

Protocol for Talos L120C Leginon imaging

Location:

DE-782 (Talos room)

General rules:

- Do not use the Talos or automated collection software unless you are accompanied by an EM core staff member or have been cleared as an independent user
- Use tools only for their intended purposes
- Remove grids from desk promptly
- Respect your reservation time
- Report any problems to Caleigh ASAP

Sign-up protocol:

- Use iLab (base rate, \$60/hr) to reserve for the entire time you will be using the Talos
- Book supported use unless you are **entirely** comfortable with the setup for screening/collection
- Staff services can be booked under “Services” through iLab
- Door access will be granted after training

Training plan:

- Please contact Caleigh at 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 Talos use, please contact staff and booked “Supported Use” time

Training objectives:

- Successfully insert the room temperature sample holder without disrupting the column vacuum
- Find eucentric height, focus, and set appropriate imaging conditions
- Setup and run automated data collection using Leginon software

User provided materials:

- Stained cryo-EM grids
- Storage space for micrographs

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

- Sharp tweezers
- Room temperature sample holder and pin tool
- Data stored for 30-days after collection

Startup checklist:

- Turn on the filament (will take 10 min)
- Log into iLab Kiosk and “Start” your session
- Insert screen
- Check vacuum readings, LN2 level, emission, and make sure you have a beam
- Confirm stage is at 0,0,0
- Insert RT sample holder
- Turn Turbo off
- Insert C2 = 70

Leginon protocol (screen):

- Start “Leginon Client” on the Talos computer
- Login to Leginon computer and start control GUI (start-leginon.py)
- Create new session - choose project, add clients, enter C2 size (70um)
- Run the application MSI-Raster
- Node menu -> Kill -> Preview
- Import presets from trusted user
- Check presets for magnification and beam intensity
- C2 = 150 um
- Send gr preset to the microscope
- Find good square using microscope joystick
- C2 = 70um
- Send sq preset to microscope, recenter region of interest
- Send hl preset to microscope, recenter region of interest
- Objective = 100um
- Z-Focus node, “Simulate Target”
- Subsquare node, “Simulate Target”
- Exposure targeting node, choose targets – Raster will autofill
 - Focus target – not on anything too dark
- Submit targets and queue
- Turn Manual Focus on and correct
- Collect exposures and decide whether to screen more, switch grids, or move to collection

Leginon protocol (collect):

- Same setup as screening up to C2 = 150
- Send gr preset to the microscope
- Mark good + alignment squares in microscope “Search” box
- Go to square with medium/large piece of junk (alignment sq)
- C2 = 70um
- Align presets en -> hl, hl -> sq
- Go to good square

- Objective = 100um
- Square “Simulate Target”

(iterate until all square images are collected)

- Subsquare targeting node, choose subsquare targets and Z-focus spot
 - Z-focus away from edge of square
- Submit targets and queue

(iterate until all subsquare images are submitted)

- Exposure targeting node – Raster will autofill
 - Unselect acquisitions too close to edge
 - Make sure focus spot isn't on something dark
- Submit targets **NOT** queue

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

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

(can continue choosing exposure targets and submitting targets after this)

- Leave Manual Focus on for at least one subsquare to check it and then disable in Focus Sequence list
- Continue until all exposure targets are in the queue and then you can monitor progress from webviewer LOI outside of microscope room
- Leave TIA opened on the microscope computer so people can see the microscope is in use
- Come back promptly to do shutdown when collection stops

Shutdown checklist:

- Exit and logout of Leginon computer
- Retract Ceta camera
- “Reset” holder
- Close column valves
- Remove sample holder and store grid safely
- Store sample holder in cabinet
- End session on iLab Kiosk
- If you're the last user** turn off filament

**unless someone else is coming in the next 1hr

Finished! 😊