Before starting check microscope settings in SerialEM

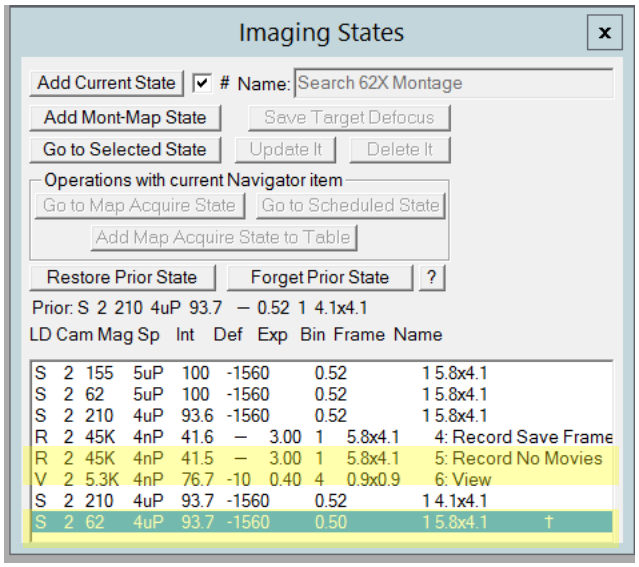
1. Load Settings file **20250725_MultiGrid_SPA_45K**.

2. Align the microscope, **complete direct alignments**, and objective lens corrections (astigmatism, comma free alignment and comma vs image shift calibrations in SerialEM).

3. Check the View, Focus, Record, and Search parameters. Set the position of Focus on the tilt axis over the carbon and make the beam very small so it does not illuminate the Quantitfoil hole.

4. After completing comma vs IS in low-dose mode **check offsets for View and Search**.

5. **Check each of the imaging states used for Multiple Grid Operations.** Make sure the beam conditions and camera parameters are correct. If you notice that the beam in 62X and 210X is off center reset "Additional Beam Shift" in the low-dose panel.



Imaging States Used in Multiple Grid Operation.

Record Save Frames to take data by saving movies

Record No Movies to take micrographs no movies saved

Search 210 MMM to take Medium Mag Maps

Search 62 Montage to take low mag full grid montages

Highlighted in yellow

6. If you need to update the image states close Multiple Grid Operation window.

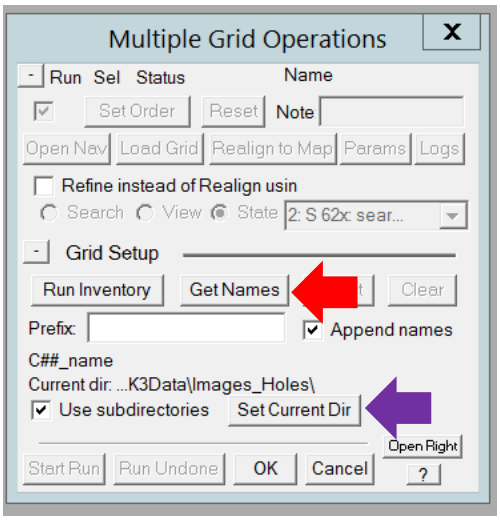
7. At this point the microscope has been aligned, the serialEM setting file loaded and updated, all imaging states are updated with the beam centered.

Document created by Dr. Joshua Strauss

Collect Low Mag Full Grid Montages

1. **Clear or end the previous Multi-grid session.**
   a. SerialEM Navigator→Montaging & Grids → Clear Multi-grid session.
   b. On the Multiple Grid Operations Window click “End run” if it is still running.

2. **Open Multiple Grid Operation window.**
   a. SerialEM Navigator→Montaging & Grids → Multiple grid operation.

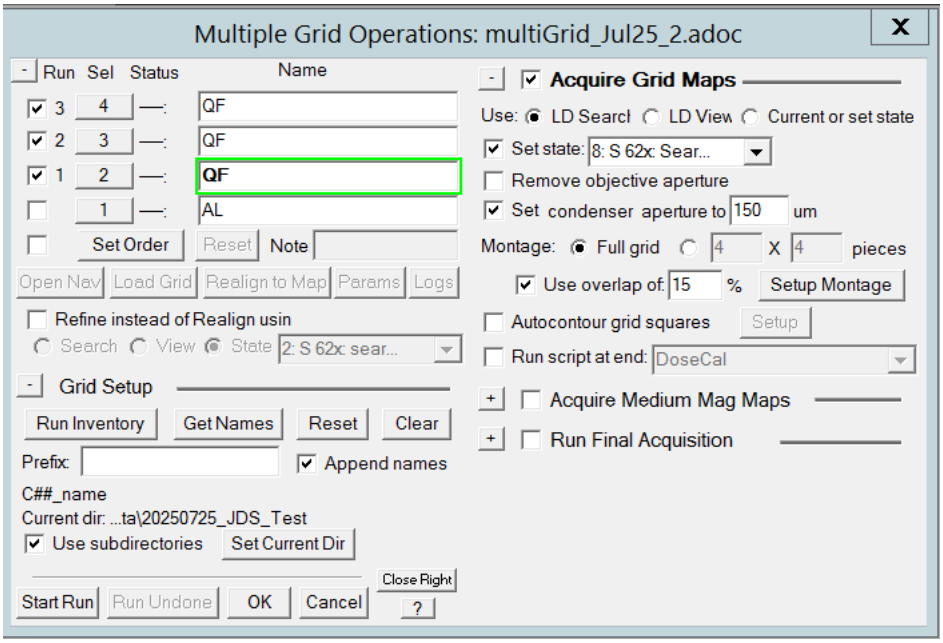


3. **Set Current Directory** to X:\K3Data\date_initials (Purple arrow). Here I will set the directory to 20250725_JDS_Test.

4. On the microscope computer in the Autoloader tab **edit slot state** and **Run Inventory.** Make sure to enter the names of each of the cartridges that will be displayed on the SerialEM Multiple Grid Operation window. The names can be edited or changed if you like.

5. On the Multiple Grid Operation Window click **Get Names** (Red Arrow).

6. Check **Acquire Grid Maps** as show in image.
   a. Use LD Search
   b. Do not check Auto contour grid squares.
   c. Do no check Remove objective aperture
   d. Set condenser aperture to 150 um.
   e. Check Full grid or specify number of pieces in X and Y if you want to image a smaller LM montage.



7. **Check cartridges that you want to montage**. Click "Set Order" to change the ordering of the grids that are to be imaged. As shown in the above image cartridges 4, 3 and 2 will be collected.

8. Click **Start Run** it will take ~ 15 mins to acquire a full grid montage of each grid.

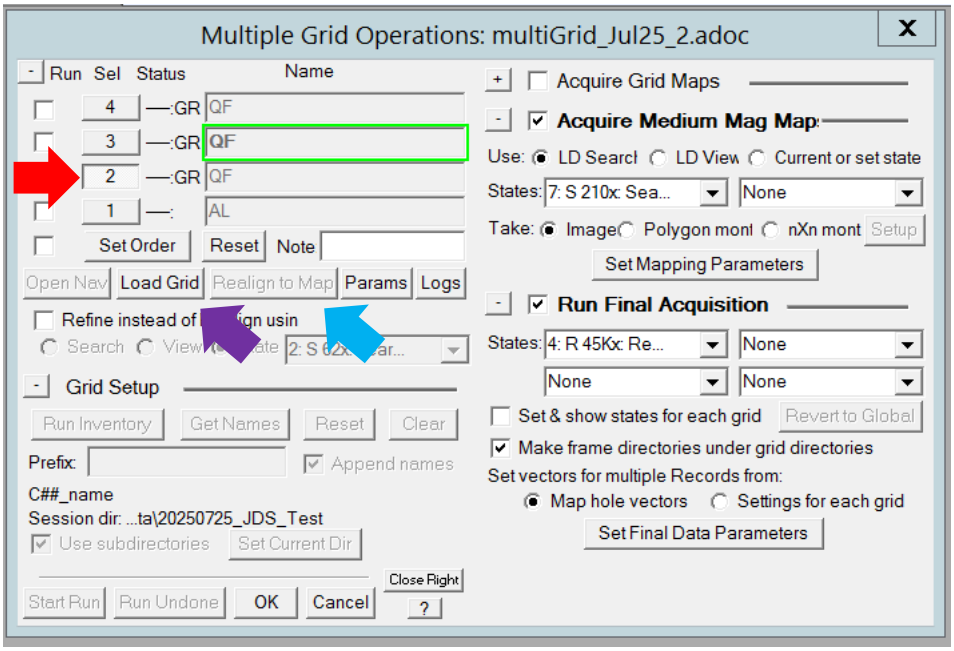
9. **Click Ok on pop-up**. Don't need to change anything just make sure Parameters to use in Low Dose Mode are set to Search.

10. **Watch the first one to make sure there are no errors**. If that happens stop the run to fix the error and repeat.

11. SerialEM (or Dummy version on microscope computer or gaming PC) open each montage. **Add points to grid squares** with thin ice that you want to image. Then save each of the navigator files. You can do this as the montages are collecting or after all of the grids have been montaged.

Collect Medium Mag Montages and Data using BIS Method

1. In SerialEM **open that navigator that you want to collect data on**.
   a. Click on the grid (as shown below for #2 – red arrow)
   b. Open Nav to open navigator (or use Navigator tab – grey in image)
   c. Load grid onto the stage shown by purple arrow.

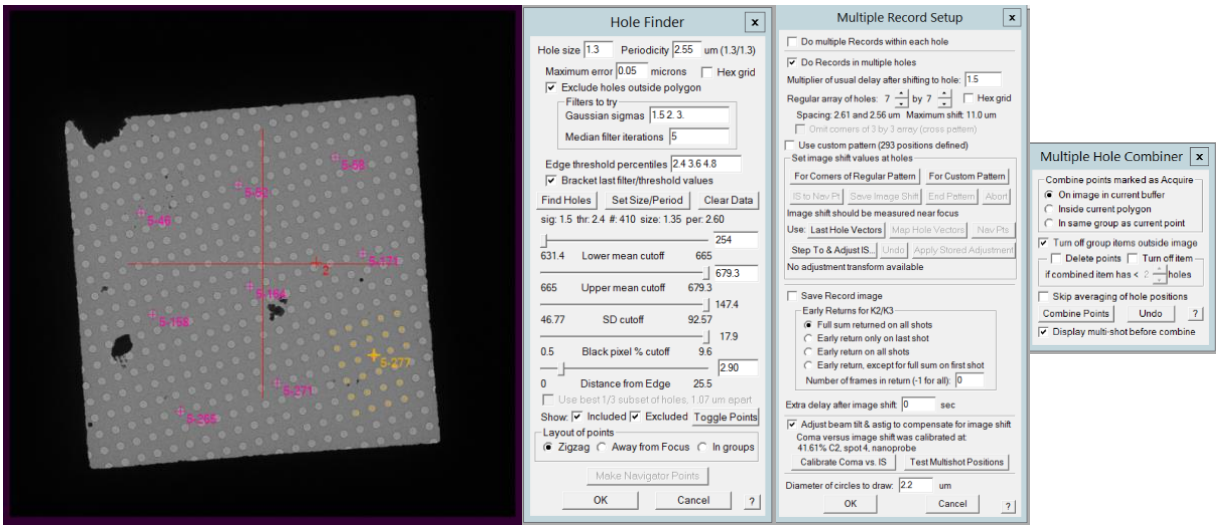


2. Run **Realign to Map** on the Multiple Grid Operation window (greyed out – blue arrow). Before running realign to make take an image with the 62X imaging state to make sure the beam is centered before doing this.

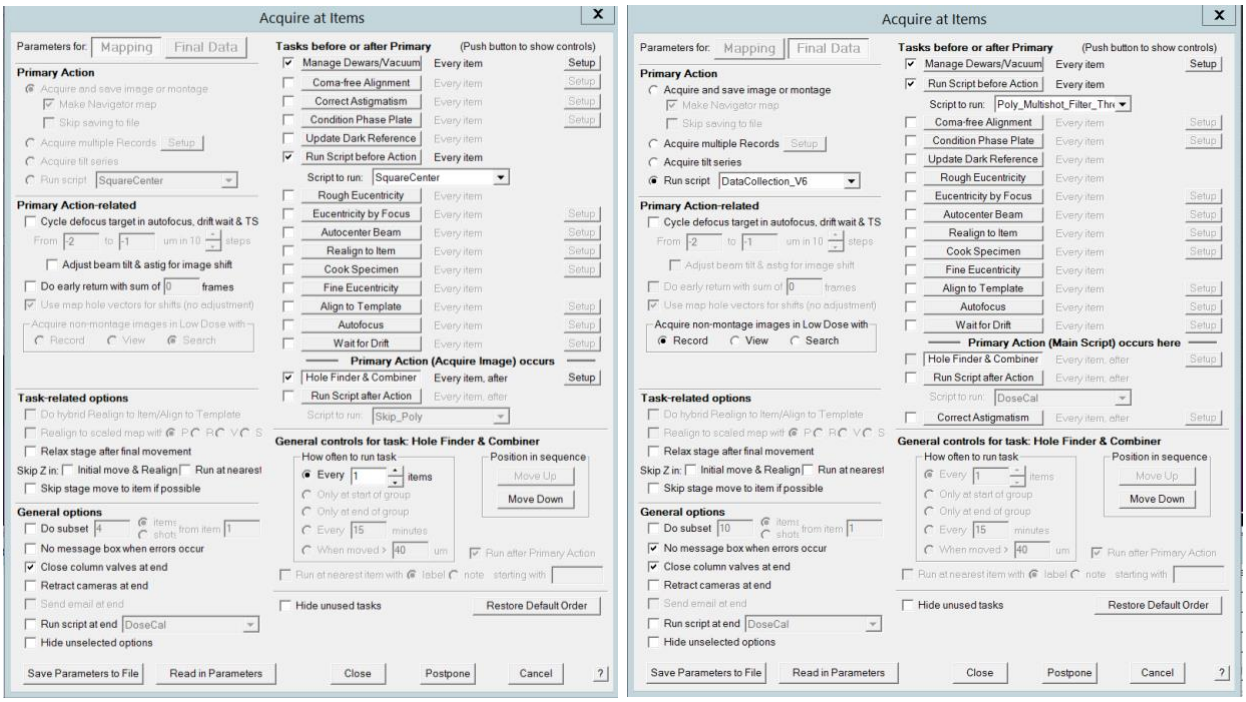
3. After completing Realign to **Map double check**. Pick point on the Navigator and go to XYZ on to move the state to that point. Then take a search 210X image to verify it worked.

4. Take Search image of that grid square, center it in the main window and save the **Search Image to buffer M and N.**

5. Use this Search image to **test the hole finder** and **combine multishot**. Adjust Hole Finder Hole size, Periodicity, and sliders. It should look like the image below if done correctly.



6. On Multiple Grid Operation Window click on Set **Mapping Parameters**, and **Set Final Data Parameters to check settings.**



Check the DataCollection_V6 script and modify if you like. *Normally you don't need to do this. Do not change any of the other scripts if you are taking a 7x7 multishot or tilting the stage.*

12. Before running **take an image of a hole and copy to buffer P**. Reduce image by factor of 3. Or load an image of a grid hole., read, and copy to buffer P. Images are located in the folder "Images_Holes" under K3Data.

13. On the Multiple Grid Operations Window. Check marker on the grid and click **"Start Run".**

14. Popup – no is fine for apply marker shift – I did not see any difference for this.

15. **Watch microscope** and if there is an error stop the run by clicking on Camera & Script panel "Stop" and "PauseN". Resume the run after you fix error.