Arctica Data Collection Protocol V1.0

Low Mag Maps (LMM)

- Spot size 6, C2 150um, EFTEM LM 145x, Illumination area big enough to cover detector, test with a record pic (Bin 4, .3-5 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

lontage Setup	File Properties			
C Ceta	File type			
© Falcon 4	Save images to			
FITTING TO NAVIGATOR AREA: Change mag	 MRC stack file 			
to adjust number of pieces. Changing mag.	C HDF stack file			
binning, overlap, or "Move stage" will refit to area	C TIFF file (one image per file)			
Magnification: 100 📩 Binning: 1 📩	C Series of TIFF files listed in an Autodoc file C JPEG file (one image per file)			
Pixel size: 128 nm				
lumber of pieces in X: 🗧 🔔 Y: 🖣 📮	C None C ZIP C LZW C JPEG			
Piece size in X: 4096 Y: 4096	Image data treatment			
Overlap in X 612 Y: 522 Reset	Save non-float data as When saving 16 bit data			
	C Bytes C Truncate above 32767			
Minimum overlap: 10% - and 0.5 micron	Integers C Divide by 2			
Total Area: 18032 x 14818 pixels	C Unsigned integers C Subtract 32768			
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2301 x 1891 microns	Guisigned integers			
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2301 x 1891 microns ✓ Move stage instead of shifting image □ Image shift in blocks of 1412 x 1435 x microns	Percent of pixels to truncate converting to bytes			
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• Save NAV after LMM completes by Nav ribbon>Save> Save as Nav in KEEP folder

Medium Mag Maps (MMM)

- Spot size 6, C2 50um, EFTEM LM320x to LM540 depending on size of squares.
- Take a record pic (Bin 1, .3-.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

Navigator	- 0	×
Label	Registration point 1 Comer point (C)	2
	<u> </u>	-
Color Red	Draw Rotate when load For anchor state	
	Note:	
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Go To XY		
Go To XYZ		
Go To Marker		
Load Map		
New Map		
Anchor Map		
Delete Item		
Realign to Item		

1

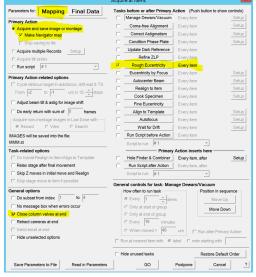
- Shift To Marker LMM to Medium Mag Open LMM (double click) from Nav> Search for distinct feature inside a square>On Nav select Add Points> Click on feature and remember where on feature point is made>On Nav click Stop Adding>on Nav click Go to XYZ> Take Record pic> Feature should be on pic but not exactly 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

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

change noi				
Navigator		_		\times
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Add Polygon	Label Color X Y Z T	ype Reg	j. Acq. N	ote

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



When MMS finish>Nav ribbon>save

Created by Jose Adrian Martinez 8.2.23

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, C2 50um
- Record beam EFTEM, NanoProbe, SA79kx or 100kx, Energy Slit in @20ev
- Trial and Focus Copy settings from Rec to Trial and Focus, , Energy Slit in @20ev



• View - EFTEM, NanoProbe, SA1100x to SA19500 depending on hole size, Energy Slit out

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

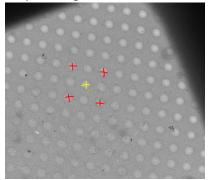
Align Record Mag to View Mag, View Shift Offset

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 ...

_ F Image Alignment & Focus ?	_ D Low Dose Control ?
Align to G To Marker Clear	✓ Low Dose Mode View: 11.5Kx nP.4 IA.11.75u Continuous update (see tooltip)
Reset Image Shift Autofocus	Cefine position of area
_ Options Def. target = -1.50 um	Position on tilt axis: 0.00 um Maximum area separation: -0.22 um
Move stage for big mouse shifts	Go to: <u>Vie.</u> Foc. Tri. Rec. Sea. Additional beam shift (and DF tilt) Set Reset Uncalibrated
Set Threshold Shift	Offsets for: View Search
 Correct backlash in stage moves Center image shift on tilt axis Adjust image shift between mags 	Defocus: -20 <u></u>
Trim dark borders in Autoalign	Copy current area settings to
Set Autoalign Trim Fraction	Balance Shifts

Multi-shot Parameters Setup

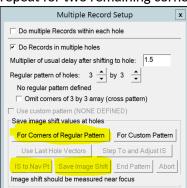
 On NAV open MMM> create 9 hole pattern highlighted below> make center point (yellow) by Add Points> Stop Adding > add 4 corner points (red) by Add Points> make 4 corners points> Stop Adding



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

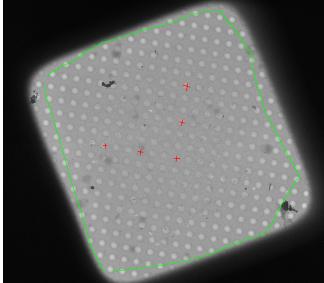


- Take VIEW pic > drag to carbon area > take RECORD pic to confirm strong thon rings on FFT > if strong rings present move on to do 3 cals under SerialEM top ribbon> Focus and Tuning >
 - 0 Correct Astigmatism by CTF
 - 0 Correct Coma Free Alignment by CTF
 - 0 Coma vs Image Shift settings for depend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



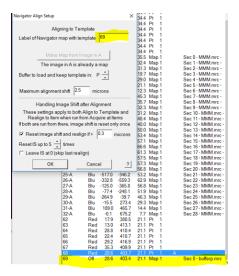
- On Navigator ribbon > Montaging and Grids > Add Grid of Points > input Polygon number (green number on MM > on keyboard hit Enter, then Enter again> acquire points will populate inside polygon in magenta color> repeat steps for about 20-30 MMMs> save NAV
- **Option 2 for selecting acquire points** > 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 A
69	Off	28.6	403.4	21.1	Мар	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



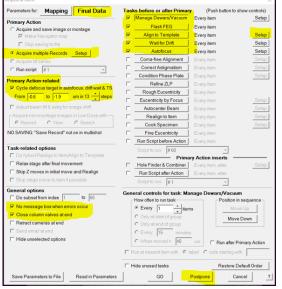
on Nav re open BufferP/View Aligned Hole > on Low Dose Panel find Define Position of Area >



 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

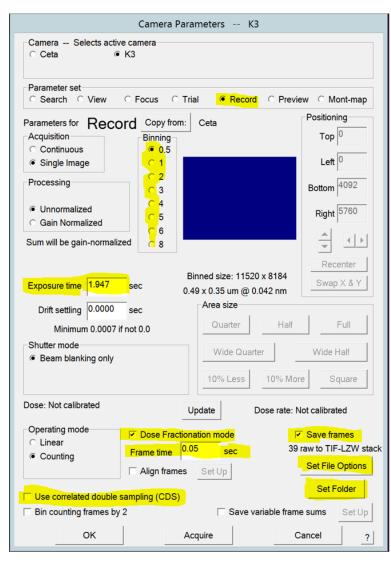
Created by Jose Adrian Martinez 8.2.23

Final Data Camera Setup

• Go to the Camera Tile > click Setup

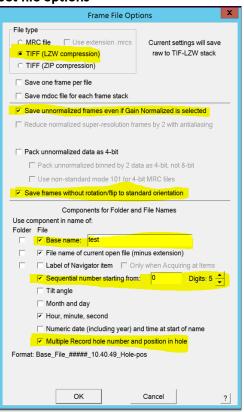


• Go over highlighted areas below



• 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