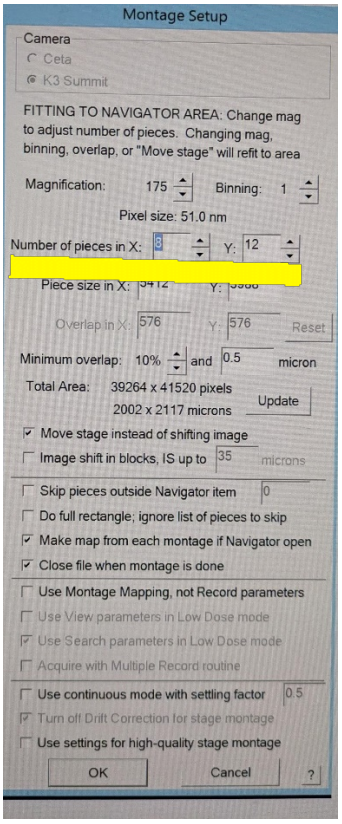
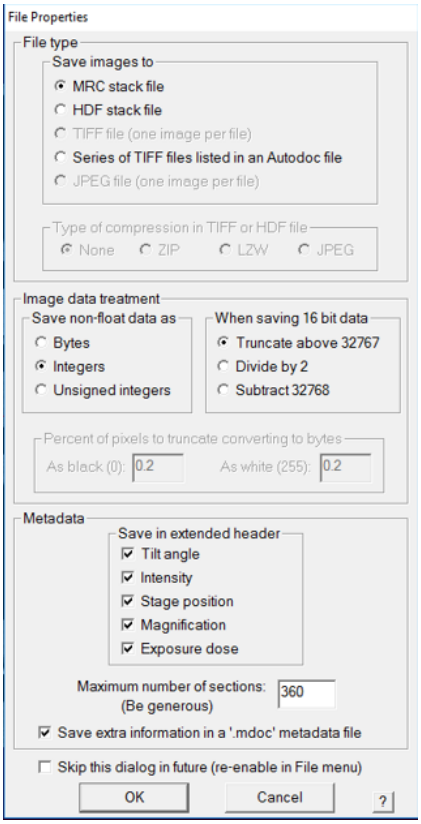
North Krios Data Collection Protocol v1.3 FFI

Low Mag Maps (LMM)

- Spot size same as collection (see cheat sheet for specific parameters), C2 70µm, EFTEM LM175×, Illumination area big enough to cover detector, test with a record pic (Bin 4, .5 secs, longer if CDS mode)
- 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

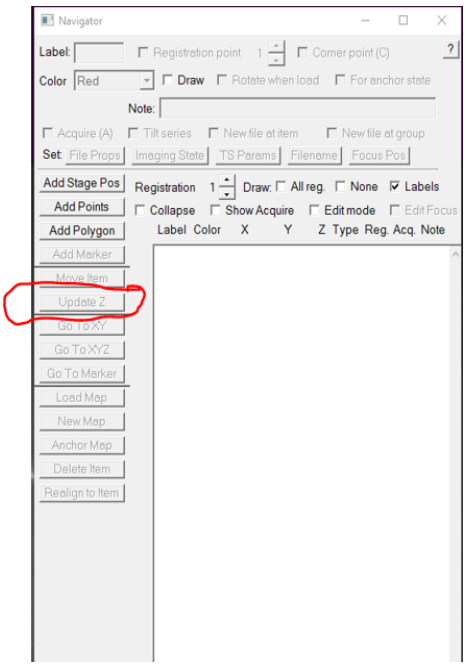




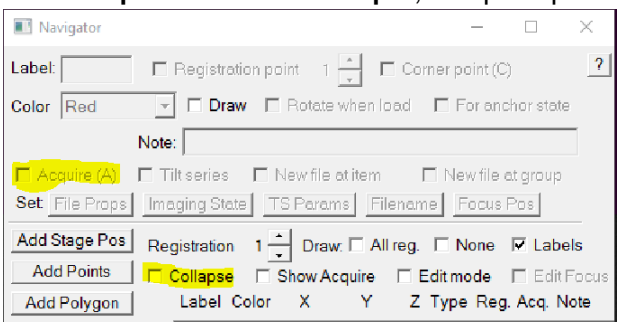
- Montage Controls > Start
- Save NAV after LMM completes by Nav ribbon > Save > Save as Nav in KEEP folder

Medium Mag Maps (MMM)

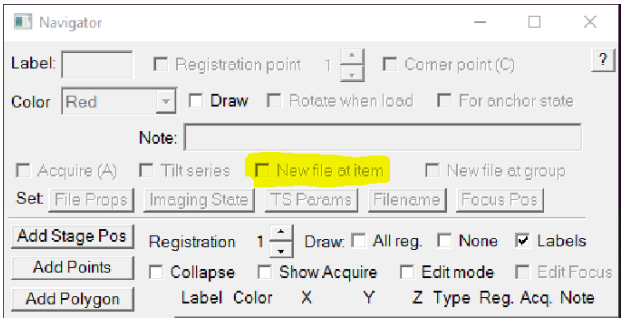
- Spot size same as collection (see cheat sheet for specific parameters), C2 70µm, EFTEM LM580× or whatever fits a single grid square in one shot
- Take a record pic (Bin 1, .5 secs) of a centered square at selected Medium Mag, then Tasks Ribbon > Eucentricity > Rough Eucentricity > Wait for finished message on log window > On Nav window click “Update Z”



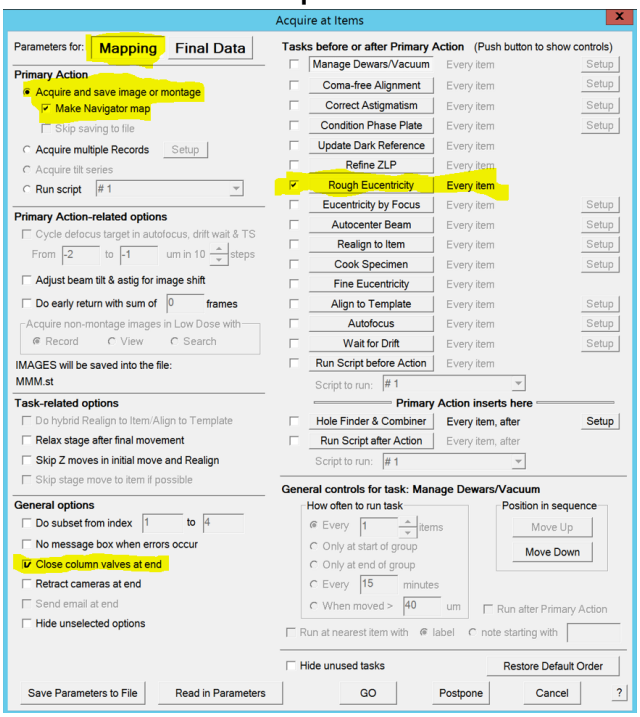
- **Shift To Marker LMM to Medium Mag -** Open LMM (double click) from Nav > Search for distinct feature inside a square > On Nav select **Add Points** > Click on feature and remember where on feature point is made > On Nav click **Stop Adding** > On Nav click **Go to XYZ** > Take Record pic > Feature should be on pic but not exactly where point was placed > Click on where point should be (green +) > Navigator ribbon > Halfway down 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 (40+ squares) > Collapse into group by checking **Collapse** > Check **Acquire** > Uncheck **Collapse**, all square points will show an A beside it



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



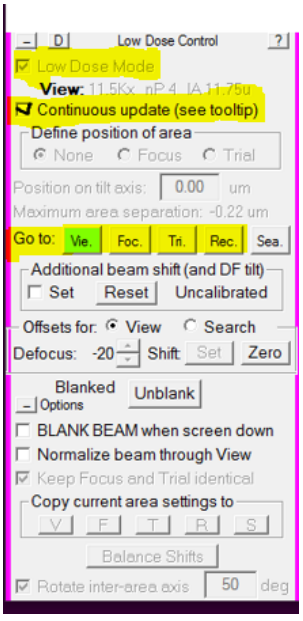
- Nav ribbon > Click on **Acquire at Items** > On new window select settings below > Go



- When MMMs finish > Nav ribbon > Save

Low Dose Beam Setup

- On Nav open MMM with distinct feature(s), we will dose 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 same as collection, C2 70µm for all beams**
- **Record beam –** EFTEM, Nanoprobe, SA81k× or SA105k×

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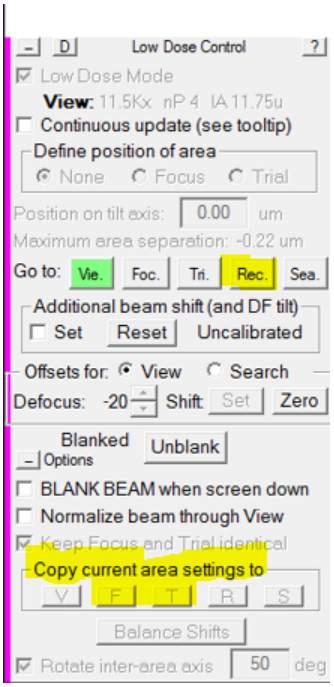
- **Trial and Focus –** Copy settings from Rec to Trial and Focus



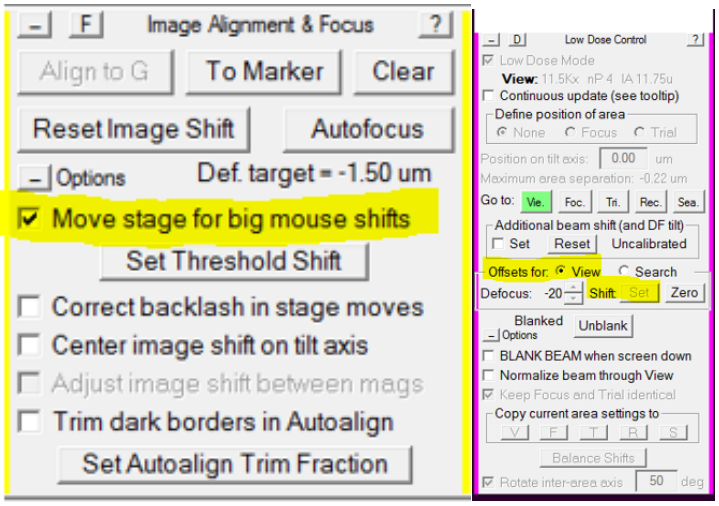
- **View –** EFTEM, Nanoprobe, SA15000× ± one mag, depending on hole size

<u>Shift to Marker MMM to View Mag</u>

- 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** > Move 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

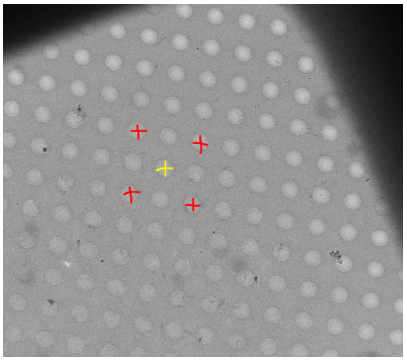
<u>Align Record Mag to View Mag, View Shift Offset</u>

- 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 FOR BIG MOUSE SHIFTS

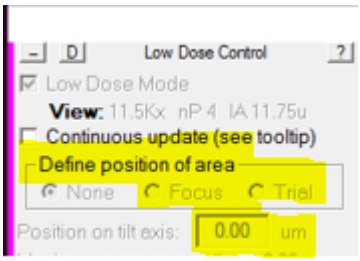


Multi-shot Parameters Setup

- 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**

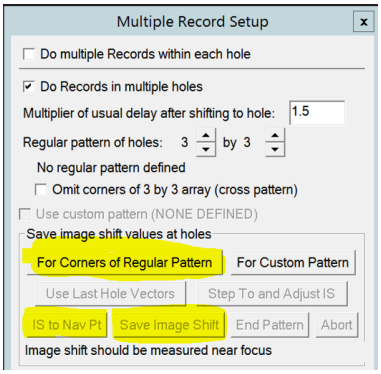


- On Low Dose Control Tile > Define Position of Area click Focus or Trial > On Position on tilt axis box change to zero



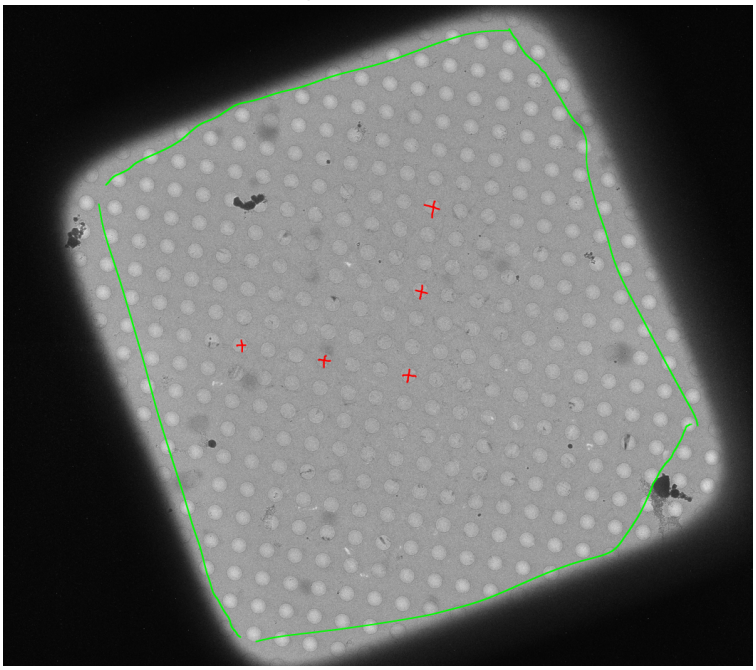
- Take VIEW pic > Drag to carbon area > Take RECORD pic to confirm strong thon rings on FFT > If strong rings present move on to do 3 cals under SerialEM top ribbon > Focus and Tuning >
    - Correct Astigmatism by CTF
    - Correct Coma Free Alignment by CTF
    - Coma vs Image Shift – settings for depend on hole size
- On NAV select center point > **Go To XYZ** > Take VIEW pic > Drag to align hole, retake VIEW pic > Repeat until 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, usually 4 at 165kx
- 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



<u>Set up Acquire Points</u>

- 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**

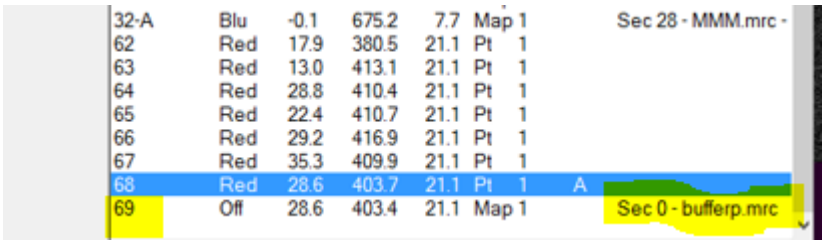


- On Navigator ribbon > Montaging and Grids > Add Grid of Points > Input Polygon number (green number on MMM > On keyboard hit Enter, then Enter again > Acquire points will populate inside polygon in magenta color > Repeat steps for about 20-30 MMMs > Save NAV

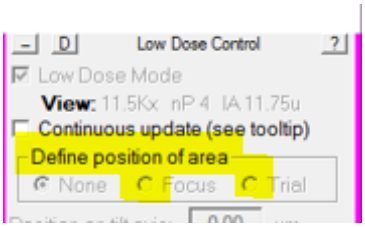
- **Option 2 for selecting acquire points** > See Hole Finder Protocol in Binder

Align to Template Setup and Define Area of Focus/Trial

- 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



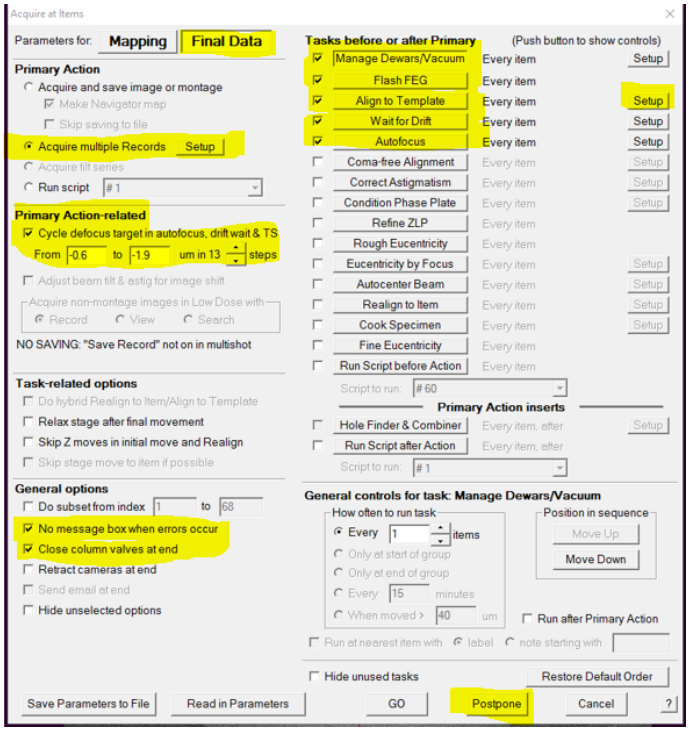
- On Nav re open BufferP/View Aligned Hole > On Low Dose Panel find Define Position of Area



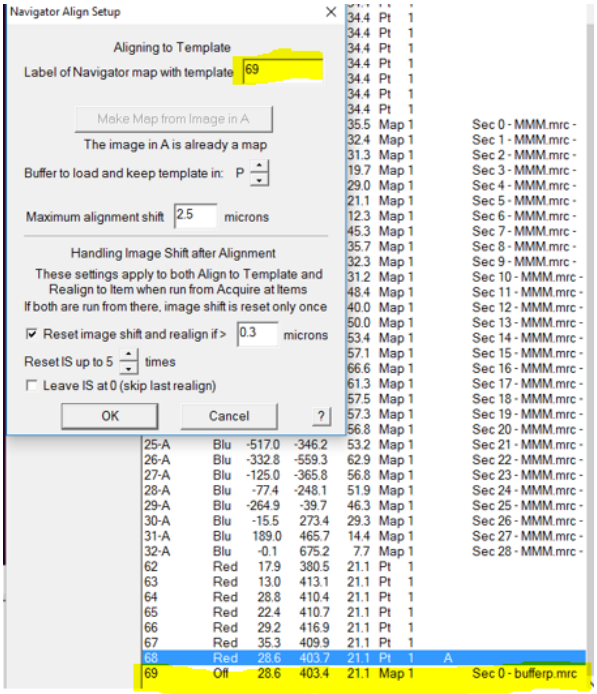
- Click Focus or Trial > F/T area will appear on image > Click on biggest area of carbon visible on image > Area of F/T will move to where you click > Select **none** on low dose panel when desired area is selected

Final Data Setup

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



- Click setup on Align To Template > 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

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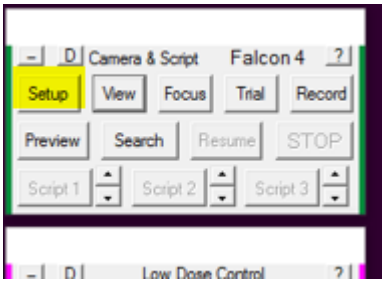
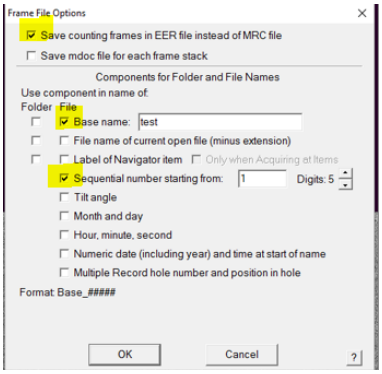


- Go over highlighted areas below

- Record settings bin 4, 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

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