

Multi-Records (Multi-shots/Multi-hole) Setup under SerialEM

- when setting up the "pattern" under the view mag, one has to adjust the defocus close to what's used under data collection under "record" mode, which is closer to zero. Normally we set this at -100 um or similar under view by default (for hole centering), and if not changed, this would seriously mess up the setup that may lead to mismatch between view and record mags.

- Center the "center point/hole" (Point C) under -100 um defocus;
- then switch to ZERO defocus under View mode. (your view image for Point C, if taken again, may drift/off-center, do NOT re-center under zero defocus).
- Setting up the four corner hole/points (Point 1 through 4) at ZERO defocus.
- When finished, reset defocus for View mode back to -100um.

- what's registered (in XY) under the MMM map is often misaligned under the View mag, no matter how carefully one tried to align the two mags beforehand. This is more so when one uses a low mag (in LM lens group) such as 210x or 510x to capture MMM maps (avoiding the time consuming MMM maps using M/SA lens group). What Weiwei did was to further refine positions of the four "corner" points between MMM and View mags as follows:

- before setting up the multi-shots: "Go to XYZ" on one of the 4 points,
- take a view at -100um defocus;
- it's likely not matching the hole well;
- add a marker to the center of the hole under view;
- Nav->Shift to marker.
- He did this for all 4 corner points, before setting up the multi-shots.

- when not sure, it's always a good idea, after image shift to the center of a hole under view during the setup (before click on the "save image shift" button), to switch to "preview" just to make sure that it's hitting the hole. one could even fine tuning between preview and view modes here to register additional image shift if needed. we didn't have to do this, yet.

- I start keeping the "move stage for big mouse shifts" unchecked, and using strictly SHIFT+right mouse click for "stage move" and right click alone for image shift. because only image shift should be used during the multi-shots setup (most of the time), it's important that one doesn't "accidentally" trigger the stage shift.

- there's an "IS" field on one of the panels to the left. one should keep a close eye on that to make sure it's at zero unless an image shift is warranted. **Reset IS back to zero otherwise.**

- always run, under "Calibration" -> "Focus and tuning" -> "Image Shift vs. coma", before start of multi-shots data collection. One may have to increase binning, change the defocus, or increase exposure time to get the Thon rings to show up properly. **we used 2.5 seconds exposure, binning of 4, under 45k mag ss=6. For R1.2/1.3 grids, the 3x3 setup has a max image shift about 3.6um. for R2/2 grids, this becomes 5.7um. After finishing this calibration, make sure to change the "Record" settings back to the original exposure time and binning.**

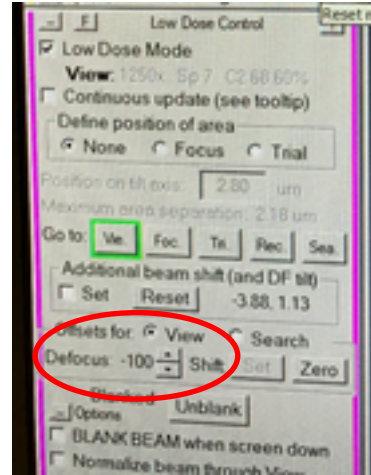
Highly recommended:

After one finishes setting up the mutl-shots pattern:

- Add a point to the MMM map (testing MMM map).
- Make it “Acquire”.
- check “Record” camera settings, make it simple (1s exposure, no dose fraction)
- Navigator -> Acquire at points
 - o uncheck “Save Frames”
- Make sure hole centering and multi-shots works before adding grid points to acquire.

Weiwei's protocol for setting up 9-hole (3x3) pattern:

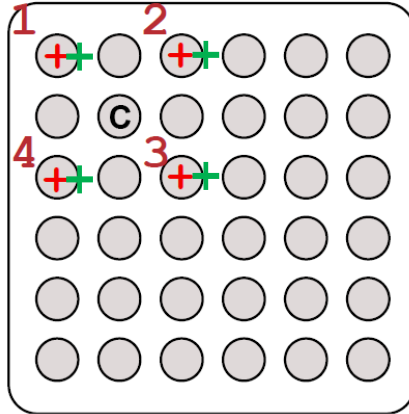
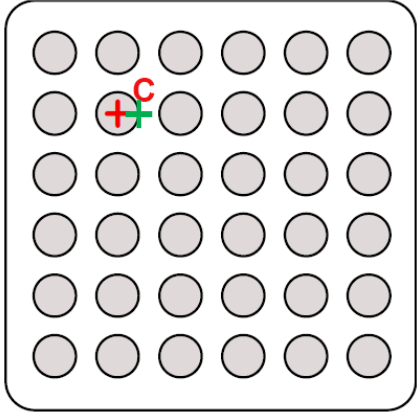
- a. Pick an MMM map of one square in the navigator (either go to the square line or the MMM map of it) and click "go to XYZ" (Note: better if the ice is not too thin to see the holes well on view image)
- b. Set the defocus to **-100um**, identical to the value used during hole centering step during data collection.



- c. Add a point to the center of the 3x3 pattern (hereafter referred to as **Point C**). Take a view shot, if not seeing the hole well, adjust the view setup to 2s (usually at 1s).
- d. Click "Add points" in the navigator window and pick the four corner holes of a 3X3 array in a clockwise order.
- e. "Stop adding" and better note the 4 points (e.g. template pt1/2/3/4), so that they will not be mistakenly used.

- what's registered (in XY) under the MMM map is often misaligned under the View mag, no matter how carefully one tried to align the two mags beforehand. This is more so when one uses a low mag (in LM lens group) such as 380x or 700x to capture MMM maps (avoiding the time consuming MMM maps using M/SA lens group). What Weiwei did was to further refine positions of the four "corner" points between MMM and View mags as follows:

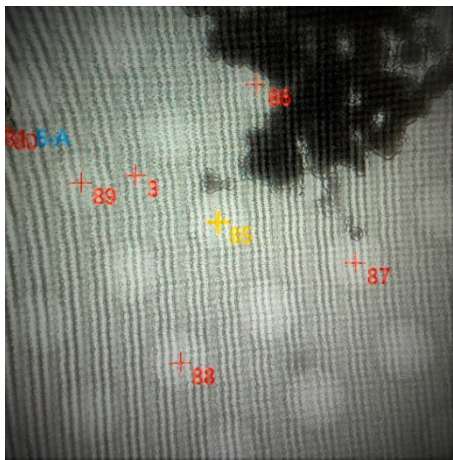
- before setting up the multi-shots: "Go to XYZ" on one of the 4 points,
- take a view at -100um defocus;
- it's likely not matching the hole well;
- add a marker to the center of the hole under view;
- Nav->Shift to marker.
- Do this for all 4 corner points, before setting up the multi-shots.



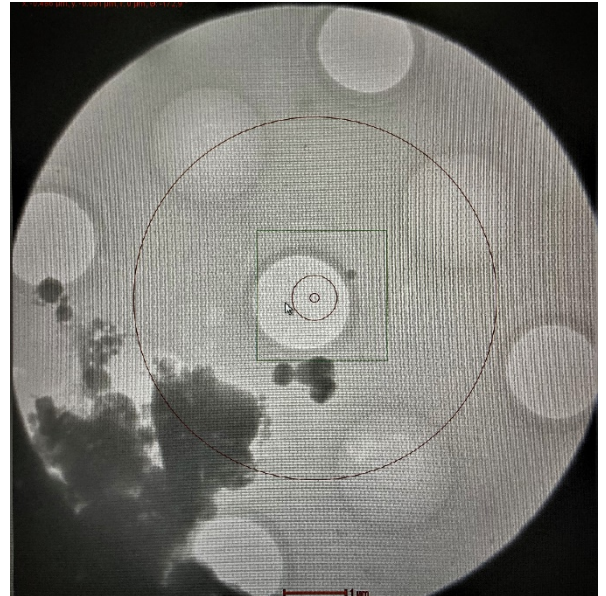
*for more precisely
alignment between "view"
and "record/preview", add
the four corner points to
the edge of the holes
(green markers)*

*****. burn with beam the 4 corner positions. *** (noticed that the image flips between the LM and SA lens groups, so your MMM images looks upside-down from the “view” mag images at 6700x)**

84	Red	228.0	-69.3	226.5 Pt	1	
85	Red	216.0	-74.2	226.5 Pt	1	ct
86	Red	219.5	-74.9	226.5 Pt	1	1
87	Red	215.3	-77.7	226.5 Pt	1	2
88	Red	212.5	-73.5	226.5 Pt	1	3
89	Red	216.8	-70.7	226.5 Pt	1	4

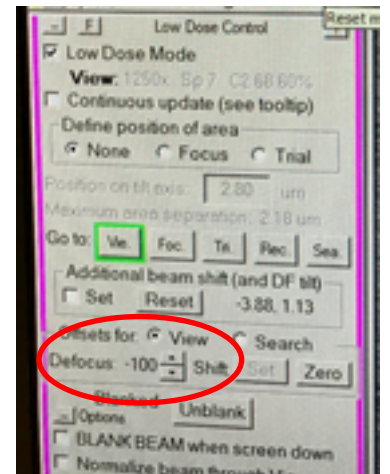


MMM map showing the 5 points selected (taken with LM lens group)



View mag (at 6700x) showing the 5 points intentionally “burned” empty

*****. Adjust the defocus from -100 um to 0 using downward arrowhead. *****



g. Highlight Point C line in the navigator

h. Navigator -> Montage & grids -> set multi-shot parameters

i. A “Multiple Record Setup” window will pop out:

a). Click ”For corners of regular pattern” -> Then the two panels below it will be clickable.

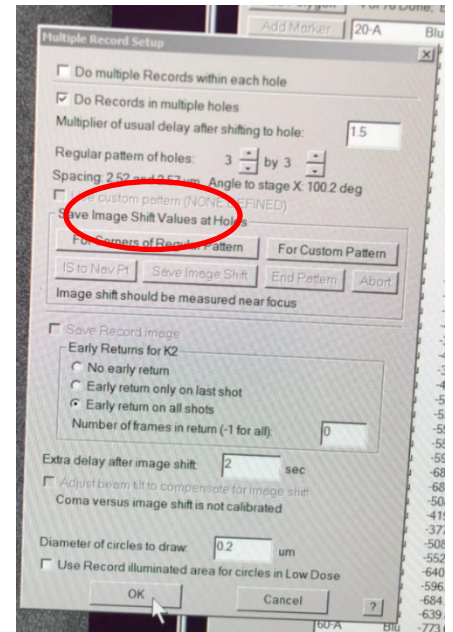
b). First click “IS to Nav Pt” (note: IS = image shift)

c). Ignore the warning and hit OK.

it would read underneath “Go to first corner”

d). The First of the 4 picked points will turn from red to yellow (see the figure below)

e). Take a view shot and center to the hole using **image shift (right-click, manually drag to the target Point 1), using the burned beam mark as reference.** confirm centering with another view shot.



- when not sure, it's always a good idea, after image shift to center of hole under view during the setup (before click on the "save image shift" button), to switch to "preview" just to make sure that it's hitting the hole. one could even fine tuning between preview and view modes here to register additional image shift if needed.

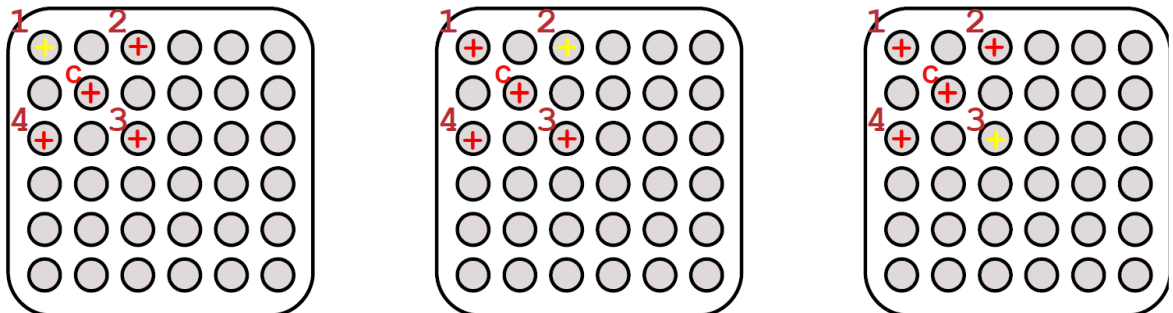
f). Then click “Save image shift”.

Click “IS to Nav Pt”, now **Point 1** would turn back to red, **Point 2** would become yellow.

now it would read underneath “Shift by 2 holes in first direction”

follow step e) to image shift to Point 2

g). repeat steps f), for Point 3 and Point 4.



h). When done with all four holes, will see an update of the numbers in one of the lines above the “For corners of regular pattern” button: Spacing: 3.8 and 3.6 um Angle to stage X: 100 deg

make sure to check the box to “Adjust beam tilt to compensate....”

i). Click “ok” at the bottom of the Multiple Record Setup window.

j). Very important: adjust the defocus back to **-100 um** using upward arrow head! And change the view setup back to **1s**.

k). Save navigator.

- **Reset Image Shift!!! Make sure “IS” is zero!!!**
- Click on “Show Acquire area” will bring up the pattern.
- **Calibration -> Focus & Tuning -> Coma vs. Image Shift**, Set the **MAX** amount of image shift between the furthest two hole centers (could be measured by **SHIFT+left click and drag**)
- One may have to increase binning, change the defocus, or increase exposure time to get the Thon rings to show up properly. **we used 2.5 seconds exposure, binning of 4, under 45k mag ss=6. For R1.2/1.3 grids, the 3x3 setup has a max image shift about 3.7um (set Max at 4 um). for R2/2 grids, this becomes 5.7um (set Max at 6**

um). Watch out for beam “clipping” during the process.

- **After finishing this calibration, make sure to change the “Record” settings back to the original exposure time and binning.**
- check then uncheck “Continuous update” when done.

