Common Data Collection with SerialEM Standard Operating Procedure

version 1.0

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1. Purpose

1.1. Automated collection of screened 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
- ❏ TEM UI
- ❏ Cross-Grating Replica Grid
- ❏ Vitrified CryoEM Grids

4. Procedure:

4.1. 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
4.1.2.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 a grid square
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. Perform Direct Alignments on Cross Grating Replica Grid
4.2.1. You should have a grid square centered from step 4.1.2
4.2.2. Run Eucentric Rough
4.2.3. Send Record Imaging State to microscope or Adjust microscope magnification to ~1Å/pixel
4.2.4. Press Eucentric Focus button on hand panel
4.2.5. Insert FluScreen
4.2.6. Open Direct Alignments OCX and perform:
4.2.6.1. Pivot Points X
4.2.6.2. Pivot Points Y
4.2.6.3. C2 Aperture Centering
4.2.6.4. Rotation Center

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4.2.6.5. Beam Center
4.2.6.6. Click “Done”

4.3. Set your Record Beam / Record Imaging State
4.3.1. Navigate to a grid square on the cross grating
4.3.2. Adjust magnification to your desired magnification / Å-pix
4.3.3. Adjust beam diameter
4.3.3.1. Multi-shot: >= 100nm larger than camera FOV
4.3.3.2. 1 shot per hole >100 nm larger than hole
4.3.4. Choose C2 aperture size that your beam is within its parallel range
4.3.4.1. 150µm = 866µm to 11 (Don’t use for data collection)
4.3.4.2. 100µm = 577µm to 10.9
4.3.4.3. 70µm = 404µm to 10.8
4.3.4.4. 50µm = 289µm to 10.8
4.3.5. Adjust Dose rate of Record Imaging state to between 15 and 20 e^-/pixel/second
4.3.5.1. Spot Size (C1 lens strength) will either double or halve dose rate
4.3.5.2. Intensity/Beam Diameter (C2+C3 lens strength) for fine adjustment
4.3.5.3. Choosing a different C2 aperture may help
4.3.5.4. Check and uncheck “Continuous Update” in SerialEM to store optics settings
4.3.6. Acquire a test image by pressing Record and adjust beam / settings if required.
4.3.7. Copy record imaging state to focus imaging state via Low Dose Panel (see fig X)

4.4. Gain Collection
4.4.1. Check the status of the current gain image on file
4.4.1.1. Navigate to a broken grid square and acquire a Record image over vacuum
4.4.1.2. Open Digital Micrograph and click “Capture” to take an image
4.4.1.3. Go to Process > Auto-correlate
4.4.1.3.1. If the correlation image is featureless, current gain image is up-to-date and you may skip ahead to section 4.5; if there are notable features, a new gain image should be collected.
4.4.2. Go to Camera > Collect Gain Reference
4.4.2.1. Double check that “continuous update” is no longer checked in SerialEM
4.4.2.2. Use the TEMUI to insert the 150-micron aperture
4.4.2.3. Adjust dose to the specified value (~1500) with the Spot Size and IA controls in SerialEM, using the flu screen while changing spot size
4.4.2.4. Once target dose is achieved, click “Collect”
4.4.2.5. Adjust dose to new specified value (~15) with the Spot Size and IA controls in SerialEM, using the flu screen while changing spot size
4.4.2.6. Once target dose is achieved, click “Collect”
4.4.2.7. Once procedure is complete, check autocorrelation again (as in 4.4.1.3) to verify gain reference quality

4.5. GIF Alignment and Tuning
4.5.1. GIF Tuning requirements vary based on GIF type. See appendix for more details
4.5.2. Make sure that “Slit In” is selected in the Energy Filter box in Record mode in SerialEM
4.5.3. Collect a Record image
4.5.3.1. If the image is dark or if a dark edge is visible within the field of view, center the slit by going to Digital Micrograph and clicking “Center ZLP”
4.5.3.2. Once procedure is complete, return to SerialEM and click “Clear Adjustment” in the Energy Filter box
4.5.4. Collect another Record image to verify that the slit is not blocking signal

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4.6. Perform final tuning steps on cross grating before loading sample grid
- 4.6.1. Navigate to a grid square and run Rough Eucentric
- 4.6.2. Correct Objective Astigmatism: Focus/Tune menu > Correct Astigmatism by CTF
- 4.6.3. Correct Coma: Focus/Tune menu > Correct Coma by CTF
- 4.6.4. Correct Coma vs. Image Shift: Calibration menu > Focus and Tune > Coma vs. Image Shift

4.7. Load Data Collection Grid
- 4.7.1. You can load a grid from either the Microscope PC or the SerialEM PC
- 4.7.2. MPC TEM UI: Autoloader OCX: Click Slot, Click Load
- 4.7.3. SerialEM: Menu: Script > One-Line Scripts: LoadCartridge # (1-12)

4.8. Collect Low Magnification Montage – Whole Grid Overview
- 4.8.1. Open Navigator window (Menu: Navigator > Open)
- 4.8.2. Create and save LMM.mrc (Menu: Navigator > Montaging & Grids > Setup Full Montage)
- 4.8.3. Find eucentric height of central grid square. (Menu: Tasks > Eucentricity > Eucentric Rough)
- 4.8.4. Start montage collection (Montage Panel: Start)

4.9. Setup Multishot and Focus Position
- 4.9.1. Double-click LMM in Navigator to view it
- 4.9.2. Navigate to grid-square you will not collect on (too thin and/or dry if available)
- 4.9.3. Get Z-height of grid square by running Eucentric-Rough
- 4.9.4. Calibrate View-Record offset
  - 4.9.4.1. Navigate to a spot on the substrate and go into Preview until signs of charging are visible
  - 4.9.4.2. Take a View image; a circular burn mark should be visible from the previous step
  - 4.9.4.3. If View image is not centered over burn mark, Image Shift to the center of the mark
  - 4.9.4.4. Save calibrated shift by selecting the Offset for View radio button and click Shift: Set
- 4.9.5. Center stage over a hole using Stage Shift
- 4.9.6. Set autofocus position via “Define Position of Area” in Low Dose Panel (see figure x)
  - 4.9.6.1. Click “Focus” radio button
  - 4.9.6.2. Click appropriate focus position on the substrate next to the hole
  - 4.9.6.3. Click None when complete
- 4.9.7. Note current defocus value in View and adjust to -10µm via Low Dose Panel
- 4.9.8. Acquire View image and re-center stage over hole
- 4.9.9. Use “Add Points” to place 4 points in the corners of the multishot pattern
- 4.9.10. Navigator menu > Montaging & Grids > Set Multishot Parameters:
  - 4.9.10.1. Click “For Corners of Multishot Pattern”
  - 4.9.10.2. Verify first point (of 4) is selected in Navigator
  - 4.9.10.3. Click “IS to Nav Pt”
  - 4.9.10.4. Click “Save Image Shift”
  - 4.9.10.5. Repeat for each corner (3 more times), the software will automatically advance to next point
  - 4.9.10.6. Click the center of your pattern and verify the locations of your multishot pattern
  - 4.9.10.7. Adjust any of the points and rerun procedure (4.7.9.1 to 4.7.9.5) if they’re inaccurate
- 4.9.11. Adjust Multishot Parameters (see appendix 7.2)
- 4.9.12. Adjust View Defocus back to original defocus value
- 4.9.13. Test multishot pattern:
  - 4.9.13.1. Center a different hole
  - 4.9.13.2. Run Autofocus
  - 4.9.13.3. In One-line Scripts: MultipleRecord

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4.9.13.4. If the pattern is off, you most likely need to adjust Record-View offset

4.10. Prepare and Acquire Medium Magnification Montages (MMM)

4.10.1. Double-click the LMM in Navigator window to view it
4.10.2. Identify the area of your grid you successfully screened in / would like to start
4.10.3. Use "Add Points" in Navigator window and click the center of all the grid-squares you would like to collect on.
4.10.4. Select the group of points and assign "Acquire" tag via checkbox in Navigator window
4.10.4.1. The points will all have an A next to them
4.10.5. Zoom into the largest selected grid-square and draw polygon around it
4.10.5.1. Select the largest grid-square because this defines the size of the MMM
4.10.5.2. In Navigator window, press "Add Polygon" button
4.10.5.3. Left-click the corners of grid-square, use right click to adjust last laid point
4.10.5.4. In Navigator window, click "Stop" button (was Add Polygon) to close the polygon
4.10.6. With polygon selected in Navigator window: Navigator menu > Montaging & Grids > Setup Polygon Montage
4.10.6.1. Check "Use View parameters in Low Dose Mode"
4.10.6.2. Check "Always ask"
4.10.6.3. Uncheck "Move stage …."
4.10.6.4. Click ok
4.10.6.5. For file save settings, make sure you save an MRC
4.10.6.6. Click ok
4.10.6.7. Navigate to correct path to save file MMM.mrc
4.10.7. Acquire the Medium Magnification Montages (see fig X)
4.10.7.1. Navigator menu > Acquire at Items
4.10.7.2. Click Mapping button
4.10.7.3. Primary Task: Acquire map or montage
4.10.7.4. Check "Make Navigator Map"
4.10.7.5. Tasks before or after primary: Check Rough-Eucentric
4.10.7.6. Click "Go" and wait for procedure to complete

4.11. Add data collection targets to Medium Mag Montages

4.11.1. Open Hole Finder: Navigator Menu > Montaging & Grids > Find Holes in Regular Grid
4.11.2. Open Multishot Combiner: Navigator Menu > Montaging & Grids > Combine Points for Multishot
4.11.3. Double-click an MMM to view it
4.11.4. Input Hole Size and Periodicity (hole spacing) in Hole Finder
4.11.5. Click "Find Holes"
4.11.6. Adjust the sliders so that all holes you would like to collect are pink
4.11.7. Click "Make Navigator Points"
4.11.8. Click Combine Points in Multiple Hole Combiner
4.11.9. Manually adjust point selection as needed
4.11.9.1. Check "Edit Mode" box in Navigator window
4.11.9.2. Uncheck "Collapse"
4.11.9.3. Select any points that are next to the grid bar by clicking on them and click "Delete" in the navigator window to remove them
4.11.9.4. Select and delete any points that are over contamination, over broken areas, or in otherwise undesirable areas of the grid
4.11.9.5. If there are areas which are desirable for collection which were not automatically detected by the Hole Finder, points may be manually added by clicking "Add Points" in the navigator window and clicking in the center of a hole

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4.11.9.5.1. Note that this will automatically collect in your multishot pattern; the points you select will be the center points in the pattern

4.11.10. Make sure "Collapse" is checked after manual adjustments and drag group of points under the corresponding map

4.11.11. Repeat steps 4.9.4 to 4.9.10 for every MMM you want to collect on

4.11.12. Make sure to uncheck "Edit Mode" in the navigator window when done

4.12. Adjust the defocus range in LD script (Appendix 7.3)

4.12.1. Open LD script: Script menu > Edit > Edit 1

4.12.1.1. Use arrows to find LD script (usually 4)

4.12.1.2. Adjust the defocus range settings (in µm):

4.12.1.2.1. TD_high = high end of acceptable defocus targets

4.12.1.2.2. TD_low = low end of acceptable defocus targets

4.12.1.2.3. TD_inc = size of increment the script will adjust current defocus by

4.12.1.3. Press "OK" to save and close script

4.13. Start Automated Data Collection

4.13.1. Adjust Camera Properties for Record

4.13.1.1. In SerialEM, click "Setup" button in Camera & Script (Green)

4.13.1.2. Click Record radio button to see Record Imaging State camera settings

4.13.1.3. Adjust exposure time to reach between 50 and 60 $e^-/Å^2$ total dose

4.13.1.3.1. Use equation found in Appendix (section number)

4.13.1.4. Divide your exposure time by the number of frames you want to save and enter into Frame Time. Make sure Dose Fractionation is checked.

4.13.1.5. Check "Save Frames" and set the folder to the appropriate directory

4.13.1.6. Verify "Align Frames" is unchecked

4.13.1.7. Press OK

4.13.2. Open Acquire at Items to set collection parameters (Navigator Menu > Acquire at Items):

4.13.2.1. Click "Final Data" button

4.13.2.2. Primary Task: Run script LD

4.13.2.3. Task related options: check "Skip stage move to item if possible"

4.14. Shutdown

4.14.1. After data collection is complete,

4.14.2. Uncheck Save Frames

4.14.3. Close Navigator Window

4.14.4. Close all open maps with Menu: File > Close

4.14.5. Close column valves and load grid 1, the cross grating.

5. Chemicals: N/A

6. Waste Disposal: N/A

7. Appendix:

7.1. For detailed instructions on tuning a Gatan Imaging Filter, see video linked in the Center Specific section of the Data Collection Merit Badge website.

7.2. For detailed instructions on correcting the Record to View Offset, see video linked in the Center

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Specific section of the Data Collection Merit Badge website.

7.3. The PNCC LD Script can be found in the Center Specific section of the Data Collection Merit Badge website.

7.4. Glossary

7.4.1. 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".

7.4.2. 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:

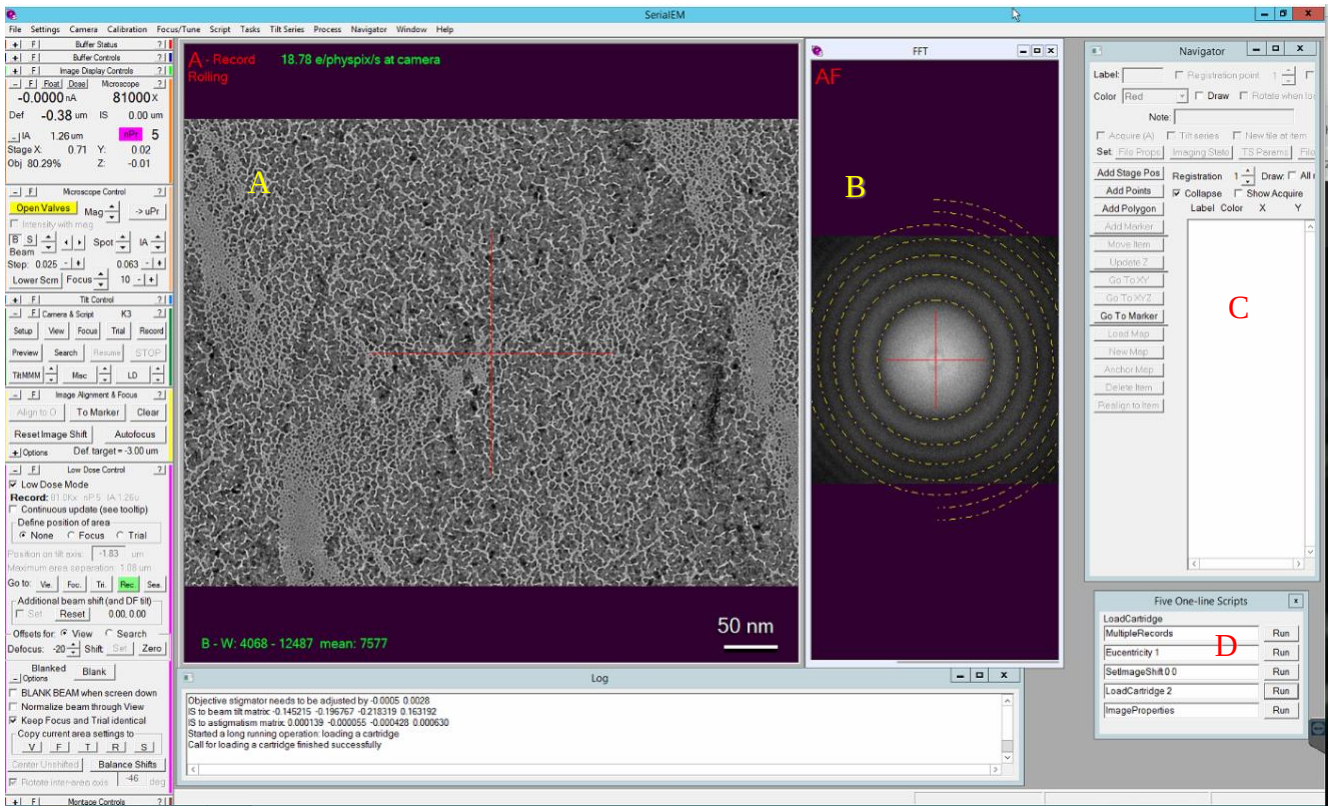

Figure 8.1 – SerialEM User Interface – Left side of the software vertically lists the various control panels (A) image display window (B) FFT window with thon ring based CTF estimation (C) Navigator window contains LMM and gridsquare points. Use the buttons to navigate (D) One-line Scripts window can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

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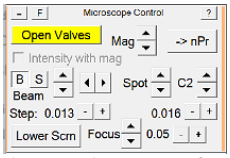

Figure 8.2 – Microscope Control Panel. – Buttons for Column Valve Open/Close, Beam & Stage Shift, C2 Lens/Beam Diameter, Spot Size, Magnification, Nano/Microprobe, Objective Lens/Focus. Step adjusts the size of each click

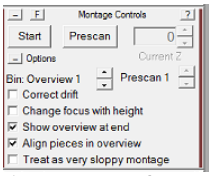

Fig 8.3 – Montage Panel – Press start to collect LMM

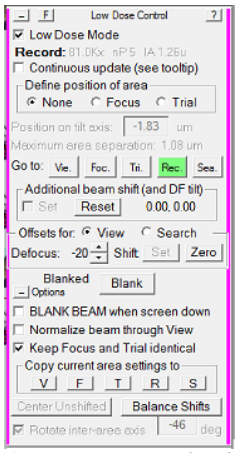

Figure 8.5 – Low Dose Control Panel – Low Dose Mode must be checked in order for the software to link the optic and camera settings for Imaging States in the Camera panel. Define position of area is used to mark the area the software will image shift to autofocus.

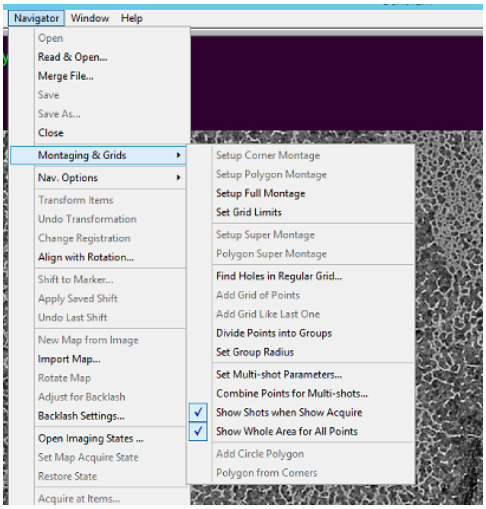

Figure 8.6 – Setup Full Montage is nested in the Navigator Menu under Montaging & Grids.

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