



Cryo-EM Merit Badge Standard Operating Procedure

A Collaboration Among the NIH-Funded Cryo-EM National Centers

An operations checklist: Am I ready to collect high-resolution data?

version 1.1

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Purpose

This is a high-level checklist meant for an experienced microscope operator to verify everything is optimized for data collection on an autoloader TEM. It does not provide instructions for how to perform each step. Many steps can be done in a different order. SOPs or checklists for each step could supplement this checklist for training purposes. For national center users, this is what center staff are checking ahead of your data collection session.

Stage one: You just sat down in front of the control station - initial checks

- ☐ Are there error messages on the TEM User Interface?
- ☐ Is the Gun on and set correctly
 - High Tension is ON and set to correct voltage
 - FEG Control Operate is ON and gun lens/extractor values are correct
 - FEG Power is ON
- ☐ Are the autoloader and column at LN₂ temperature?
- ☐ Is the vacuum level good?
- ☐ Can I see the beam on the flu screen?

Stage two: Can we collect images?

Microscope checks:

- ☐ Is the autoloader Turbo off before starting the data collection?
- ☐ Is the beam stable, i.e., not shaking/drift? (general health check)
- ☐ Is the beam centered over the camera at the magnifications used? (beam shift check)
- ☐ Is the beam on the flu screen the expected shape at the magnifications used? (condenser stigmation check)
- ☐ Is the beam diameter at high mag preset set to a proper size? (intensity check)

Camera checks:

- ☐ Is the camera in? (sanity check)
- ☐ Is the camera at the correct temperature?
- ☐ Is the correct mode/binning chosen for the camera? (acquisition parameters check)
- ☐ If any, is the energy filter tuned? (tuning checks)
- ☐ Are hardware gains updated? (flat field check)

Software checks:

- ☐ Can the image be acquired using the acquisition software (EPU, SerialEM, Leginon, etc.)?

Stage three: Is the microscope aligned properly for my experiment?

- ☐ Are the pivot points in alignment? (prevents: beam tilt eucentric focusing issues)
- ☐ What is the dose rate over the vacuum of my high mag preset, and is it set in software? (prevents: improper sample dose estimation)
- ☐ Are there artifacts in the image over vacuum or in the FFT? (prevents: bad gain correction)
- ☐ Is astigmatism minimized at zero beam tilt? (prevents: excessive astigmatism)

- ☐ Is an identifiable object centered across different presets? (prevents: inaccurate targeting)



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- ☐ Is the objective aperture centered? (prevents: uneven frequency transfer)
- ☐ Was some sort of coma correction done if necessary? (prevents: coma astigmatism for image shifts)
- ☐ Are pixel sizes/mags set appropriately for my experiment? (final mag setup check)
- ☐ Is the beam in the range of Parallel Illumination?
- ☐ Do Thon rings extend to high enough resolution? (quality check)

Stage four: Site and software-specific experiment setup

- ☐ Is the data collection software (Leginon, SerialEM, or EPU) set up properly?
- ☐ Have I completed any additional site-specific experiment logging?

Stage five: Automated data collection is running and you're ready to leave - final checks:

Physical:

- ☐ Nitrogen tank pressure (should be ~20 psi)
- ☐ Nitrogen tank has enough LN₂ left (~50 lbs)
- ☐ Nitrogen tank valves are open
- ☐ Krios door is closed
- ☐ Turbo pump is off (yes, again)

Session specific settings:

- ☐ Correct C2 is in
- ☐ High-mag pixel size is correct
- ☐ Dose fractions are set correctly
- ☐ If an energy filter is present, is the slit inserted for high-mag acquisition
- ☐ Objective aperture is set
- ☐ Gain is copied into the data collection directory
- ☐ Total dose is correct
- ☐ Pre-processing is running
- ☐ Data transfer is running