

## Common Screening with SerialEM Standard Operating Procedure

version 0.1

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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 grids are loaded and Autoloader is inventoried.
- 4.1.1.2. Confirm SerialEM server is running
- 4.1.1.3. Confirm 150 $\mu$ 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. Open Navigator (Menu: Navigator > Open)
  - 4.1.2.2.3. Press “Search” in Camera Panel
  - 4.1.2.2.4. Center stage over gridsquare
  - 4.1.2.2.5. Press “View”
  - 4.1.2.2.6. Press “Autofocus”
  - 4.1.2.2.7. Press “Record”

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

#### 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”
- 4.4.4. Once the stage stops moving, acquire a View image
- 4.4.5. Find eucentric height

#### 4.5. Screen Gridsquare and Acquire High Magnification Record Images

- 4.5.1. Acquire View Image
- 4.5.2. Center the stage over a hole
- 4.5.3. Adjust Autofocus Position (First gridsquare/Once per grid)
  - 4.5.3.1. Low Dose Panel: Adjust Focus Position, select Focus
  - 4.5.3.2. View Image: Left-click focus area on support film between holes
  - 4.5.3.3. Low Dose Panel: select None
- 4.5.4. Autofocus Panel: Click “Autofocus”
  - 4.5.4.1. (optional) Adjust Defocus: Menu: Focus & Tune > Set Target (0 to -5.0μm)
- 4.5.5. Camera Panel: click “Record”
- 4.5.6. Save Record Image
  - 4.5.6.1. Menu: Window > Save Image Snapshot
- 4.5.7. Repeat on at least one hole from same gridsquare
- 4.5.8. 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

### 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.
- 7.2. Centering the Stage – the detector is at the center of the crosshairs and you need to move the microscope stage to center the feature of interest. 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. In order to save dose, the software does not automatically acquire an updated image after moving. Acquire a View image by clicking the button in Camera panel to verify the accuracy of your movement. Additional movements are sometimes necessary due to stage motor backlash.



## 8. Images:

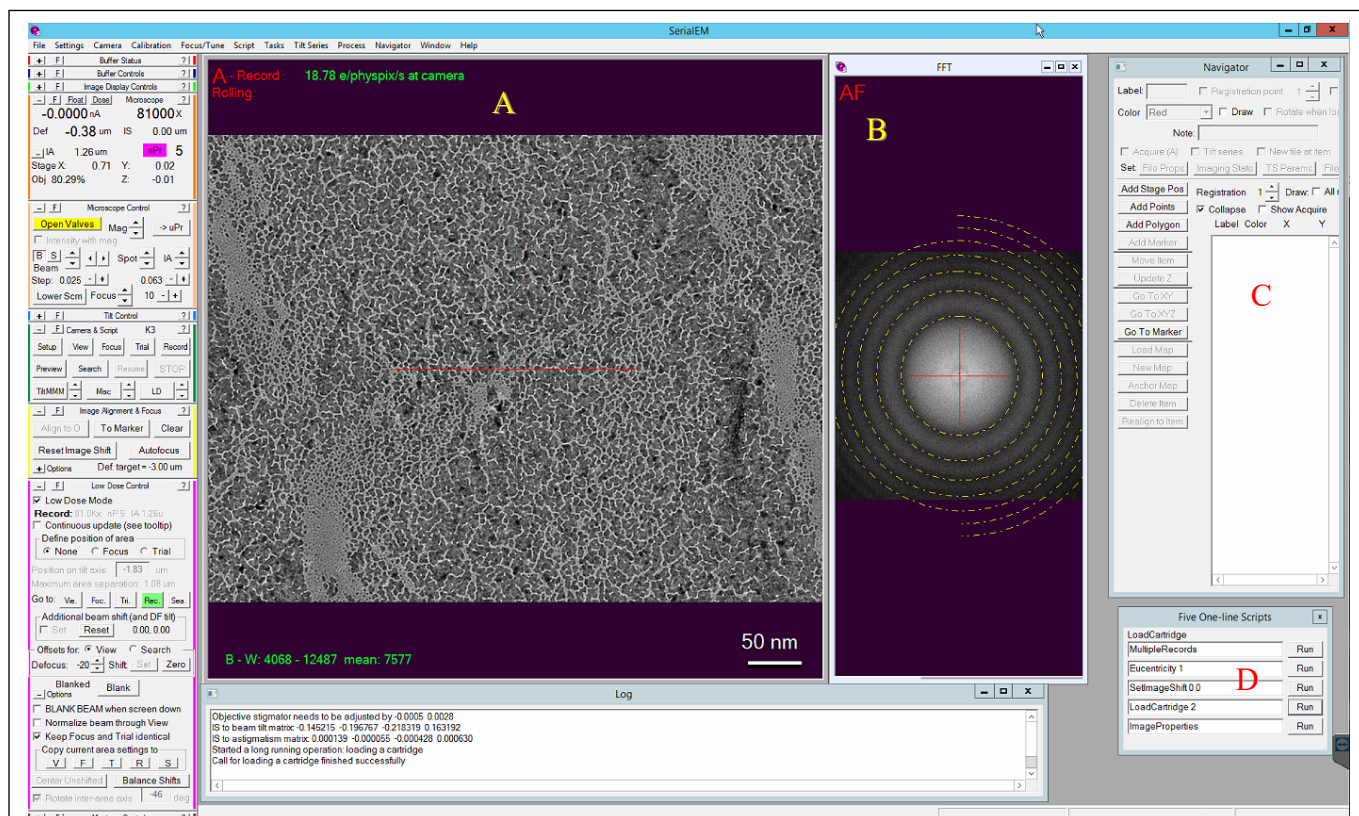


Figure 8.1 – SerialEM User Interface – Left side of the software vertically lists the various control panels (A) image display window (B) FFT window with thin ring based CTF estimation (C) Navigator window contains LMM and gridsquare points. Use the buttons to navigate (D) One-line Scripts window can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

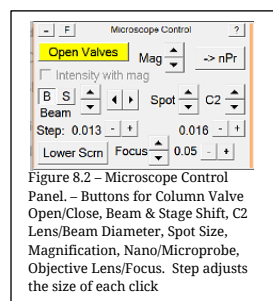


Figure 8.2 – Microscope Control Panel. – Buttons for Column Valve Open/Close, Beam & Stage Shift, C2 Lens/Beam Diameter, Spot Size, Magnification, Nano/Microprobe, Objective Lens/Focus. Step adjusts the size of each click

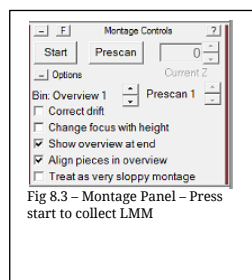


Fig 8.3 – Montage Panel – Press start to collect LMM

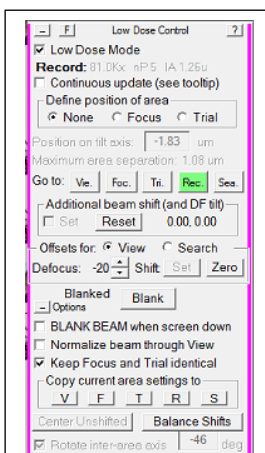


Figure 8.4 – Image Snapshot Window – Image scaling adjusts the binning. Two check boxes are for size of scalebars and markers. Click "Snapshot" to save image.

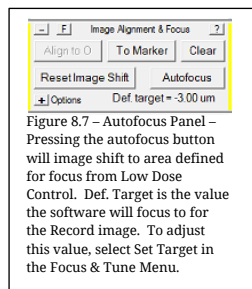


Figure 8.5 – Low Dose Control Panel – Low Dose Mode must be checked in order for the software to link the optic and camera settings for Imaging States in the Camera panel. Define position of area is used to mark the area the software will image shift to autofocus.

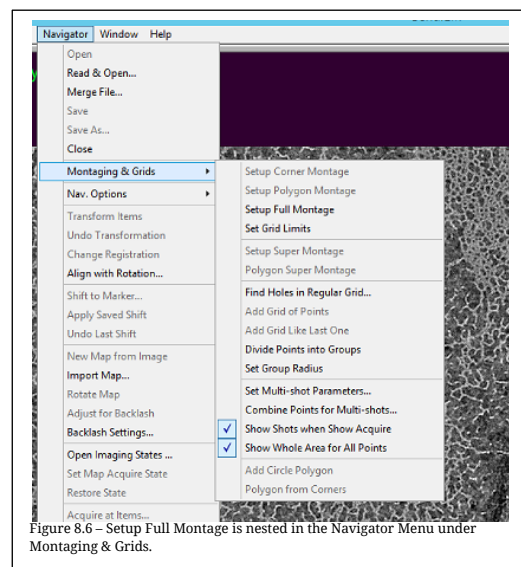


Figure 8.6 – Setup Full Montage is nested in the Navigator Menu under Montaging & Grids.