S²C² | Stanford-SLAC Cryo-EM Center

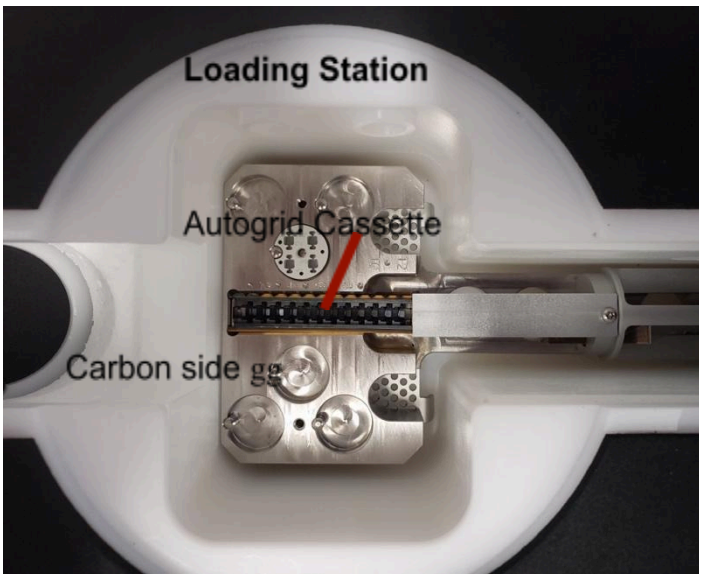
Cryo-EM Data Collection SOP-Talos Arctica with DE64 (SerialEM) MicroED only

On the day of data collection:

- Clip grids as needed
- Check microscope if cassette is in or out. If microscope is used after cryocycle, check the temperatures
- Print our safety form and imaging conditions
- Stop **Data Acquisition** of the previous user
- Close column valves
- Go to the microscope room, find the blanker control box and switch it from TEM to camera. Remember to switch it back after experiment is done
- Open DE computer, open DE server, cool down the camera. Remember to warm it up after the experiment is done
- It is suggested to bring and load a standard grid like Aluminum (EMS 80044)

Loading grids:

- Fill loading station and Nanocab with LN_2.
- Transfer grid buttons with clipped grids into loading station
- Load clipped grids into cassette in loading station.
- Carbon side should face the handle. Make sure that autogrid cartridges are held by in place by spring.
- Fill NanoCab with LN_2 and attach to side of loading station.
- Transfer cassette into NanoCab, making sure not to expose grids to air.
- Make sure cassette is placed firmly in bottom of NanoCab, and the pin should pop up.
- Cover with lid and place into secondary container for transferring to the microscope room.



eLogbook:

- Put the instrument to **Standby**
- Create an Experiment with the name “YYYYMMDD-CAXX”
- Add collaborators

Microscope Alignment

Direct Beam Alignment and Selective Aperture Alignment

- Go to vacuum area and align the beam pivot point
- Adjust the condenser astigmatism
- Center C2 aperture
- Go to diffraction mode, adjust the beam position
- Switch to standard grid
- Check the focusing in diffraction mode, focus the beam if necessary
- Go to a location on the grid, center a feature and apply a high defocus in diffraction mode
- Adjust the SA aperture so that the feature is in the middle of the field of view
- Go to view mode (defocused diffraction), use marker tool in flu cam to note the location of SA aperture in view mode

eLogbook:

- Add sample
- Add a sample, choose method “other”
- Do not use automated pipeline processing, the elogbook is only used to upload the grid maps to the central server

- Switch the experiment on and activate the sample
- The MicroED datasets will be saved on DE computer, and need to be pre-processed before manually upload into central server using scp command

Start Data Acquisition:

- The two SerialEM setting files are generally good for most applications: 670 mm for small molecule and 1900 mm for proteins
- Use SerialEM view mode to search for potential sample
- Put the sample of interest into center of SA aperture
- Insert beam stop, take a preview shot (usually 1s in diffraction mode)
- Check diffraction quality
- If going to proceed, adjust the eucentric height in view mode, make sure the crystal doesn't move out of field of view during high-tilt.
- Make sure beam stop is inserted, change parameters on MicroED-DE.script to current setting (tilting range, exposure time)
- Hit "run" and wait it finish, the final projection diffraction pattern on SerialEM usually can tell the quality of this collection

Data Transfer:

- All raw frames are saved in D drive in DE computer
- Use DE pre-processing exe script to process all the files together, which takes several minutes
- Transfer sub-summed frames into F drive for long term storage
- Only sync those sub-summed frames into central server or copy into your portable drive