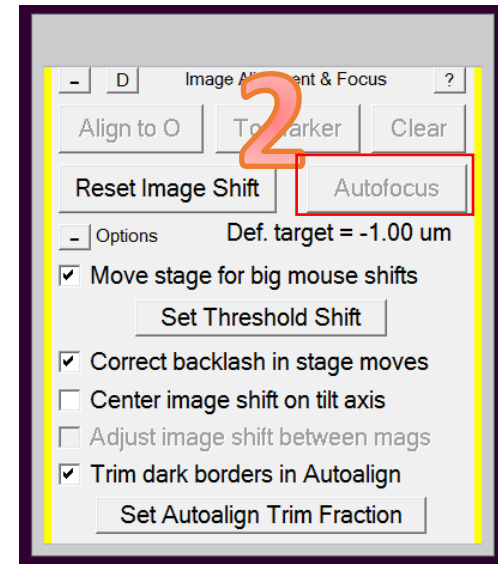
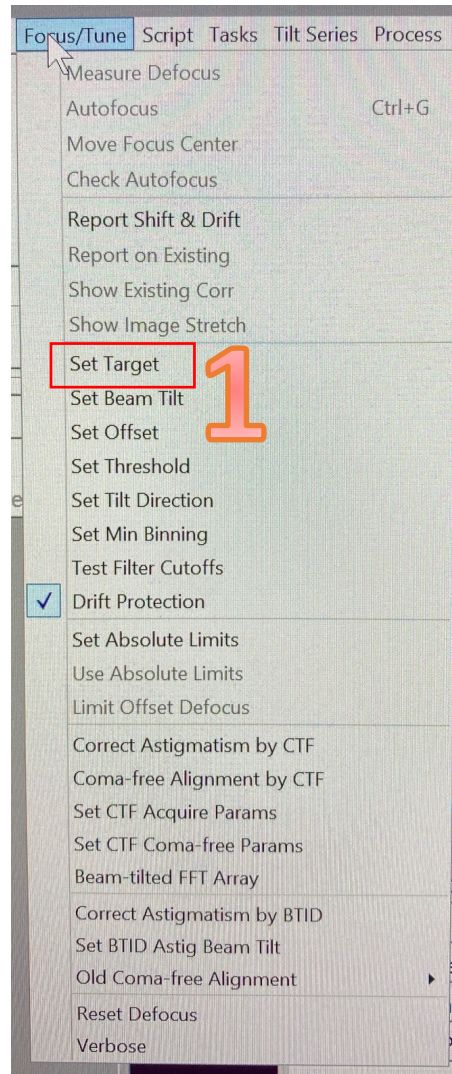
1. Shift + right click and drag to move your stage until you are centered over a hole
2. Click "View" in **Camera** a few times to make sure your stage isn't drifting away from this position
3. Select "Define position of area: Focus" in **Low Dose**
4. Left click to drop focus position (yellow) onto carbon next to the hole

The image shows a microscope control interface. At the top, a grayscale image of a grid of circular holes is displayed. A red crosshair is centered on one of the holes. A yellow grid icon is positioned next to the hole. The number '4' is written in orange above the hole, and the number '1' is written in orange to the right of the crosshair. Below the image, there are two control panels. The left panel, titled 'D Camera & Script', has a red box around the 'View' button, with the number '2' written in orange above it. The right panel, titled 'Low Dose Control', has a red box around the 'Focus' radio button under 'Define position of area', with the number '3' written in orange to its right. Below the 'Low Dose Control' panel, there is a blue arrow pointing to the text 'Change with each new grid'.

Change with each new grid

# Autofocus

1. Focus/Tune -> Set Target (-2.0)
2. Click Autofocus in Image Alignment and Focus



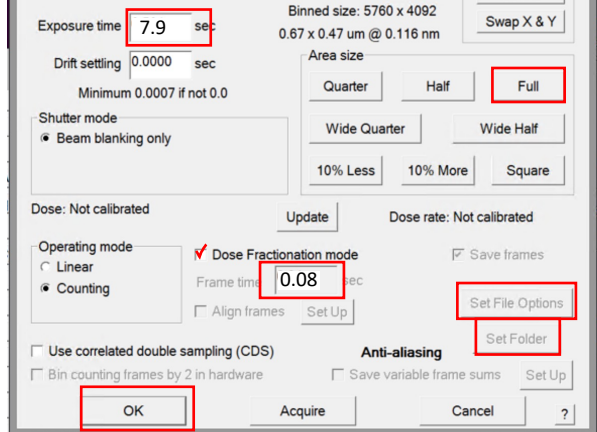
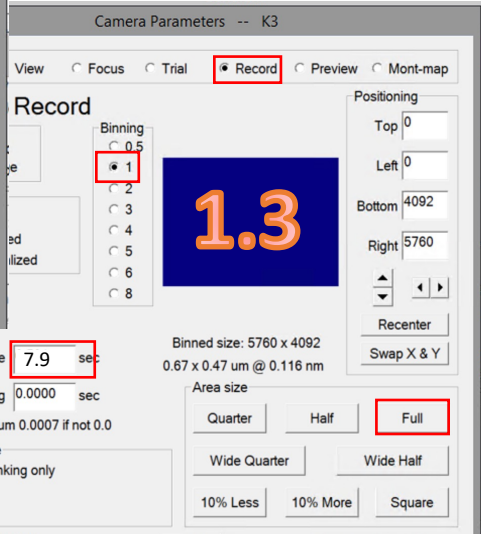
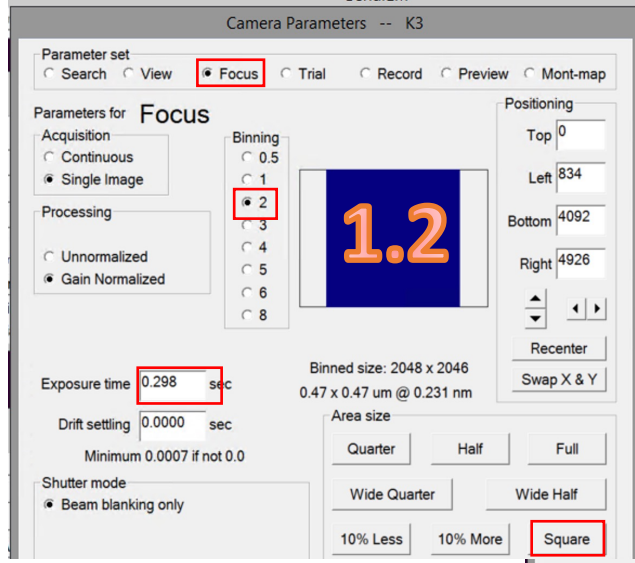
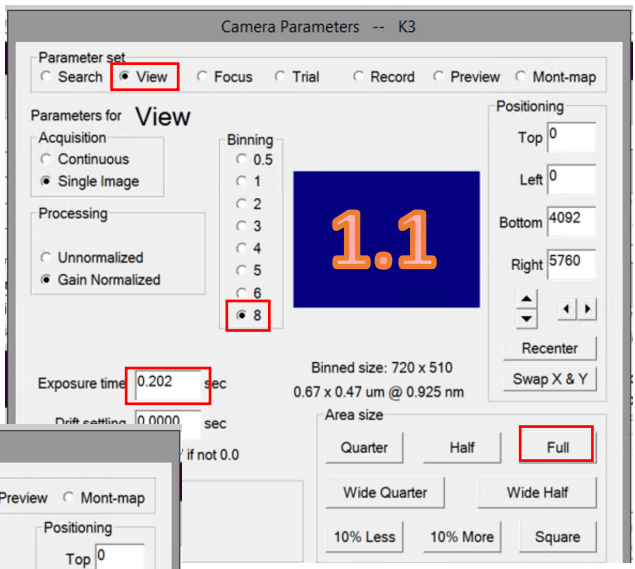
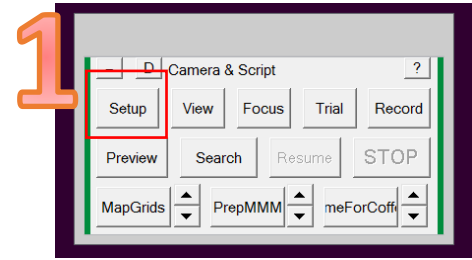
# Set imaging settings

1. Click “Setup” in Camera and set parameters

1. View: bin = 8, area size = full, exposure = 0.2s
2. Focus: bin = 2, area size = square, exposure = 0.2-0.5s
3. Record: bin = 1, area size = full, exposure = (from settings sheet), dose fractionation ON – frame time: (from settings sheet), Set File Options: (YYYYMMDD\_username\_sample\_grid), Set Folder (X:/SEM\_frames)

Change with each new grid

1



# Take exposures and start data transfer

1. Click “Record” in Camera
2. Click “View” in Camera
3. Click “Add Stage Position” on Navigator
4. Double click on “transfer\_serialEM.bat”

1. Enter username
2. Enter session name (from Leginon screening/fake session i.e. 21sep14f)

Just once per session

Camera & Script

Setup View Focus Trial Record

Preview Search Resume STOP

meForCoff

Navigator: nav\_dummy.nav

Label: 1 Registration point 1 Corner point (C) ?

Color Blue Draw Rotate when load For anchor state

# 1 Note: Sec 0 - LMM.st

Acquire (A) Tilt Set New file at item New file at group

Set: File Props In State TS Params Filename Focus Pos

Add Stage Pos Registration 1 Draw: All reg. None Labels

Add Points Collapse Show Acquire Edit mode Edit Focus

Add Polygon

Add Marker

Move Item

Update Z

Go To XY

Go To XYZ

Go To Marker

Load Map

New Map

Anchor Map

Delete Item

Realign to Item

Label	Color	X	Y	Z	Type	Reg.	Acq.	Note
1	Blu	-38.9	0.7	-19.6	Map	1		Sec 0 - LMM.st
2	Blu	-38.9	0.7	-0.0	Map	1		Sec 1 - LMM.st
3	Blu	-38.9	0.7	-0.0	Map	1		Sec 2 - LMM.st
4	Blu	-38.9	0.7	-0.0	Map	1		Sec 3 - LMM.st
5	Red	-681.7	-581.5	-0.0	Pt	1		
6	Red	-817.3	-353.7	-0.0	Pt	1		
7	Red	-740.7	-640.5	-21.6	Pt	1		
8	Red	-796.5	-699.7	-26.7	Pt	1		
9	Red	-738.3	-753.6	-26.8	Pt	1		
10	Red	-559.1	-574.7	-15.6	Pt	1		
11	Red	-743.5	-523.8	-17.7	Pt	1		
12	Red	-619.4	-519.3	-15.4	Pt	1		
13	Red	-684.1	-462.8	-15.1	Pt	1		
14	Red	-622.3	-401.2	-12.3	Pt	1		
15	Red	-689.8	-347.8	-13.1	Pt	1		
16	Red	-748.6	-292.7	-14.5	Pt	1		
17	Red	-807.3	-352.3	-18.3	Pt	1		
18	Red	-868.0	-297.9	-19.9	Pt	1		

transfer\_leq...

transfer\_ser...

4



# Open WARP on 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

A red ring with the number '3' inside, indicating the current processing status.

#### Astigmatism (use up to 3.0 $\sigma$ )

A diagram showing concentric circles representing astigmatism levels, with labels for 0.2  $\mu\text{m}$  and 0.4  $\mu\text{m}$ .

#### Defocus (use 0.10–3.50 $\mu\text{m}$ ) — average |CTF|:

A plot showing the average |CTF| with a scale from 0.00 to 0.000 and a label '1/1.1 A'.

#### Estimated resolution (use better than 4.6 $\text{\AA}$ )

A plot showing estimated resolution with a scale from 0.00 to 1.03.

#### Average motion per frame in first 1/3 (use up to 2.0 $\text{\AA}$ )

A plot showing average motion per frame with a scale from 0.00 to 1.03.

#### (use up to 10 %)

A plot showing motion with a scale from 0.00 to 1.03.

### Input

Input: M:\cryoem\cazumaya\frames\21may14\rawdata\ — \*.tif  
Pixel X/Y: 0.5610/0.5610  $\text{\AA}$ ,  $\infty$  0.0°  
Bin: 1.00x (1.1220  $\text{\AA}/\text{px}$ )  
Exposure: 0.51 e/ $\text{\AA}^2$ /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  $\text{\AA}$   Use Movie Sum  
Voltage: 200 kV C<sub>s</sub>: 2.70 mm  Phase Shift  
Amplitude: 0.07 Defocus: 0.0–5.0  $\mu\text{m}$

Motion  
Consider 44.9–9.0  $\text{\AA}$ , weight with B = -500  $\text{\AA}^2$

### Models

Defocus: 5 x 5 x 1

Motion: 7 x 5 x 99

Pick Particles  
Use Select BoxNet model...  
Expect 200  $\text{\AA}$  cryo particles; use scores above 0.95  
Maintain a minimum distance of 0  $\text{\AA}$  from   
 Extract 128 px boxes, 1.1220  $\text{\AA}/\text{px}$ ,  invert,  normalize  
 Maintain a separate list of the latest 10000 particles

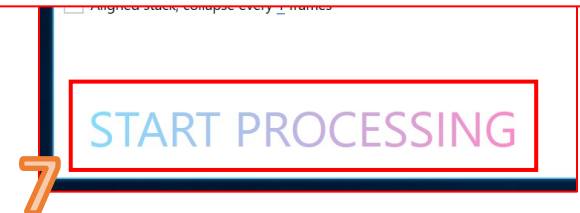
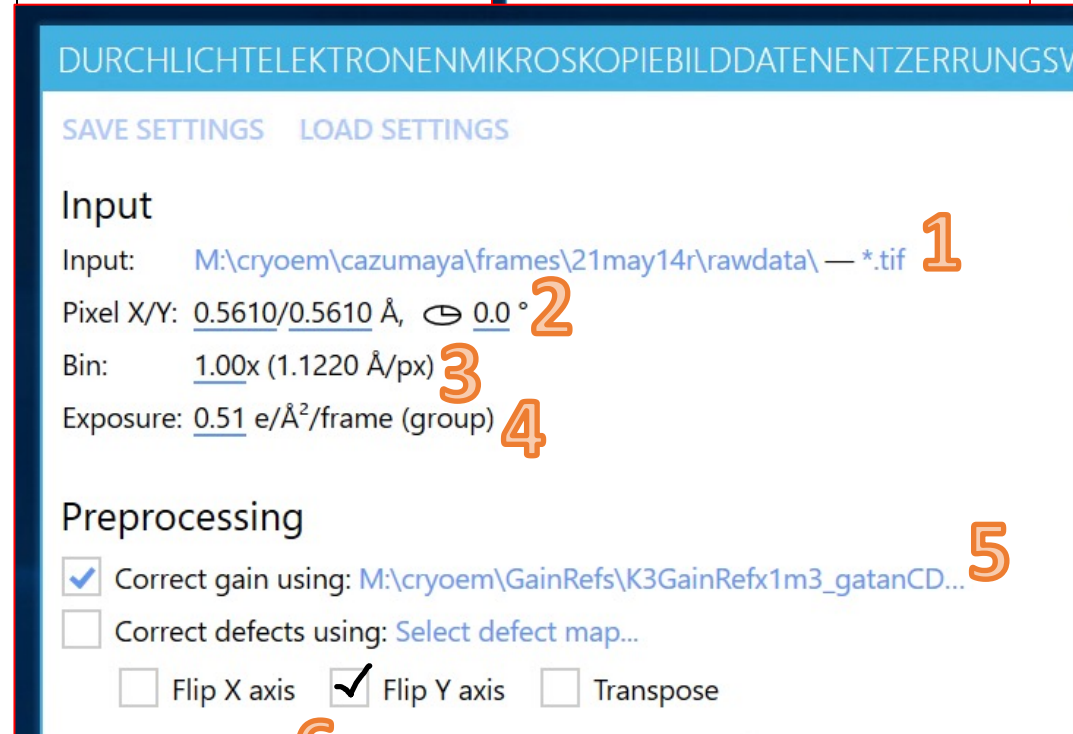
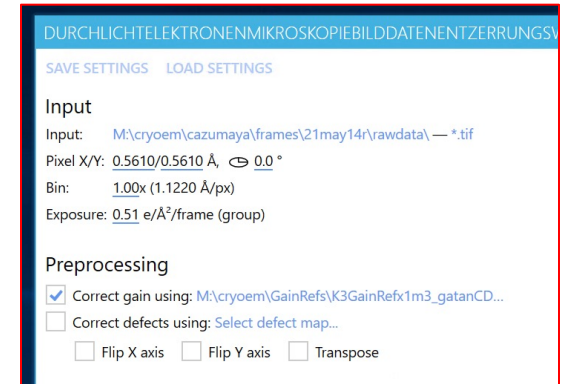
### Output

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

**START PROCESSING**

# Setup WARP

1. Input: Browse for movie folder
2. Pixel size: (see Teams) usually 0.561
3. Bin: 1.00x (usually)
4. Exposure: (see Teams) usually 0.51
5. Correct gain using: Browse for gain image
6. Flip Y axis for gain
7. Start Processing



Setup collection on this grid (SerialEM Protocol)

OR

Screen more

(repeat slides 13,14,16 to screen *in same square*)

(repeat slides 10,12,13,14,16 to screen *in new square*)

(repeat slides 3,10,12,13,14,15,16 to screen *on new grid*)

OR

Shutdown

(next slide).

Do shutdown without  
collection Glacios check list!

End iLab time and sign out!