



Cryo-EM Merit Badge Standard Operating Procedure

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

Common Screening with SerialEM Standard Operating Procedure

version 1.9

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1. Purpose

- 1.1. Screening grids with SerialEM.

2. Definitions:

- 2.1. SerialEM is a data collection software from UC Boulder
- 2.2. TEM UI is the microscope user interface located on the microscope PC
- 2.3. Autoloader Inventory is an automated procedure that identifies filled/empty slots in the cassette

3. Supplies & Equipment

- Microscope
- SerialEM

4. Procedure:

4.1. Pre-screening Software and Microscope Checks (optional)

4.1.1. TEM UI/Microscope PC:

- 4.1.1.1. Confirm Autogrids are loaded
- 4.1.1.2. Perform Autoloader Inventory
- 4.1.1.3. Confirm SerialEM server is running
- 4.1.1.4. Confirm 150 μ m C2 aperture is inserted

4.1.2. SerialEM PC:

- 4.1.2.1. Confirm “Low Dose Mode” is enabled
- 4.1.2.2. Verify Imaging States are returning images:
 - 4.1.2.2.1. Open Column Valves
 - 4.1.2.2.2. Press “Search” in Camera Panel
 - 4.1.2.2.3. Center stage over gridsquare
 - 4.1.2.2.4. Press “View”
 - 4.1.2.2.5. Press “Focus”
 - 4.1.2.2.6. Press “Record”
- 4.1.2.3. If any of the Imaging States are in error, contact center staff for assistance.

4.2. Load Screening Grid

- 4.2.1. You can load a screening grid either from the Microscope PC or the SerialEM PC
- 4.2.2. TEM UI: Autoloader OCX: Click Slot, Click Load
- 4.2.3. SerialEM: Menu: Script > One-Line Scripts: LoadCartridge # (1-12)

4.3. Collect Low Magnification Montage – Whole Grid Overview

- 4.3.1. Open Column Valves
- 4.3.2. Acquire “Search” image
- 4.3.3. Open Navigator window (Menu: Navigator > Open)



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- 4.3.4. Create and save LMM.mrc (Menu: Navigator > Montaging & Grids > Setup Full Montage)
- 4.3.5. (optional) Find eucentric height of central gridsquare. (Menu: Tasks > Eucentricity > Rough Eucentric)
- 4.3.6. Start montage collection (Montage Panel: Start)

4.4. Identify Grid Squares to Screen

- 4.4.1. Identify at least two grid squares to screen; one thick, one thin
- 4.4.2. Left-click the center of a grid square and in the navigator window, press “Add At Marker”
 - 4.4.2.1. Repeat for each grid square
- 4.4.3. In the navigator window, select a grid square’s marker and click “Go To XY”

4.5. Screen Gridsquare and Acquire High Magnification Record Images

- 4.5.1. Acquire View Image
- 4.5.2. Find eucentric height
 - 4.5.2.1. For AuFoil grids, center stage with partial grid bar for accuracy.
- 4.5.3. Center the stage over a hole (see 7.2 for procedural information)
- 4.5.4. Adjust Autofocus Position (first grid square/once per grid)
 - 4.5.4.1. Low Dose Panel: Adjust Focus Position, select Focus
 - 4.5.4.2. View Image: Left-click focus area on support film between holes
 - 4.5.4.3. Low Dose Panel: select None
- 4.5.5. Autofocus Panel: Click “Autofocus”
 - 4.5.5.1. (optional) Adjust Defocus target: Menu: Focus & Tune > Set Target (0 to -5.0µm)
- 4.5.6. Camera Panel: click “Record”
- 4.5.7. Save Record Image
 - 4.5.7.1. Menu: Window > Save Image Snapshot
- 4.5.8. Repeat on at least one hole from same grid square
- 4.5.9. Navigate to next grid square of interest and repeat section 4.5

4.6. Save LMM with Targets / Screened Squares

- 4.6.1. Double click LMM in Navigator window to load it
- 4.6.2. Save Image Snapshot

4.7. Repeat for each grid, starting at 4.2

- 4.7.1. Load next grid, acquire LMM, identify grid squares, acquire and save high magnification images

4.8. Shutdown

- 4.8.1. After screening is complete,
- 4.8.2. Close Navigator Window
- 4.8.3. Close all open maps with Menu: File > Close
- 4.8.4. Close column valves and load grid 1, the cross grating.

5. **Chemicals:** N/A

6. **Waste Disposal:** N/A



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7. Functions:

- 7.1. Eucentric Height – Calculate the Z-Height of the grid square using an automated function. The function is launched from the Tasks menu or macro “Eucentricity 1”.
- 7.2. Centering the Stage – Move the crosshairs over feature of interest using mechanical movements. Use the mouse to left-click and place the green, temporary marker where you would like to go. In the Navigator window, use the “Go To Marker” button to move. Additional movements are sometimes necessary due to stage motor backlash.

8. Images:

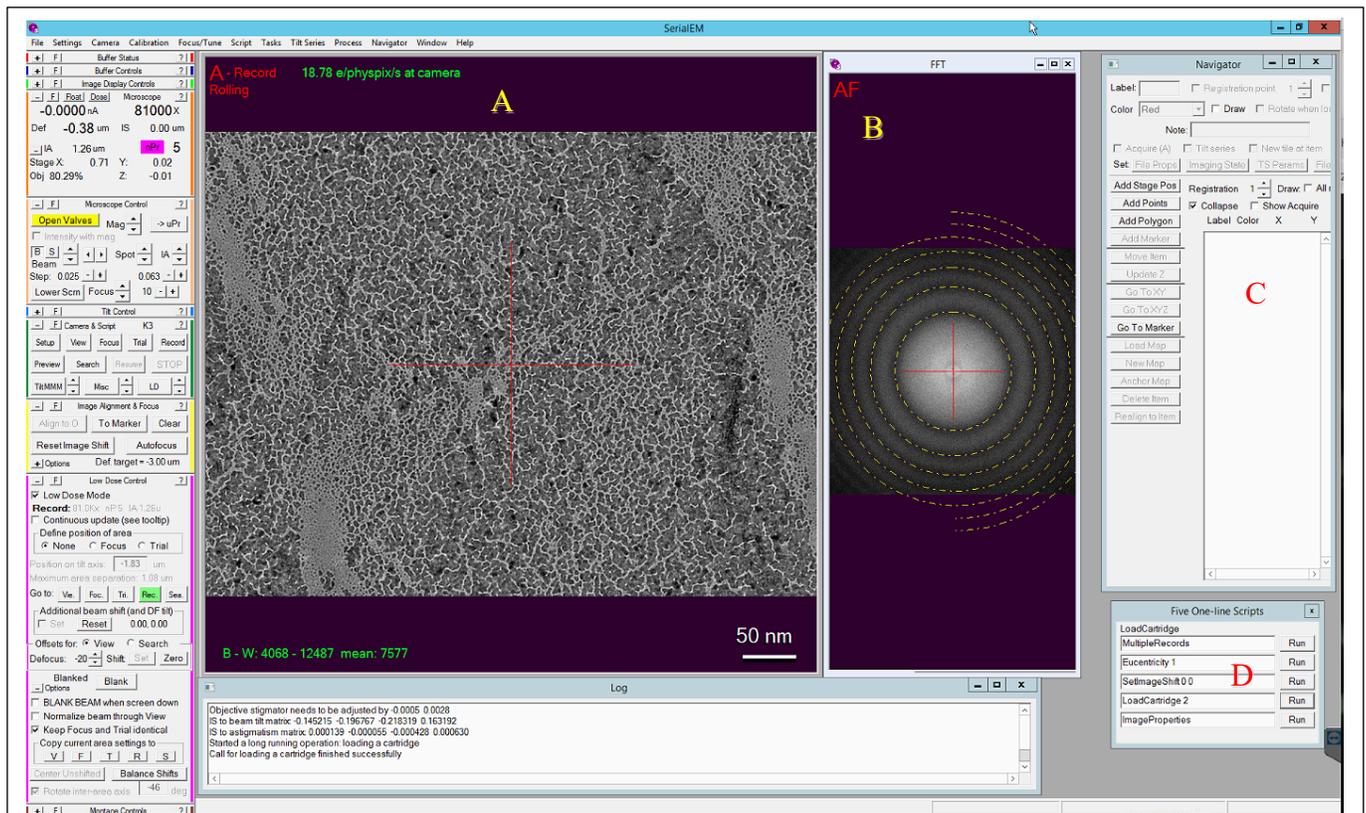


Figure 8.1 – SerialEM User Interface – Left side of the software vertically lists the various control panels (A) image display window (B) FFT window with thin ring based CTF estimation (C) Navigator window contains LMM and gridsquare points. Use the buttons to navigate (D) One-line Scripts window can run macros like eucentric height and grid exchange.

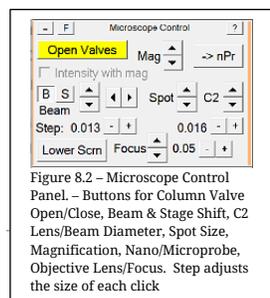


Figure 8.2 – Microscope Control Panel. – Buttons for Column Valve Open/Close, Beam & Stage Shift, C2 Lens/Beam Diameter, Spot Size, Magnification, Nano/Microprobe, Objective Lens/Focus. Step adjusts the size of each click

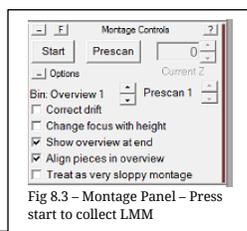
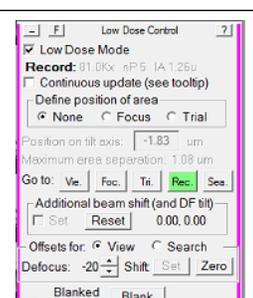


Fig 8.3 – Montage Panel – Press start to collect LMM



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