Electron Microscopy Shared Resource
Manager: Caleigh Azumaya
Version 1.1, 18-Apr-22

emsr@fredhutch.org
206-667-4289
DE-780 (Thomas Building)

Protocol for Talos L120C imaging

Location:
DE-782 (Talos room)

General rules:
- Do not use the Talos 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 staff at emsr@fredhutch.org to schedule training
    - Two 1-hour Talos 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
- Convert files to tifs and transfer to lab storage

User provided materials:
- Sample grids
- Storage space for images

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

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Startup:

- Turn on the Filament – allow approx 13min to saturate (status: beam emission stable)
- Sign in at Kiosk and click Start to begin recording filament time
- Insert specimen rod (*EM staff will do this unless user is trained*)
- Set apertures
  for biological samples C2 Aperture – 100, Objective aperture – 70, Spot Size 6
- Open Column Valves (Setup tab)
- Insert FLU screen (Screen Up/Down)
- Find beam and move to area away from ROI to reduce beam damage (screen on left monitor)
- Center beam in LM and M/SA.
  Under Direct Alignments tab, select Beam Shift, center beam with MF X / Y, press Done to save for session
  Quick alignment can be done with Roller Ball
- Determine Eucentric (Z) Height for each sample using a recognizable feature
  - Mag @ 13,500x, Wobbler on (flapout on Search tab), set recognizable feature to swing less than 500nm using Z +/- button. Easiest to do on FLU screen (Mesh grids close to zero, slot grids +80-85)

Imaging:

- Joystick moves sample stage, +/- settings to increase or decrease stage movement speed
- Focus image
  True focus can be determined using the FFT function (either on the FLU screen or with camera). Click Reset Defocus to save Eucentric.
  Focus by setting the focus step (bottom collar on focus knob) to 4 or below. The higher the focus step number, the coarser the focus step. Focus the image by turning the focus knob (top) until FFT image is diffuse (no Thon rings). Most biologicals read best a little under focused. Underfocus by turning focus counter clockwise until image appears sharp but not grainy (can you recommend a value here?).
- Camera
  Open Camera tab, Insert camera, retract FLU screen (if not already retracted), select Search. Once ROI is determined and in focus (FFT), select Preview for acquisition resolution image (can fine tune focus here), select Acquire to save image (see Saving Images set up below).

Saving Images to a Folder:

- Create a new folder on the D drive, under TIA data: (e.g. yourHutchNetID_today's date)

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- To setup Autosave Insert the Camera, select Preview and then deselect Preview to activate Autosave.
- Go to the Autosave icon on TIA software screen (or under File select autosave) and click until you get the dialog box, browse to find your folder in D:TIA Data
  To save numbered images, make sure Save Sequentially is checked (bottom left)
- Open folder (can drag box to move to left monitor).
- Select the file name box (.emi), enter the title for image series (e.g. SM_5432_2).
- Acquire image and click Save in the dialog box. Check to see that it goes into selected folder and/or subfolder and is titled correctly. Continue to acquire images until you are done with that sample.
- Repeat for each new sample.

Batch Converting Images:

- Blank the beam (Camera tab, Blank) while you are doing the batch convert
- Select Folder Export from left sidebar on TIA screen, open Settings
- To save converted images to the same folder, the “Source” and “Target” line names must be the same:
- In TIA Data select ”yourFolder” (or subfolder), activate and copy the path line from the top left. Paste into both Source and Target
- Select Image Format as “PC tiff with scale marker (full Res)” to include scale bar
- Say “OK” (data box disappears unless there is an error with the path).
- Click “Export” to start conversion.
- Once everything is converted to tiffs, “Close All (pages icon with x)”.
- You will get a dialog box – Say “No to all”
- Close all documents and discard changes = “Yes”

How to Separate TIFFs:

- Open Folder and/or subfolder
- View
- Group by
- Type
- Control, Shift – click on first and last image and move TIFFs into a TIFF folder that you have made in the subfolder or into homelinks

Insert the new sample:

- Click off autosave icon before you focus for another image
- Focus, and Preview
- Turn Preview off

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- Click on Autosave
- File – Autosave (click more than one time) and it will come up with the folder you were in. Make a new subfolder and click on it.
- Go to file name and put in everything before .emi. Ex. SM_5432_3
- Don't press "Save" in the dialog box until you have acquired an image.
- Acquire image
- Check to see if the images are in the subfolder.

End of Session:

- Close Column Valves (Setup tab)
- Reset Holder (flapout on Search tab), zeroing X,Y,Z
- Turn off AutoSave (!!)
- Retract Camera (deselect Insert Camera)
- Insert FLU screen
- Retract Obj Ap
- FLU screen on "Natural"
- Turn off Filament
- Remove sample – done by EM staff unless trained
- End Kiosk session and Log Out