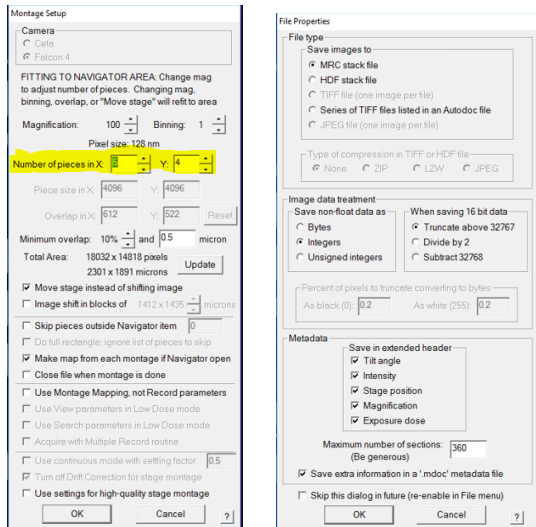


South Krios Data Collection Protocol V1.1

Low Mag Maps (LMM)

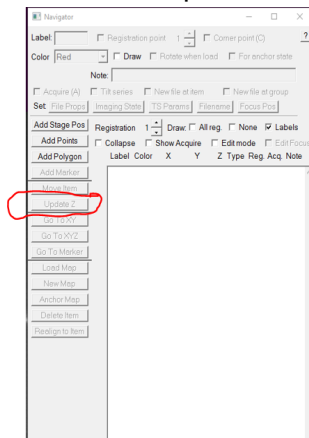
- Spot size 4-6, C2 50um, EFTEM LM100-175x depending what resolution wanted for LLM, Illumination area around 1.16mm or big enough to cover detector, test with a record pic (Bin 1, 0.5-1 secs)
- Navigator Ribbon>Open a Navigator
- Navigator Ribbon>Full Montaging and Grids>Set Up Full Montage> Default Setup and Default Montage Pieces highlighted in yellow>File Properties settings on image below>Save as LMM.st in KEEP folder



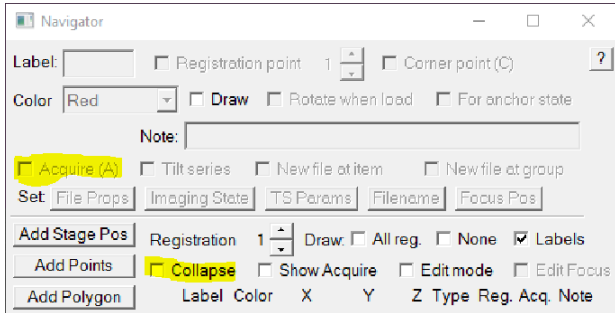
- Go to Montage Controls lower left side of serialEM tiles> hit **Start**
- Save NAV after LMM completes by Nav ribbon>Save> Save as Nav in KEEP folder

Medium Mag Maps (MMM)

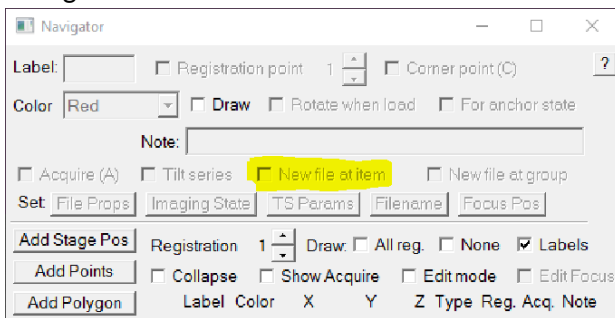
- Spot size 4-6, C2 50um, EFTEM LM600x to LM940 depending on size of squares.
- Take a record pic (Bin 1, 0.5-1 secs) of a centered squared at selected Medium Mag, then Tasks Ribbon>Eucentricity>Rough Eucentricity>Wait for finished message on log window>On Nav window click update Z



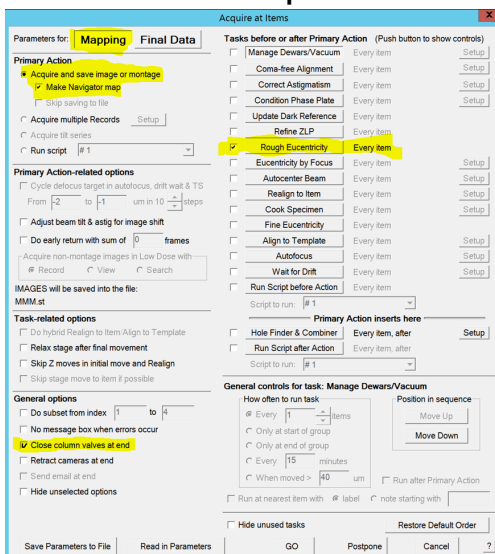
- **Shift To Marker LMM to Medium Mag** - Open LMM (double click) from Nav> Search for distinct feature inside a square>On Nav select **Add Points**> Click on feature and remember where on feature point is made>On Nav click **Stop Adding**>on Nav click **Go to XYZ**> Take Record pic> Feature should be on pic but not exactly where point was placed>click on where point should be (green +) > navigator ribbon> halfway the ribbon click **Shift to Marker**>ok> point will move to desired location
- On Nav click **Add Points**> add point to center of squares to be mapped> Select enough squares for a 24hr or 48hr data collection, 25-50 squares>collapse into group by checking **Collapse**> check **Acquire**> uncheck Collapse, all square point will show an A beside



- On Nav select first square point with an A> on Nav check **New File at Item**> selected point will change from A to AF



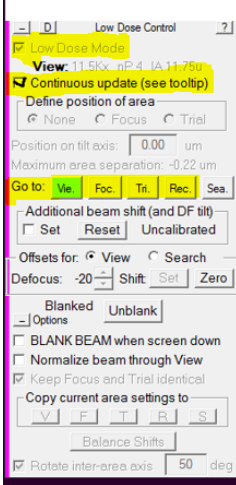
- Nav ribbon>click on **Acquire at Items**> on new window select settings below>Go



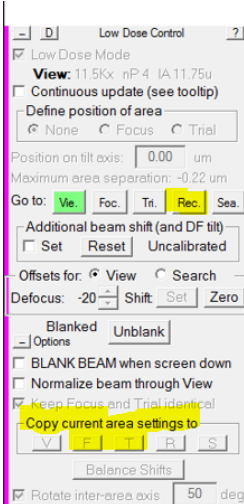
- When MMS finish>Nav ribbon>save

Low Dose Beam Setup

- On Nav open MMM with distinct feature/s, we will use this square for the next few steps
- We will now set up Record, Trial, and Focus Beams
- Activate low dose panel by checking **Low Dose Mode**>check **Continuous Update**>click on **Rec** beam first



- **Spot Size 4, C2 50um for all beams**
- **Record beam** – EFTEM, NanoProbe, SA130Kx to 275Kx, 165Kx most common, Energy Slit in @10ev
- **Trial and Focus** – Copy settings from Rec to Trial and Focus, , Energy Slit in @10ev



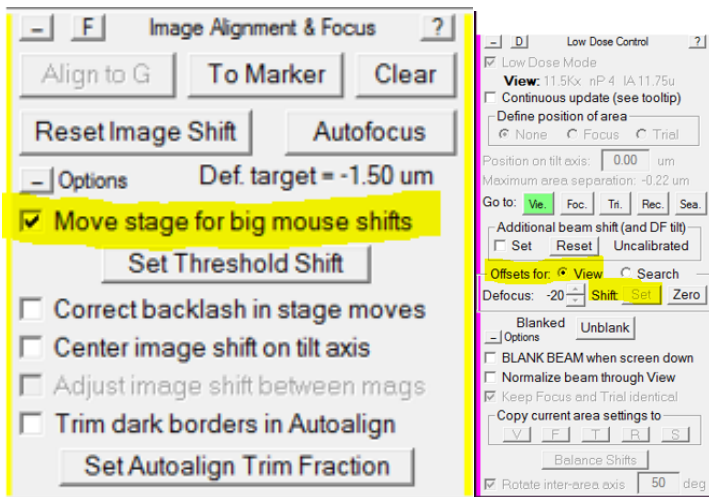
- **View** – EFTEM, NanoProbe, SA1100x to SA19500 depending on hole size, , Energy Slit in @20ev

Shift to Marker MMM to View Mag

- On NAV open MMM with features> look for distinct feature> on Nav click **Add Points**> click on feature to add point, remember where on feature > on Nav click **Stop Adding**> click **GO TO XYZ**> Take VIEW pic> if feature found on pic, click (green +) on where the point should be> Nav ribbon>**Shift to Marker**> point will move to desired location. **If feature not found on VIEW pic**>mover over to microscope flu screen and explore around till feature is found> when feature is found, center inside detector area> take VIEW pic> click (green +) on where the point should be> Nav ribbon>**Shift to Marker**> point will move to desired location.

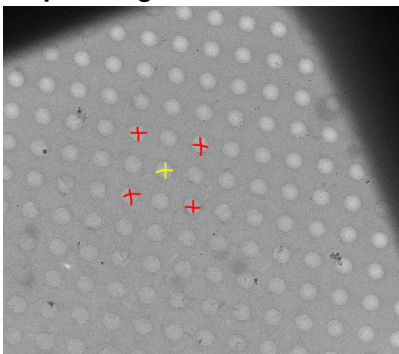
Align Record Mag to View Mag, View Shift Offset

- On NAV open MMM with features> click **Go To XYZ** > take VIEW pic> find a small feature that will fit inside a RECORD pic, drag in VIEW mag to get close to feature> take RECORD/PREVIEW pic> drag to align feature and refresh with new RECORD pic till feature is centered> take VIEW pic> locate feature, if not aligned> Uncheck **MOVE STAGE FOR BIG MOUSE SHIFTS** on SerialEM tile> Drag to align feature> in **LOW DOSE CONTROL** tile, click **SET** on offsets for view> recheck **MOVE STAGE ...**

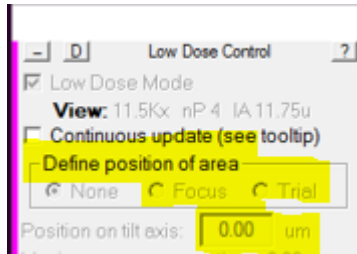


Multi-shot Parameters Setup

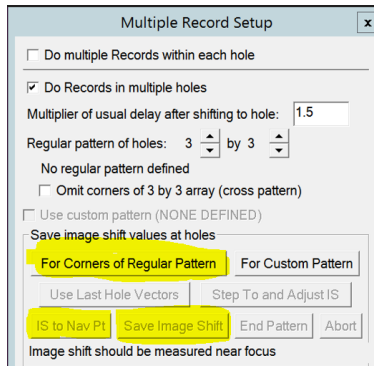
- On NAV open MMM> create 9 hole pattern highlighted below> make center point (yellow) by **Add Points**> **Stop Adding** > add 4 corner points (red) by **Add Points**> make 4 corners points> **Stop Adding**



- on Low Dose Control Tile > Define Position of Area > click Focus or Trial > on Position on tilt axis box change to zero

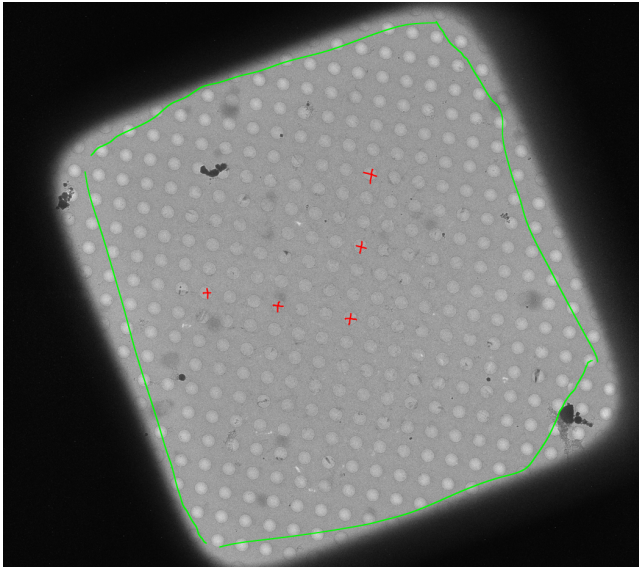


- Take VIEW pic > drag to carbon area > take RECORD pic to confirm strong thon rings on FFT > if strong rings present move on to do 3 cal's under SerialEM top ribbon> Focus and Tuning >
 - o Correct Astigmatism by CTF
 - o Correct Coma Free Alignment by CTF
 - o Take a view pic>Coma vs Image Shift – settings depend on hole size> ensure tilts fall on carbon
- On NAV select center point > **Go To XYZ** > take VIEW pic > drag to align hole, retake VIEW pic> repeat till centered
- Navigator Ribbon > Montaging and Grids > Set Multi-Shot Parameters, window will open> set up **Do Multiple Records within each hole** > select desired number of shots within hole
- on NAV select first corner point> on SerialEM left tiles Uncheck - MOVE STAGE FOR BIG MOUSE SHIFTS > Multiple record window click **For Corners of Regular Pattern**> click **IS to Nav Pt** > take VIEW PIC> drag to align hole, retake VIEW, repeat till centered> click **Save Image Shift**> first corner of pattern done > click **IS to Nav Pt to go to next corner** > take VIEW PIC> drag to align hole, retake VIEW, repeat till centered > click **Save Image Shift**> second corner of pattern done > repeat for two remaining corners



Set up Acquire Points

- On NAV open desired MMM> draw polygon around desired area by clicking **ADD POLYGON (green)** > click **Stop Adding** > create 5 point pattern below by clicking **Add Points**> make the 5 points > click **Stop Adding**



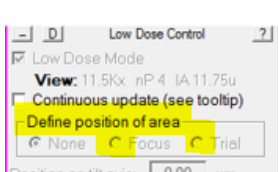
- On Navigator ribbon > Montaging and Grids > Add Grid of Points > input Polygon number (green number on MM > on keyboard hit Enter, then Enter again> acquire points will populate inside polygon in magenta color> repeat steps for about 20-30 MMMs> save NAV
- **Option 2 for selecting acquire points** > see Hole Finder Protocol in Binder

Align to Template Setup and Define Area of Focus/Trail

- We need a clean VIEW pic of an aligned hole > on NAV open desired MMM > take VIEW pic > drag to align hole > once a clean example of VIEW pic with aligned hole is found > on SerialEM windows ribbon > File > Save A > save as mrc on file properties window > name it BufferP in your KEEP folder> click save> on NAV click **New Map** > a map will be created > remember label associated with new BufferP map/Image for final data setup section

32-A	Blu	-0.1	675.2	7.7	Map 1	Sec 28 - MMM.mrc -
62	Red	17.9	380.5	21.1	Pt 1	
63	Red	13.0	413.1	21.1	Pt 1	
64	Red	28.8	410.4	21.1	Pt 1	
65	Red	22.4	410.7	21.1	Pt 1	
66	Red	29.2	416.9	21.1	Pt 1	
67	Red	35.3	409.9	21.1	Pt 1	
68	Red	28.6	403.7	21.1	Pt 1	A
69	Off	28.6	403.4	21.1	Map 1	Sec 0 - bufferp.mrc

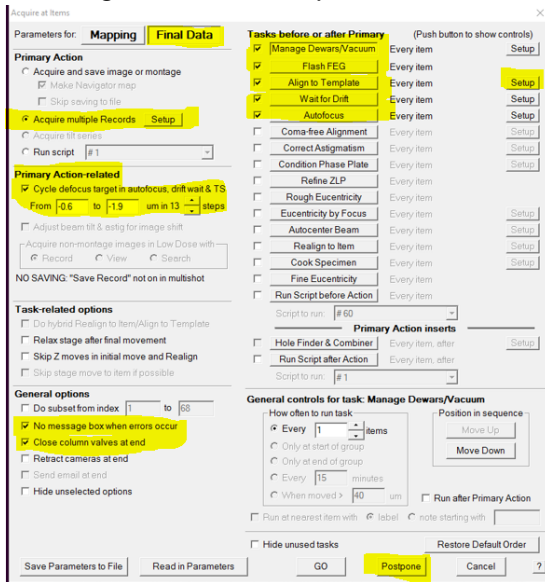
- on Nav re open BufferP/View Aligned Hole > on Low Dose Panel find Define Position of Area >



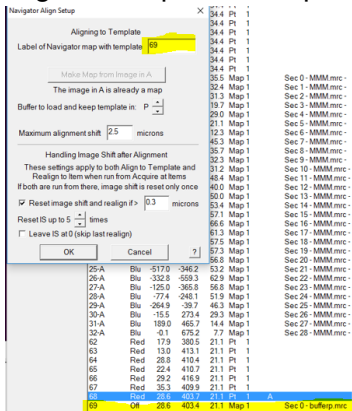
- Click Focus or Trial > F/T area will appear on image > click on biggest area of carbon visible on image > area of F/T will move to where you click > select none on low dose panel when desired area is selected

Final Data Setup

- On Navigator ribbon > Acquire at Items > below window will appear > Go over highlighted parts



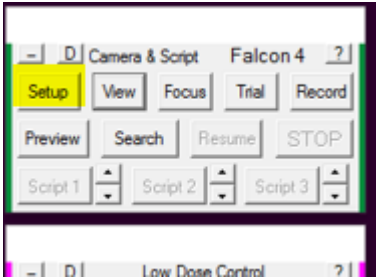
- Align To Template > Setup > new window will open > add label number of BufferP > click ok



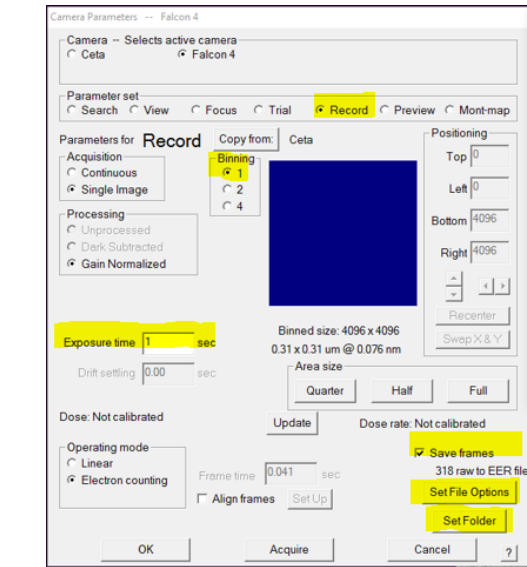
- Once all items have been checked/Reviewed > click Postpone

Final Data Camera Setup

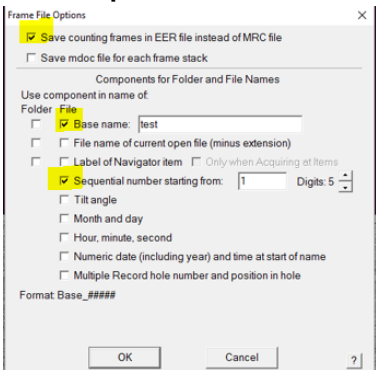
- Go to the Camera Tile > click Setup



- Go over highlighted areas below



- Record settings bin 1, exposure time to achieve desired total dose, check save frames
- Set file options**



- Set Folder** to set folder for saved images
- Go to low dose setup MMM to check beams are centered
- Go NAV ribbon>Acquire at Items>GO> data collection starts