Common Screening with SerialEM Standard Operating Procedure

version 1.7

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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
  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 150µm 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. Open Navigator window (Menu: Navigator > Open)
  4.3.3. Create and save LMM.mrc (Menu: Navigator > Montaging & Grids > Setup Full Montage)
  4.3.4. (optional) Find eucentric height of central gridsquare. (Menu: Tasks > Eucentricity > Rough Eucentric)
  4.3.5. Start montage collection (Montage Panel: Start)

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4.4. Identify Gridsquares to Screen
- 4.4.1. Identify at least two gridsquares to screen; one thick, one thin
- 4.4.2. Left-click the center of a gridsquare and in the navigator window, press "Add Marker"
  - 4.4.2.1. Repeat for each gridsquare
- 4.4.3. In the navigator window, select a gridsquare'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 gridbar for accuracy.
- 4.5.3. Center the stage over a hole (see 7.2 for procedural information)
- 4.5.4. Adjust Autofocus Position (first gridsquare/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 gridsquare
- 4.5.9. Navigate to next gridsquare 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 gridsquares, 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

7. Functions:

7.1. Eucentric Height – Calculate the Z-Height of the gridsquare 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

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to stage motor backlash.

8. Images:

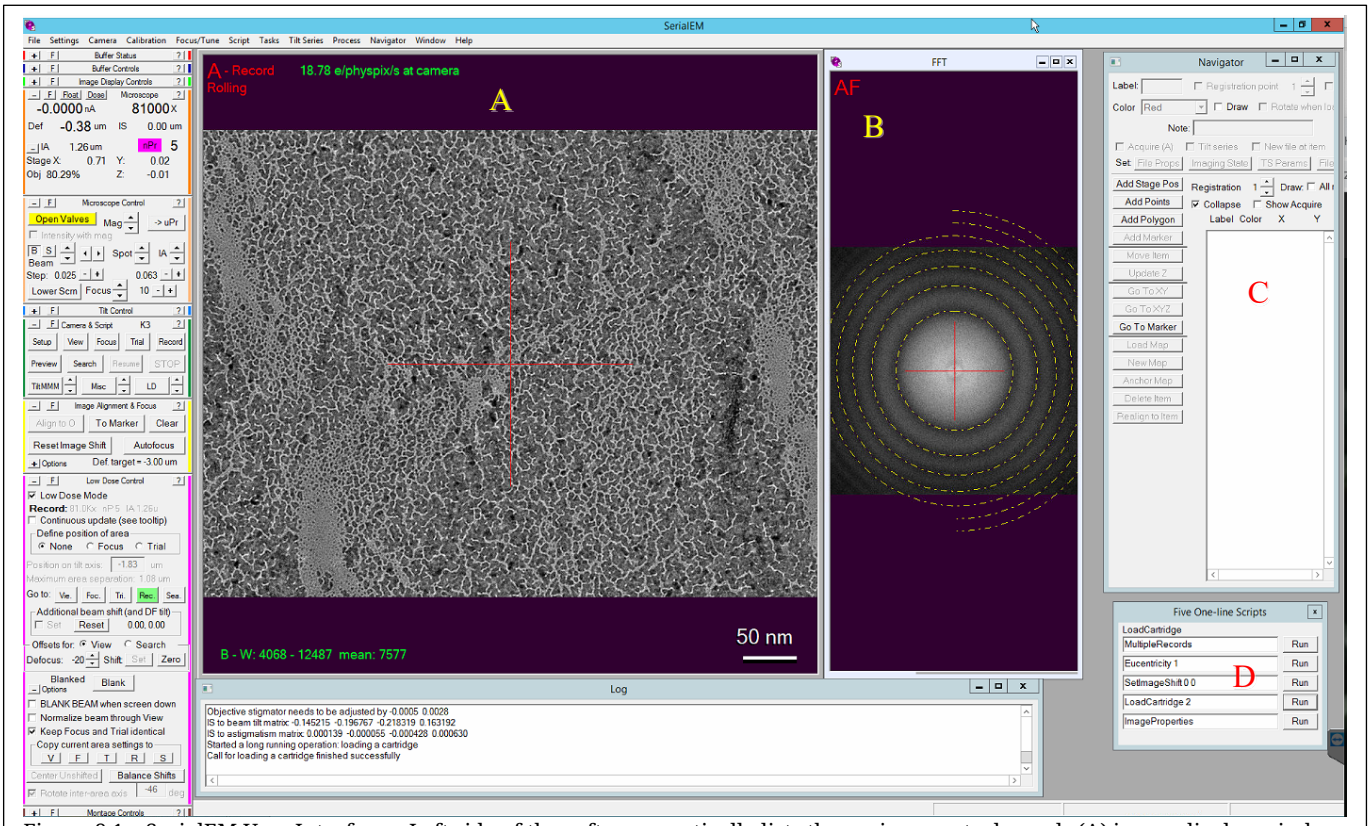

Figure 8.1 – SerialEM User Interface – Left side of the software vertically lists the various control panels (A) image display window (B) FFT window with thon ring based CTF estimation (C) Navigator window contains LMM and gridsquare points. Use the buttons to navigate (D) One-line Scripts window can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

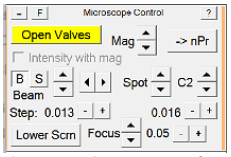

Figure 8.2 – Microscope Control Panel. – Buttons for Column Valve Open/Close, Beam & Stage Shift, C2 Lens/Beam Diameter, Spot Size, Magnification, Nano/Microprobe, Objective Lens/Focus. Step adjusts the size of each click

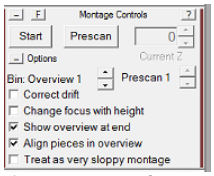

Fig 8.3 – Montage Panel – Press start to collect LMM

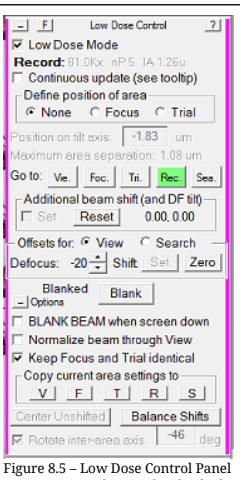

Figure 8.5 – Low Dose Control Panel – Low Dose Mode must be checked in order for the software to link the optic and camera settings for Imaging States in the Camera panel. Define position of area is used to mark the area the software will image shift to autofocus.

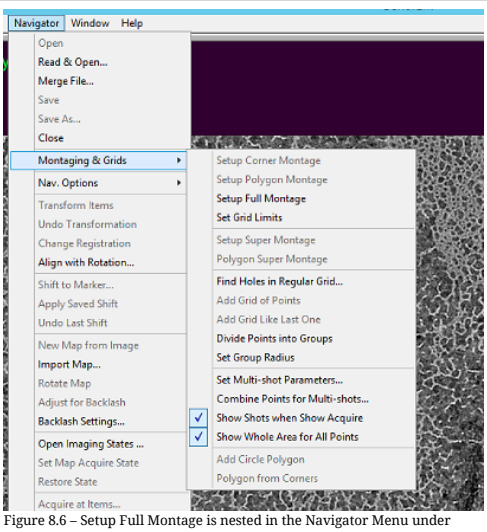

Figure 8.6 – Setup Full Montage is nested in the Navigator Menu under Montaging & Grids.

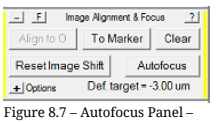

Figure 8.7 – Autofocus Panel – Pressing the autofocus button will image shift to area defined for focus from Low Dose Control. Def. Target is the value the software will focus to for the Record image. To adjust this value, select Set Target in the Focus & Tune Menu.

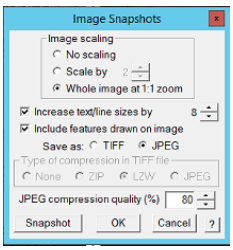

Figure 8.4 – Image Snapshot Window – Image scaling adjusts the binning, Two check boxes are for size of scalebars and markers. Click "Snapshot" to save image.

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