

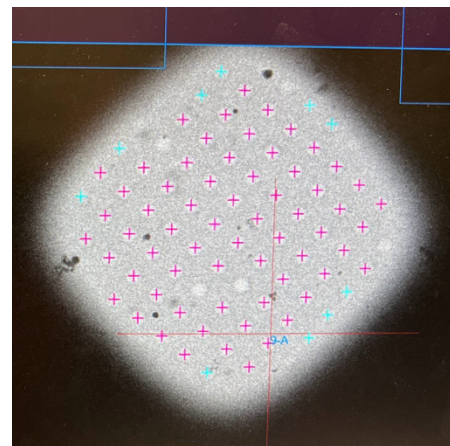
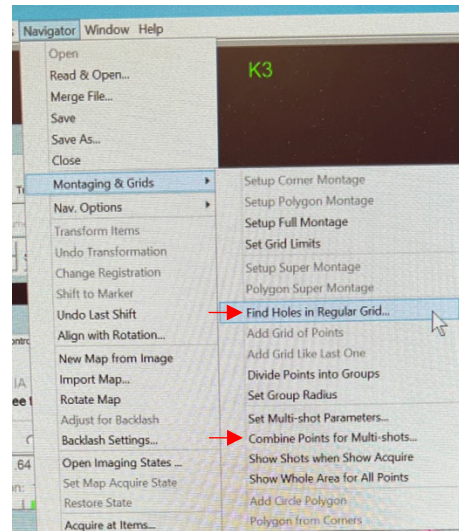
Using Hole Finder in SerialEM to set up cryo-EM data collection

References:

https://bio3d.colorado.edu/SerialEM/hlp/html/hidd_hole_finder.htm

https://bio3d.colorado.edu/SerialEM/hlp/html/hidd_multi_combiner.htm

1. Set up LMM, MMM and low dose mode as usual. Using a slightly longer exposure time for MMMs may help the Hole Finder results.
2. On an MMM image ready for target selection, open the **Hole Finder** window (*Navigator -> Montaging & Grids -> Find Holes in Regular Grid...*) and the **Multiple Hole Combiner** window (*Navigator -> Montaging & Grids -> Combine Points for Multi-shots...*).
3. In **Hole Finder**, fill in the geometry of your grid (for example, Hole size 1.2 and Periodicity 2.5 for R1.2/1.3 grids) at the top of the window (see the screenshot on the next page). Leave the *Maximum error*, *Filters to try* and *Edge threshold percentiles* values as default. One may change these values to get better results, but this needs some exploration. Refer to the above links for details.
4. Click **Find Holes**. The program will calculate the positions of holes based on the geometry of the grids you input and the filters above. If it works OK for the first MMM image (most of the holes are marked with magenta or blue/cyan crosses), click **Set Size/Period** to update the grid geometry with the calculated/measured values (one only needs to do this once).
5. Scroll the four sliders to make desired holes marked with **magenta** crosses. These will be the included targets, whereas **blue** and **cyan** ones will be excluded. Refer to the first link for details about the sliders.



6. Once the hole selection is done, click **Make Navigator Points**. This will add the magenta holes as Navigator Points in the **Navigator** window and mark them as **Acquire** targets. As lots of points will be added in **Navigator**, it is a good idea to check **Collapse** in the **Navigator** window now.

7. Click **Combine Points** in the **Multiple Hole Combiner** window. This will combine the points according to the pattern one defined before (for example, the 3 by 3 pattern for data collection using beam-image shift). Checking **Show Acquire** in the **Navigator** window (*Show Shots when Show Acquire and Show Whole Area for All Points* in the *Navigator -> Montaging & Grids* menu should also be checked) will show the

areas that will be collected on, with a full 3x3 target marked with a big square and separate polygons around individual holes if it's not a complete 3x3 target.

8. Leave both windows open and move on to the next MMM image.

9. **Hole Finder** should work fine in Dummy SerialEM as well. To use the **Multiple Hole Combiner** in Dummy, one needs to save the settings in the regular SerialEM session first and read in the setting file in Dummy. This can be done through *Settings -> Save* or *Save as*. Make sure not to overwrite an existing file.

10. Generally, **Hole Finder** works better on a square with thin ice. Sometimes it will not work properly on a thick square. One can first try to set it up on a relatively thin area and then try it on the thick squares.

