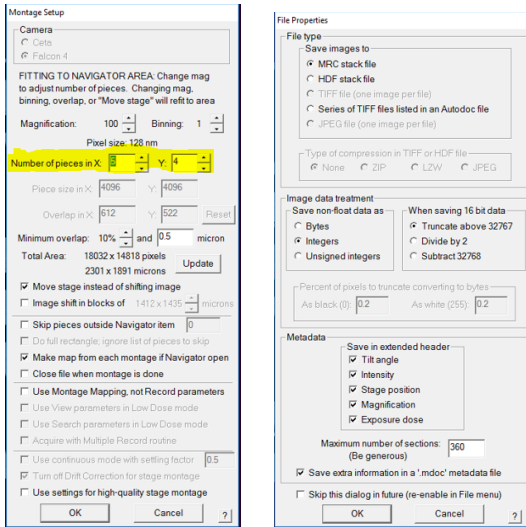


# North Krios Data Collection Protocol V1.0

## Low Mag Maps (LMM)

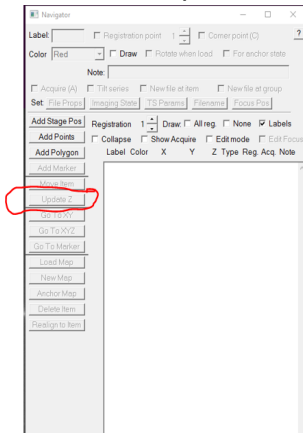
- Spot size 6-7, C2 70um, EFTEM LM100-175x depending what resolution wanted for LLM, Illumination area big enough to cover detector, test with a record pic (Bin 4, .5 secs)
- Navigator Ribbon>Open a Navigator
- Navigator Ribbon>Full Montaging and Grids>Set Up Full Montage> Default Setup and Default Montage Pieces highlighted in yellow>File Properties settings on image below>Save as LMM.st in KEEP folder



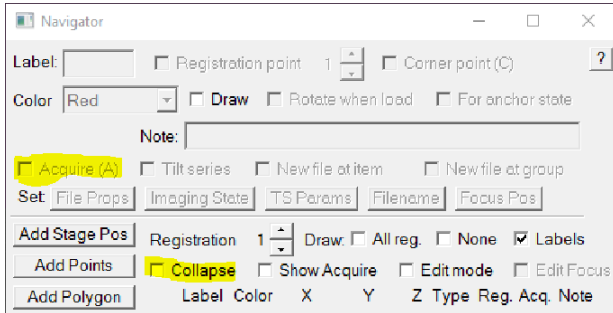
- Save NAV after LMM completes by Nav ribbon>Save> Save as Nav in KEEP folder

## Medium Mag Maps (MMM)

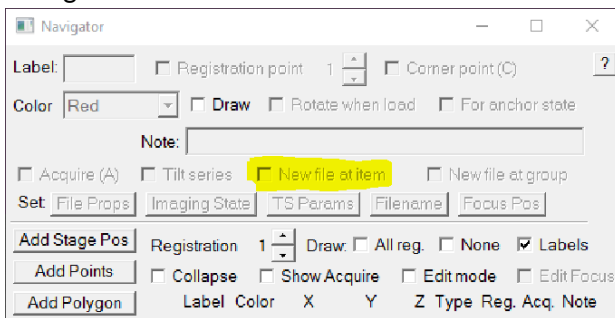
- Spot size 6-7, C2 70um, EFTEM LM540x.
- Take a record pic (Bin 1, .5 secs) of a centered squared at selected Medium Mag, then Tasks Ribbon>Eucentricity>Rough Eucentricity>Wait for finished message on log window>On Nav window click update Z



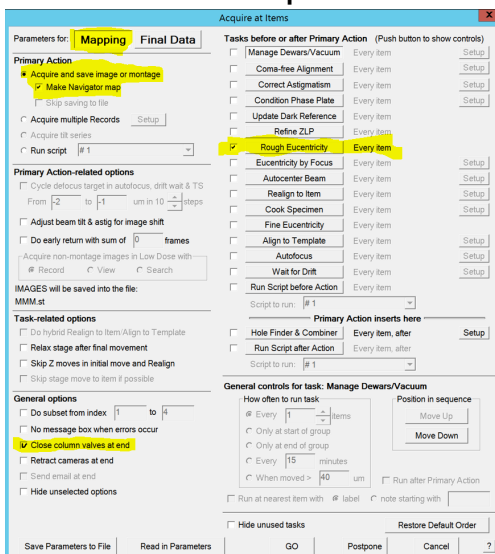
- **Shift To Marker LMM to Medium Mag** - Open LMM (double click) from Nav> Search for distinct feature inside a square>On Nav select **Add Points**> Click on feature and remember where on feature point is made>On Nav click **Stop Adding**>on Nav click **Go to XYZ**> Take Record pic> Feature should be 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



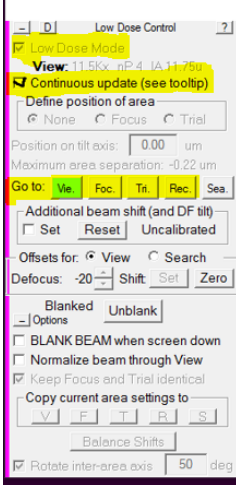
- Nav ribbon>click on **Acquire at Items**> on new window select settings below>Go



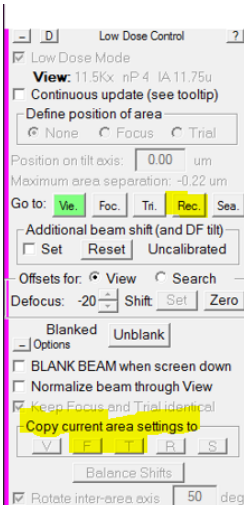
- When MMS finish>Nav ribbon>save

## 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 6-7, C2 70um for all beams**
- **Record beam** – EFTEM, NanoProbe, SA81kx or SA105K
- **Trial and Focus** – Copy settings from Rec to Trial and Focus



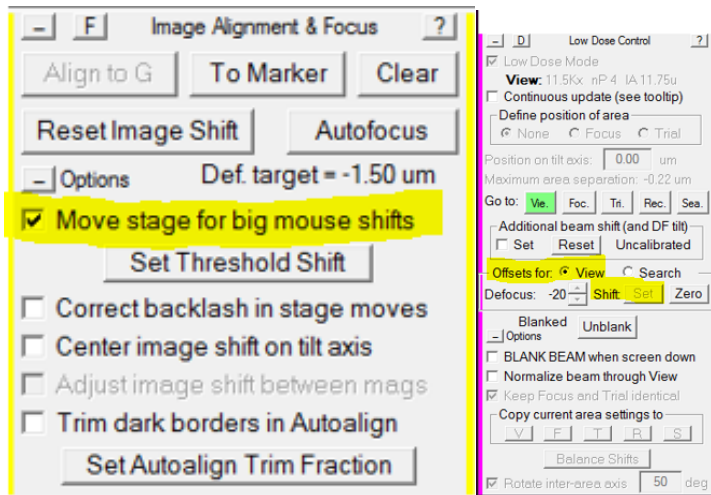
- **View** – EFTEM, NanoProbe, SA15000x +- one mag, depending on hole size

## 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.

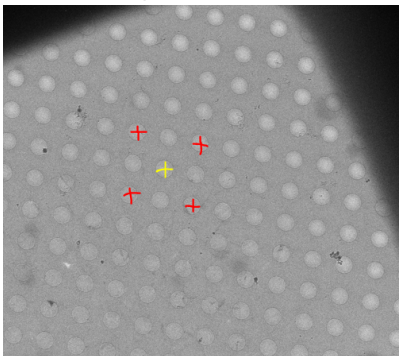
### Align Record Mag to View Mag, View Shift Offset

- 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 ...**

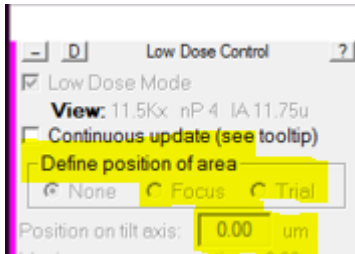


### Multi-shot Parameters Setup

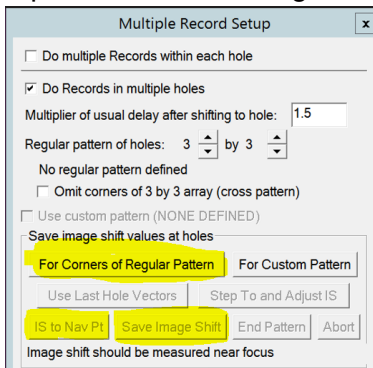
- On NAV open MMM> create 9 hole pattern highlighted below> make center point (yellow) by **Add Points**> **Stop Adding** > add 4 corner points (red) by **Add Points**> make 4 corners points> **Stop Adding**



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

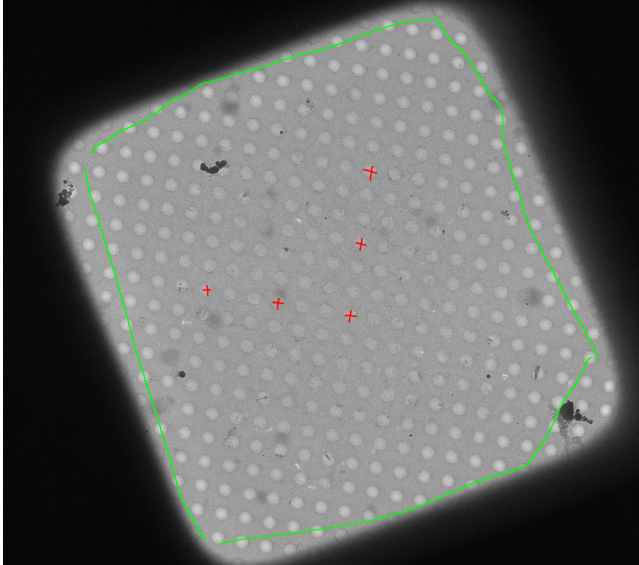


- Take VIEW pic > drag to carbon area > take RECORD pic to confirm strong thon rings on FFT > if strong rings present move on to do 3 calcs under SerialEM top ribbon> Focus and Tuning >
  - o Correct Astigmatism by CTF
  - o Correct Coma Free Alignment by CTF
  - o Coma vs Image Shift – settings for depend on hole size
- 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**



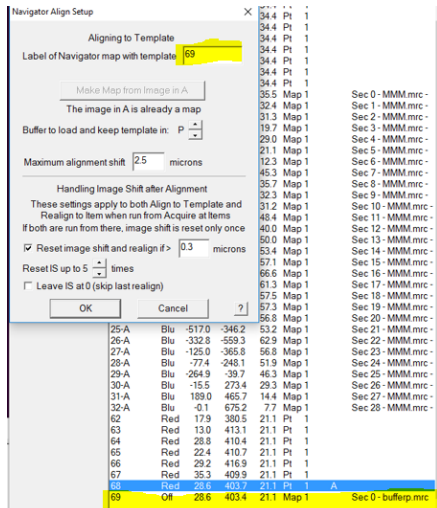
- On Navigator ribbon > Montaging and Grids > Add Grid of Points > input Polygon number (green number on MM > on keyboard hit Enter, then Enter again> acquire points will populate inside polygon in magenta color> repeat steps for about 20-30 MMMs> save NAV
- **Option 2 for selecting acquire points**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

### Align to Template Setup and Define Area of Focus/Trail

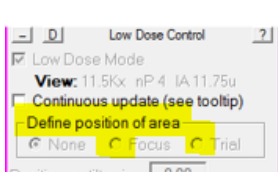
- We need a clean VIEW pic of an aligned hole > on NAV open desired MMM > take VIEW pic > drag to align hole > once a clean example of VIEW pic with aligned hole is found > on SerialEM windows ribbon > File > Save A > save as mrc on file properties window > name it BufferP in your KEEP folder> click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

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

- Navigator Ribbon> Acquire at Items> click setup on Align To Template > new window will open > add label number of BufferP > click ok



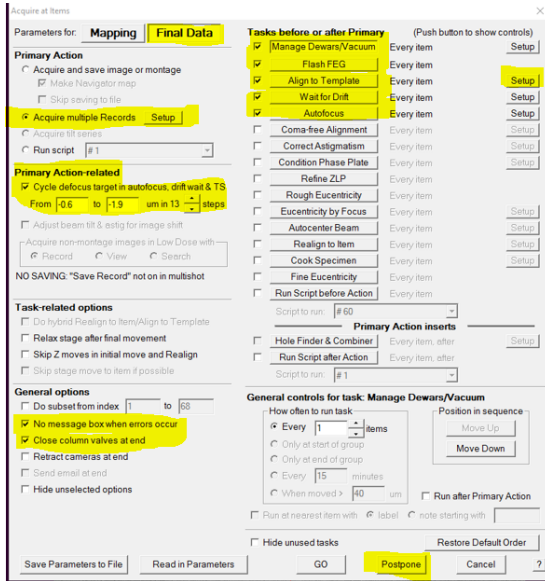
- on Nav re open BufferP/View Aligned Hole > on Low Dose Panel find Define Position of Area >



- 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

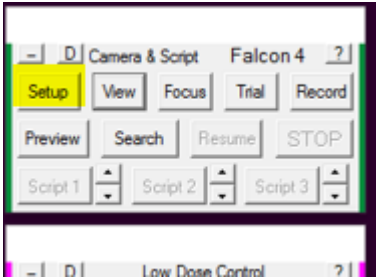
- On Navigator ribbon > Acquire at Items > below window will appear > Go over highlighted parts



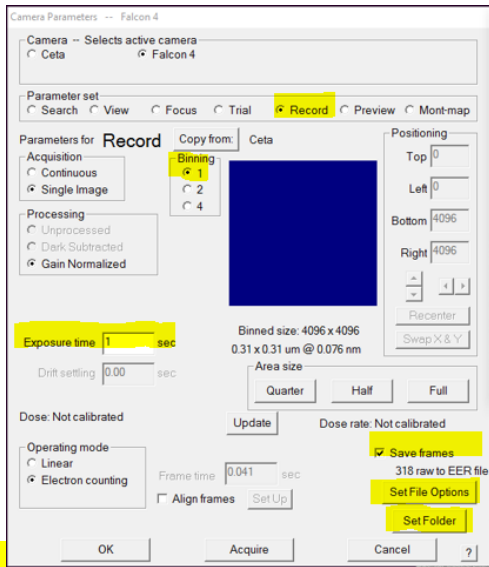
- Once all items have been checked/Reviewed> click Postpone

## Final Data Camera Setup

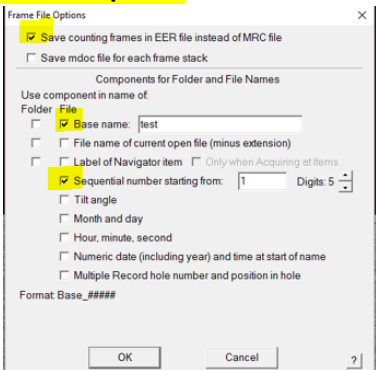
- Go to the Camera Tile > click Setup



- 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