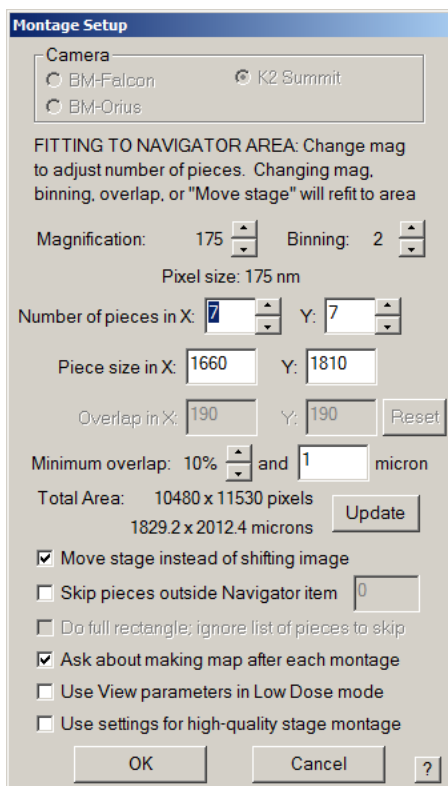


SOP: SPA data collection using SerialEM (Update in Progress)

- Open SerialEM server
- Open SerialEM
- Load settings

Creating a Low Magnification Montage

- Open **Navigator** menu and load **“Imaging States”**
- Double click on “LMM” mode (LM 145X, spot size 9, C2 100%)
- Check in TIA FluScreen viewer if the setting is ok (Obj. aperture is removed and C2 aperture is 150 um)
- Open **Camera** -> **Parameters**-> **Record**, make sure K3 is selected, **Record** is in Counting mode, mag is 145X, exposure time 1 s, Bin 2, no dose fractionations. Click **OK**
- Open **Navigator** menu, click **Montaging & Grids** and **Setup Full Montage**
- In **Montage Setup**, use “7X11” tiles, 20% overlap and make sure “Move stage instead of shifting image” and “Ask about making map after each montage” are selected



- Click **OK** and save .st file with mag and exposure dose. Save files as Atlas.mrc
- To start the montage, go to **“Montage Controls”** and click on **Start**
- Click “Yes” to make final overview of montage into a map

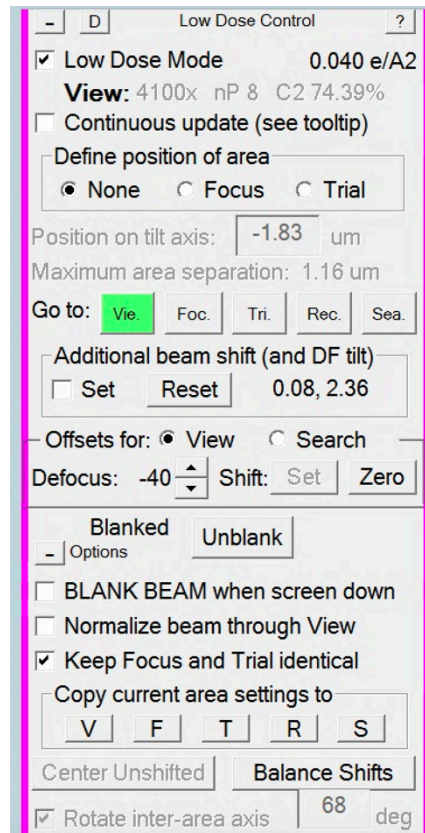
- Close the montage file
- Alternatively, you can run “Cars” and “LMMCars” scripts to create grid maps for all the grids

Image Shift:

- Double click on “**MMM**” mode (LM ~600X, spot size 7, C2 aperture 50 um, C2 lens is 50-70%)
- Identify a bad grid square with a feature, add a point on the feature and **go to XY**
- Open **Tasks** menu -> **Eucentric Rough**
- Put the screen down to find the feature
- Take a record image in “**MMM**” mode, and add a marker
- Open **Navigator** -> **Shift to marker**
- This process can be repeated until LMM and MMM are well correlated
- Same with MMM and **View**
- **If the shift is not correct, you can always undo this by clicking Navigator -> Undo last shift.**

Set up low dose imaging:

- Turn on low dose mode and move the stage to a broken square or a square can be sacrificed
- Make sure you are eucentric and in focus
- Go to **View**, check continuous updates. Open DM, adjust magnification so that you can find the samples. Adjust spot size and intensity so the dose rate of K3 is between 15-25.
- Open **Camera** -> **Parameters**, for **View** mode: 4100X, 0.5 s, bin by 2, full readout
- Uncheck continuous updates
- Go to **Search**, same optics as in the **MMM** setting
- Go to **Record**, check continuous updates, adjust optics for data collection. Make sure beam is in parallel illumination (C2 value). Measure dose.
- Calibrate dose in SerialEM (1 s exposure and bin 1), and set up exposure time in Record
- Open **Camera** -> **Parameters**, for **Record** mode: 100,000X, 2+ s, bin 1X (counting), full readout, dose fractionation on
- Copy **Record** settings to **Trial** and **Focus**.
- **Trial**: 100,000X, 0.2 s, bin by 4, full readout
- **Focus**: 100,000X, 0.2 s, bin by 4, wide half
- Not over vacuum, take a **View** and set the “define position” of area for **Focus** and **Trial**
- Take a **Preview/Record**, then **Trial/Focus** to verify the position, then **View**.



Make a Medium Mag Image (Grid Square Image):

- Add a point in the center of desirable squares (add all the good squares and then stop adding)
- Click the first added point and click “**Collapse**”
- **Navigator -> Acquire (A) and New file at group**
- Select “Single frame Image”
- Click **OK**
- **Navigator -> Acquire at items**, select “**Mapping**”, check **Rough Eucentricity**
- Click **Go** and start collecting
- Save the **Navigator** file

Fix the register between View and Record:

- Center feature in **Preview** image
- Take **View** image
- Check continuous update
- Uncheck “move stage for big mouse shifts” in the Image alignment and focus control panel.
- Right click and drag to center feature
- Under offsets for view click “Set”
- Take preview to ensure feature is centered, repeat if necessary

Set up multiple record:

- Take a **View** image.
- (9 holes) Add a nav point in the center of a group of 9 holes (for best results, the edge of the hole can be used for exact template centering) and go to it
- Make sure record beam is at -1-2 microns defocus. Go to 0 defocus offset with the view beam (usually -20 microns is used for View)
- Uncheck the “move stage for big mouse shifts” box, make sure image shift is zeroed and right click and drag to add points to the neighboring holes. Either four corners of your pattern, or all points in the pattern i.e. “for corners of a regular pattern” or “Custom pattern”
- Click **Navigator-> Montaging and grids-> Set multi-shot parameters**
- Select “**Do multiple Records within each hole**”
- Number of shots in main ring around center: **4** (depends on hold geometry and pixel size)
- Select **Do records in multiple holes**
- Select **Use record illuminated area for circles in low dose**
- Select regular pattern of holes, 3 X 3
- Select the first of the nine nav points in the **Navigator** window
- Click for corners of a regular pattern (for all 9 holes) button in the multishot setup menu
- Make sure you click **IS to NAV Pt** and the second point will turn yellow
- Take a **View** shot and center the hole by right clicking and dragging to image shift. Then retake the **View** to ensure that the hole is centered
- Press **Save Image Shift**
- Press the **IS to Nav Pt** button in this dialog, or select the next added point in the Navigator and press **Go To IS** there.
- Note* To improve accuracy of the image shift, one can go through a second round of template setup, centering template on the edge of the holes, taking a preview image after shifting the view beam to ensure that the edge of the hole is in view. If additional image shift needs to be added, add it here before saving IS. This has not been necessary for single shots/hole.
- Take a **View** and shift it to the same position as for the first point.
- Press **Save Image Shift**
- Repeat the last three steps for each other point.
- Click **OK** to exit the setup window
- Return view defocus offset to -20 (or whatever is desired).
- Save NAV.
- Recheck “Move stage for big mouse shifts”

Finding points for data collection:

- Double click a grid square map
- Under **Navigator -> Montaging & Grids -> Find Holes in Regular Grids**
- Select **Add Points**. Left click on the precise center of 5 holes, starting at a corner, with one in each direction immediately adjacent to the corner and one further down the line of holes in each direction. This establishes the geometry of the holes.

```
X 0 0 0 0
0 0 0 0 0
0 0 0 0 0
0 0 0 0 0
X 0 0 0 0
X X 0 0 X
```

- Go to **Navigator** -> **Montaging & Grids** -> **Add Grid of Points**. Enter the number of the polygon (this can be found on the overview map image or the Navigator window)
- For the next prompt, select **Away from Focus Area** to select points away from the Focus area to prevent beam damage on exposure areas prior to imaging. For the last prompt, select **Yes** to turn on **Acquire** for every point
- A grid of points will appear on the square overview, which should be centered well over all of the holes within the polygon. If this is not the case, select the points in the **Navigator** window and select **Delete Item** to remove them. Then, readjust the centering of the 5 points used for the geometry of the holes and repeat the step **Add Grid of Points**
- Repeat this procedure for each of the good square overviews
- You can also add points one by one
- Check **Acquire** in the **Navigator**

Setting the template:

- Select the best-looking hole
- Run **Eucentric rough**
- Take a **View** image with the hole in the center
- **Copy Active Image to Buffer P**

Beam alignment, Coma and Stigmatism:

- **Rough Eucentric**
- Under **Focus and Tune**
- Set defocus target to 0
- Start **Autofocus**, review **FFT**, should be at zero. If not, charge focus knob to make it to zero
- Perform direct alignment on the microscope on carbon
- **Autostigmatate by CTF**
- **AutoComa by CTF**
- **Coma vs ImageShift**

Starting data collection:

- Set up **Multiple Record** if needed:
- Take a **View** image.
- (9 holes) Add a nav point in the center of a group of 9 holes (for best results, the edge of the hole can be used for exact template centering) and go to it

- Make sure record beam is at -1-2 microns defocus. Go to 0 defocus offset with the view beam (usually -20 microns is used for View)
- Uncheck the “move stage for big mouse shifts box”, make sure image shift is zeroed and right click and drag to add points to the neighboring holes. Either four corners of your pattern, or all points in the pattern i.e. “for corners of a regular pattern” or “Custom pattern”.
- Click **Navigator-> Montaging and grids-> Set multi-shot parameters**
- Select **Do records in multiple holes**
- Select **Use record illuminated area for circles in low dose**
- Select regular pattern of holes, 3X3
- Select the first of the nine nav points in the **Navigator** window
- Click for corners of a regular pattern (for all 9 holes) button in the multishot setup menu
- Make sure you click **IS to NAV Pt** and the second point will turn yellow
- Take a **View** shot and center the hole by right clicking and dragging to image shift. Then retake the **View** to ensure that the hole is centered
- Press **Save Image Shift**
- Press the **IS to Nav Pt** button in this dialog, or select the next added point in the Navigator and press **Go To IS** there.
- Note* To improve accuracy of the image shift, one can go through a second round of template setup, centering template on the edge of the holes, taking a preview image after shifting the view beam to ensure that the edge of the hole is in view. If additional image shift needs to be added, add it here before saving IS. This has not been necessary for single shots/hole.
- Take a **View** and shift it to the same position as for the first point.
- Press **Save Image Shift**
- Repeat the last three steps for each other point.
- Click **OK** to exit the setup window
- Return view defocus offset to -20 (or whatever is desired).
- Save NAV.
- Recheck “Move stage for big mouse shifts”
- Make a polygon that fits the square
- Select **Add Points**. Left click on the precise center of 5 holes, starting at a corner, with one in each direction immediately adjacent to the corner and one further down the line of holes in each direction. This establishes the geometry of the holes.

```
X 0 0 X 0 0 X
0
0
X
0
0
0
X
```

- Go to **Navigator** -> **Montaging & Grids** -> **Add Grid of Points**. Enter the number of the polygon (this can be found on the overview map image or the Navigator window)
- **Navigator** -> **Acquire at items**
- Run SPA script

Set up the Multi-shot pattern

Select holes-Find Holes

Combine points