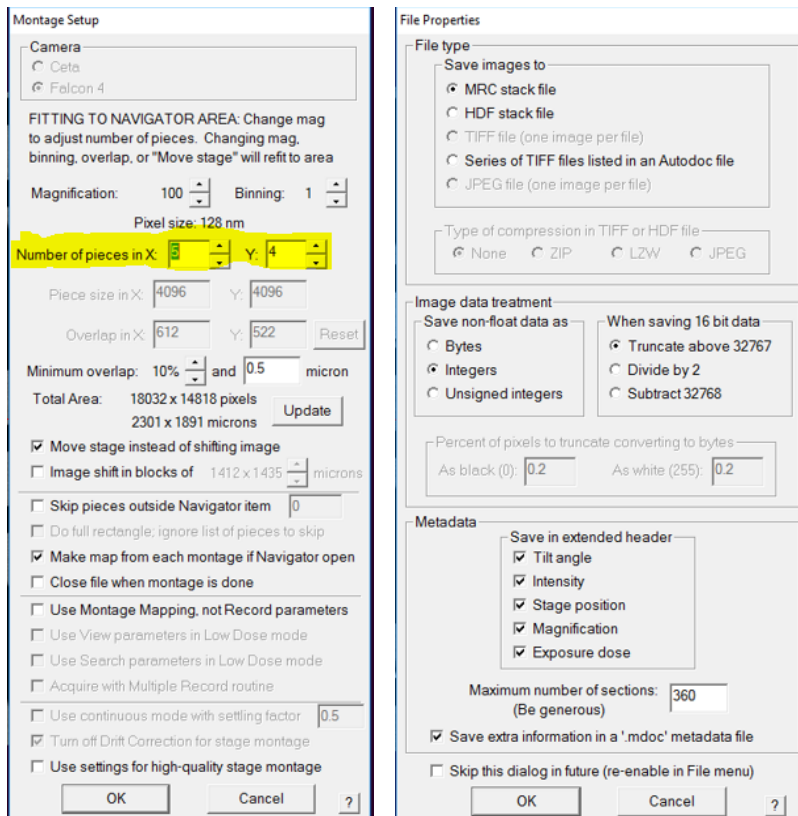


# North Krios Data Collection Protocol v1.1

## Low Mag Maps (LMM)

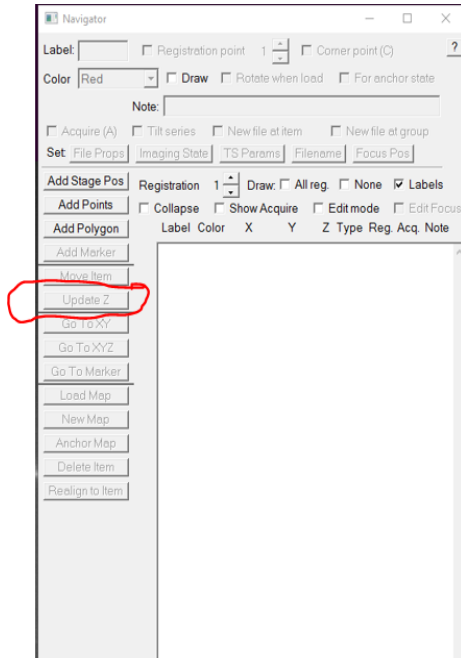
- Spot size same as collection (see cheat sheet for specific parameters), C2 70µm, EFTEM LM175x, Illumination area big enough to cover detector, test with a record pic (Bin 4, .5 secs, longer if CDS mode)
- Navigator Ribbon > Open a Navigator
- Navigator Ribbon > Full Montaging and Grids > Set Up Full Montage > Default Setup and Default Montage Pieces highlighted in yellow > File Properties settings on image below > Save as LMM.st in KEEP folder



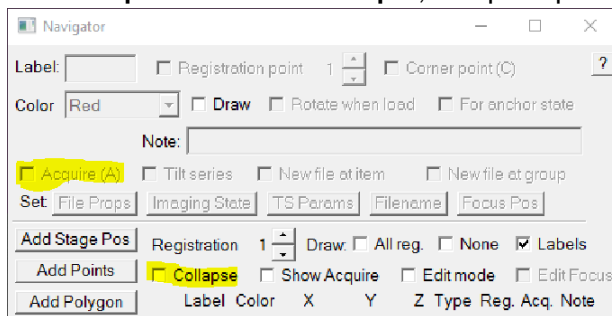
- Montage Controls > Start
- Save NAV after LMM completes by Nav ribbon > Save > Save as Nav in KEEP folder

## Medium Mag Maps (MMM)

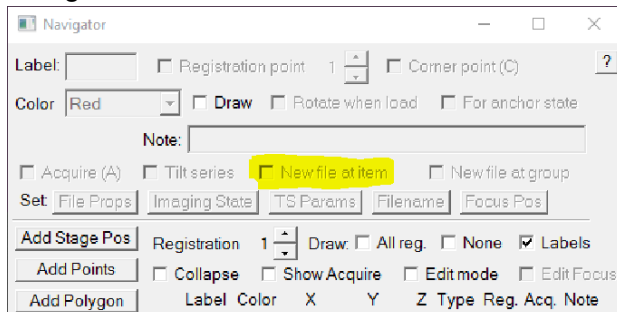
- Spot size same as collection (see cheat sheet for specific parameters), C2 70µm, EFTEM LM540x or whatever fits a single grid square in one shot
- Take a record pic (Bin 1, .5 secs) of a centered square at selected Medium Mag, then Tasks Ribbon > Eucentricity > Rough Eucentricity > Wait for finished message on log window > On Nav window click "Update Z"



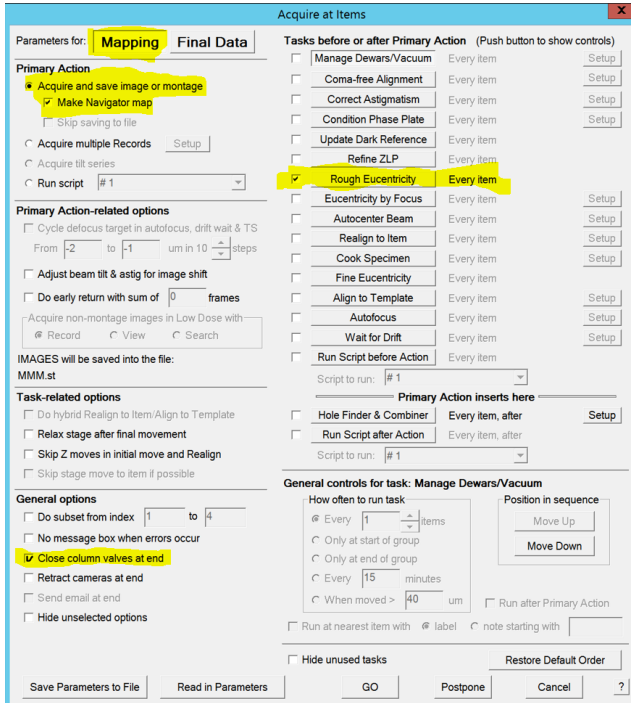
- **Shift To Marker LMM to Medium Mag** - Open LMM (double click) from Nav > Search for distinct feature inside a square > On Nav select **Add Points** > Click on feature and remember where on feature point is made > On Nav click **Stop Adding** > On Nav click **Go to XYZ** > Take Record pic > Feature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



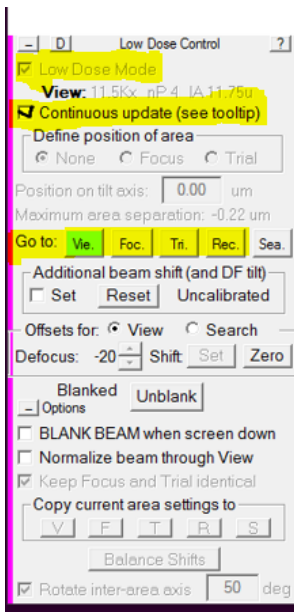
- Nav ribbon > Click on **Acquire at Items** > On new window select settings below > Go



- When MMMs finish > Nav ribbon > Save

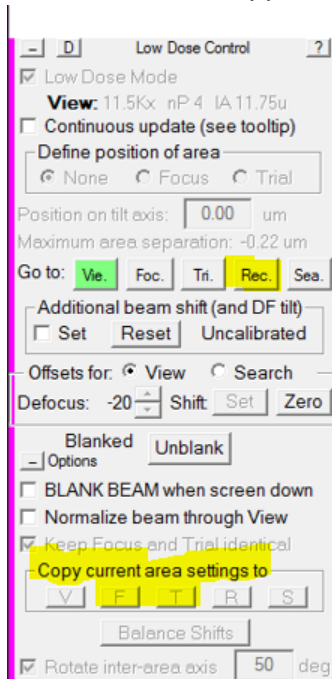
## Low Dose Beam Setup

- 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 70 $\mu$ m for all beams
- **Record beam** - EFTEM, Nanoprobe, SA81k $\times$  or SA105k $\times$

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



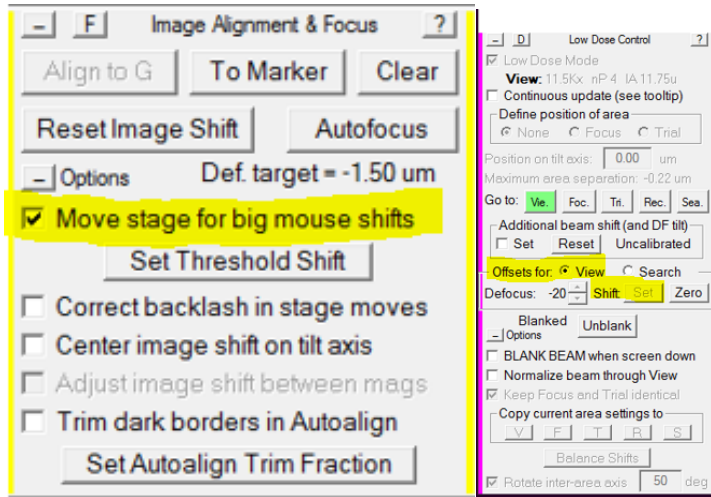
- **View** – EFTEM, Nanoprobe, SA15000× ± one mag, depending on hole size

### 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** > 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

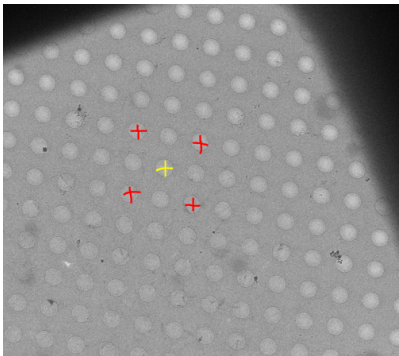
### Align Record Mag to View Mag, View Shift Offset

- 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 FOR BIG MOUSE SHIFTS

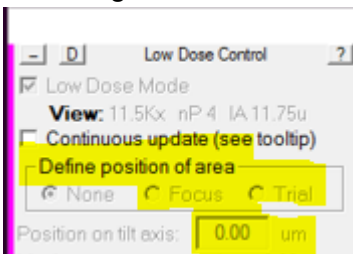


### Multi-shot Parameters Setup

- 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**

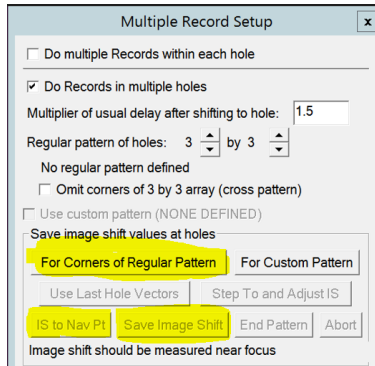


- On Low Dose Control Tile > Define Position of Area click Focus or Trial > On Position on tilt axis box change to zero



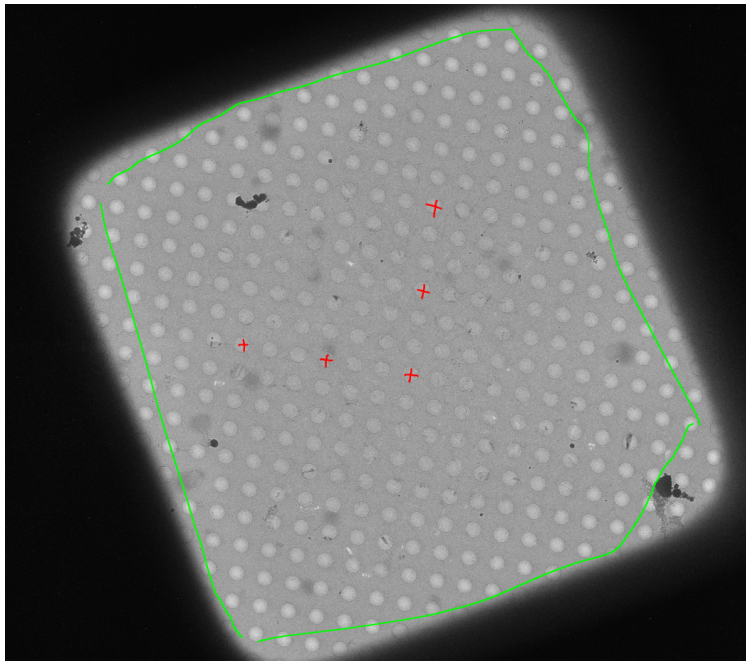
- Take VIEW pic > Drag to carbon area > Take RECORD pic to confirm strong thon rings on FFT > If strong rings present move on to do 3 cals under SerialEM top ribbon > Focus and Tuning >
  - o Correct Astigmatism by CTF
  - o Correct Coma Free Alignment by CTF
  - o Coma vs Image Shift – settings for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



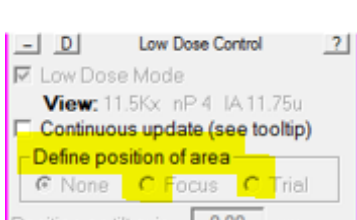
- On Navigator ribbon > Montaging and Grids > Add Grid of Points > Input Polygon number (green number on MMM) > On keyboard hit Enter, then Enter again > Acquire points will populate inside polygon in magenta color > Repeat steps for about 20-30 MMMs > Save NAV
- **Option 2 for selecting acquire points** > 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

| Item | Color | X    | Y     | Z    | Type  | Label               |
|------|-------|------|-------|------|-------|---------------------|
| 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

Parameters for: Mapping Final Data

Primary Action

- Acquire and save image or montage
- Make Navigator map
- Skip saving to file
- Acquire multiple Records **Setup**
- Acquire tilt series
- Run script #1

Primary Action-related

- Cycle defocus target in autofocus, drift wait & TS  
From 0.6 to -1.9 um in 13 steps
- Adjust beam tilt & astig for image shift
- Acquire non-montage images in Low Dose with: Record View Search

NO SAVING. "Save Record" not on in multishot

Task-related options

- Do hybrid Realign to Item/Align to Template
- Relax stage after final movement
- Skip Z moves in initial move and Realign
- Skip stage move to item if possible

General options

- Do subset from index 1 to 68
- No message box when errors occur
- Close column valves at end
- Retract cameras at end
- Send email at end
- Hide unselected options

Tasks before or after Primary (Push button to show controls)

- Manage Dewars/Vacuum Every item **Setup**
- Flash FEG Every item **Setup**
- Align to Template Every item **Setup**
- Wait for Drift Every item **Setup**
- Autofocus Every item **Setup**
- Coma-free Alignment Every item **Setup**
- Correct Astigmatism Every item **Setup**
- Condition Phase Plate Every item **Setup**
- Refine ZLP Every item
- Rough Eucentricity Every item
- Eucentricity by Focus Every item **Setup**
- Autocenter Beam Every item **Setup**
- Realign to Item Every item **Setup**
- Cook Specimen Every item **Setup**
- Fine Eucentricity Every item
- Run Script before Action Every item

Script to run: #60

Primary Action inserts

- Hole Finder & Combiner Every item, after **Setup**
- Run Script after Action Every item, after
- Script to run: #1

General controls for task: Manage Dewars/Vacuum

How often to run task

- Every 1 items
- Only at start of group
- Only at end of group
- Every 15 minutes
- When moved > 40 um

Position in sequence

- 
- 

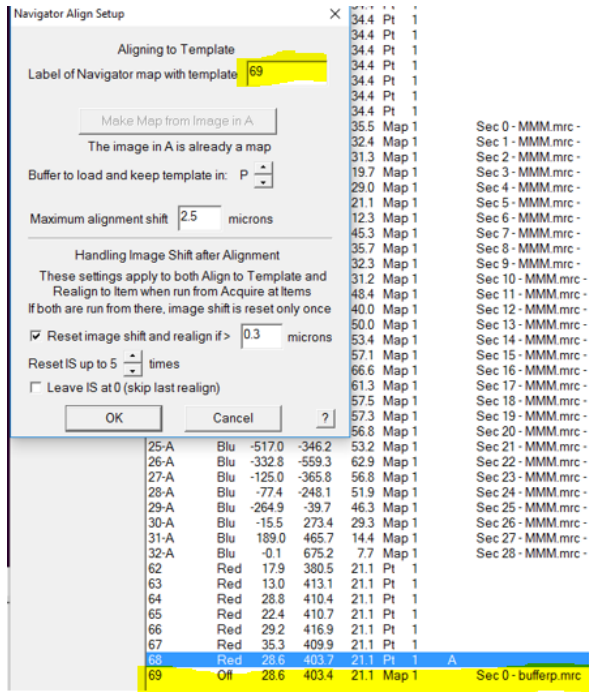
Run after Primary Action

Run at nearest item with label note starting with

Hide unused tasks **Restore Default Order**

Save Parameters to File Read in Parameters GO **Postpone** Cancel ?

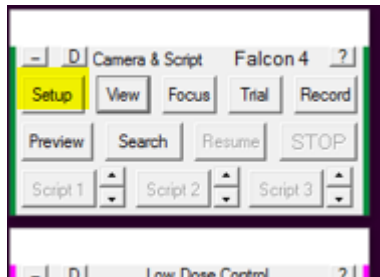
- Click setup on Align To Template > New window will open > Add label number of BufferP > Click ok



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

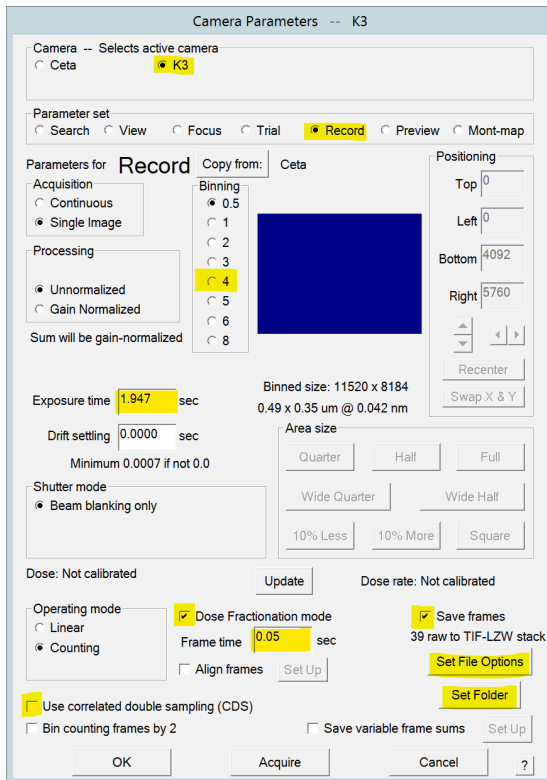
### Final Data Camera Setup

- Go to the Camera Tile > click Setup

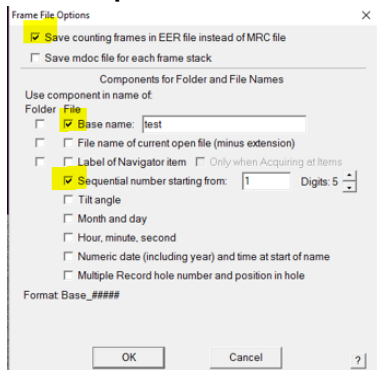


- Go over highlighted areas below





- 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