

## Protocol for Glacios cryo-EM imaging (Leginon)

### Location:

DE-788 (Glacios room)

### General rules:

- Do not use the Glacios or automated software unless you are accompanied by an EM core staff member or have been cleared as an independent user
- Facility staff will clip, load, and unload all autogrids
- Use computers and programs only for their intended purposes
- Respect your reservation time
- Report any problems to Caleigh ASAP

### Sign-up protocol:

- Use iLab (base rate, \$60/hr): reserve for the entire time you plan to use the Glacios – 3hr minimum
- Book supported use for the first 3hrs unless you are **entirely** comfortable with the setup for screening
- Staff screening can be booked through “Services” tab on iLab
- Door access will be granted after training

### Training plan:

- Please contact Caleigh at [cazumaya@fredhutch.org](mailto:cazumaya@fredhutch.org) to schedule training
  - One 30-min introduction to the microscope and two 2-hour Leginon training sessions
  - If returning >30 days after last Glacios use, please contact (via email) for refresher

### Training objectives:

- Perform startup and shutdown procedures responsibly
- Find eucentric height, focus, and set appropriate imaging conditions
- Setup and run automated data collection using Leginon software

### User provided materials:

- Frozen cryo-EM grids
- Storage space for movies and micrographs

### Shared resources tools list: if anything is missing/out of the ordinary, please contact Caleigh ASAP

- Clipped and loaded cryo-EM grids
- Data stored for 30-days after collection

### **Startup checklist:**

- Log into iLab Kiosk and “Start” your session
- Insert screen
- Check vacuum readings, LN2 level, and make sure you have a beam
- Turn Turbo to “Auto off”
- Insert grid you want to start screening
- Insert C2 = 50

### **Leginon protocol (screening):**

#### **Startup**

- Start “Leginon Client” on the Glacios computer **AND** K3 computer
- Login to Leginon computer and start Leginon GUI
- Create new session
- Run the application MSI-T2
- Node menu -> Kill -> Preview
- Import presets from trusted user

#### **Choose good square**

- Take the objective aperture out
- Send gr preset to the microscope
- Find a square where you want to image using microscope joystick and jpgs

#### **Follow Leginon nodes Square -> Exposure**

- Send sq preset to microscope, recenter region of interest
- Insert Objective = 100um
- Go to Square node, “Simulate Target”
- Go to Hole targeting node, choose targets, submit
  - Choose targets to show holes with varying ice thickness if there is variability in the square
  - Place focus target away from square edge
- Go to Exposure targeting node, choose targets, submit
  - Choose targets in holes with varying ice thicknesses
  - Place focus target on carbon
- Enable Manual Focus and correct if needed

#### **Motion correction/Monitoring**

- Can check sq/hl on [emweb.fredhutch.org](http://emweb.fredhutch.org) – hard to see en
- Start Leginon transfer script on K3 computer
- Start WARP on K3 computer
- Check exposures and decide whether to screen more, switch grids, shutdown or move to collection (likely in SerialEM)

### **Leginon protocol (collect):**

- If you have not already, take correction images (above)
- Send gr preset to the microscope
- Find an alignment square (distinctive contamination at all magnifications)

- Insert C2 = 50um
- Center piece of junk in sq, hl, and en magnifications
- Align presets pr -> hl, hl -> sq, sq -> gr
- Insert C2 = 150 um
- Send gr to scope
- Take atlas of grid
- Choose squares to image in square targeting and start queue
- Begin trying to optimize hole finder in hole targeting – template, threshold, blobs, lattice, acquisition – keep focus away from grid bar
- Submit targets – do **NOT** submit queue until **squares** are finished collecting

When all square images have been collected – check webviewer LOI to confirm

- Insert C2 = 50 um and Obj = 100 um
- Submit hole queue
- Begin trying to optimize hole finder in exposure targeting – template, threshold, blobs, lattice, acquisition – one focus spot per image
- Submit targets – do **NOT** submit queue until **holes** are finished collecting

When all hole images have been collected - check webviewer LOI to confirm

- Go to Manual Focus, find zero, reset defocus and get eucentric from microscope
- Submit the exposure queue

(continue accepting exposure targets and submitting targets after this)

- Leave Manual Focus on for at least one round of focusing to check it and then disable in Focus Sequence list (no orange boxes!!)
- Start WARP on remote desktop from K3 PC
- Continue until all exposure targets are in the queue and then you can monitor progress from webviewer/WARP outside of microscope room

### **Shutdown checklist:**

- Mark grids to save/discard on Notepad
- Close column valves if not collecting
- Confirm Leginon and WARP are running
- Confirm manual focus is OFF in Leginon
- Set column valves to close when collection is finished

Finished! 😊