SerialEM Note: Multiple Record Setup Dialog (Saving Accurate Image Shift Values for Holes at LD View)

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In order for image shift positions to correspond well enough between this View mag and the Record mag, this operation must be done near focus (within about 20 microns: e.g. -10 um). Assuming that you use View images, the steps are:

- 1. Naviogator>Montaging&Grids>Set Multi-shot Parameters, multiple record setup dialog appears, keep this dialog open for the following steps.
- 2. Reduce the View defocus offset to the lowest level (e.g. -5 um or -10 um) while you still can see the holes.
- 3. Take a View image.
- 4. If image shift range is a concern, first shift the image with the right mouse button drag to center the pattern, and Reset Image Shift if necessary.
 - i) If setting up 2 by 2 pattern, please center on the film where is the center of four holes;
 - ii) If setting 3 by 3 pattern, please center on the middle holes.

Note: no matter what pattern is selected, always center the pattern in this step.

- 5. Add a set of Navigator points at the centers of the holes, in the order required when shifting to the holes (see below). 4 points at four corners are required if a regular pattern is selected later. If necessary, take a new View image to see the required positions.
 - i) All four holes (at four corners of a regular pattern) may not be visible in one View image, one can use right mouse button drag, Reset Image Shift, and take View image to make the necessary holes visible in View images.
 - ii) I use clockwise for the order. four pt items will be in Navigator. Select the first item of the four.

Note: Center of hole may not be accurately located, using the edge of hole is recommended for experienced users to increase accuracy.

- 6. Press the relevant button to start saving image shifts. e.g. `for corners of the regular pattern`
- 7. Press the 'IS to Nav Pt' button in this dialog, (or select the first added point in the Navigator and press 'IS To XY' there)
- 8. Take a View image, center with the right mouse button drag (**NO** Reset Image Shift), and take another View image to check the centering. Take a Preview image to more accurately check the centering, do additional IS via right mouse drag in Preview image if necessary.
- 9. When centered, press the 'Save Image Shift' button.
- 10. Press the `IS to Nav Pt` button in this dialog, (or select the next added point in the Navigator and press `IS To XY` there)
- 11. Take a View and center the image. (This is a repeat of step 7)
- 12. Press 'Save Image Shift'. (This is a repeat of step 8)
- 13. Repeat the last three steps (9-11) for each other point.
- 14. Restore the View defocus offset (e.g. -200 um). Done and close dialog.