## **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	<ul> <li>MRC stack file</li> </ul>			
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			
Total Alea. Toos2 X 14010 pixels	Consigned integers Coublact 52766			
2301 x 1891 microns	Guisigned integers			
Lindate	Percent of pixels to truncate converting to bytes			
2301 x 1891 microns Update				
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-			
2301 x 1891 microns Update ✓ Move stage instead of shifting image □ Image shift in blocks of 1412 x 1435 - microns □ Skip pieces outside Navigator item 0	Percent of pixels to truncate converting to bytes As black (0): 02 As white (255): 02			
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			
2301 x 1891 microns Update Vote stage instead of shifting image Image shift in blocks of 1412 x 145 $\frac{1}{2}$ microns Skip pieces outside Navigator item Do full rectoring: ingree Stof bytes to skip Vote store store store shore store shore sho	Percent of pixels to transfer converting to bytes As block (0) 02 As white (255) 02 Metadata			
2301 x 1831 microns Update	Percent of pixels to truncate converting to bytesAs black (0): 02As white (255): 02			
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2301 x 1831 microns         Update           Ø Move stage instead of shifting image         Image shift indicks of 1142 x 145 microns           Image shift indicks of 1142 x 145 microns         Skip pieces outside Navigator item           D to full nectongle ignore is for pieces to skip         Make map forn each montage if Navigator open           Close file when montage is not Record parameters         Use Wontage Mapping, not Record parameters           U use Vene parameters in Low Dose mode         Use Sector parameters in Low Dose mode	Percent of pluets to tuncete converting to bytes - As black (0): [92] As white (25): [92] Metadata Save in extended header [97] Tit angle [97] Itangle [97] Itangle			
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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:	
C Acquire (A)	🗖 Tilt series 🖉 New file at item 👘 New file at group	
Set File Props	Imaging State TS Params Filename Focus Pos	
Add Stage Pos	Registration 1 + Draw: TAll reg. T None 🔽 Labe	ls
Add Points	Collapse Show Acquire Editmode Edit	
Add Polygon	Label Color X Y Z Type Reg. Acq. N	ote
Add Marker		1
Move Item		
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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

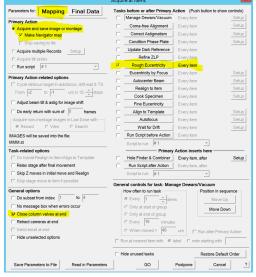
- Shift To Marker LMM to Medium Mag Open LMM (double click) from Nav> Search for distinct feature inside a square>On Nav select Add Points> Click on feature and remember where on feature point is made>On Nav click Stop Adding>on Nav click Go to XYZ> Take Record pic> Feature should be on pic but not exactly 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		-		$\times$
Label:	🗖 Registration point 🛛 📑 🗖 Corne	r point (C	)	?
Color Red	🚽 🗖 Draw 🔲 Rotate when load 🛛	For and	chor state	9
	Note:			
Acquire (A)	🗖 Tilt series 🛛 🗖 New file at item 👘 🗍	Newfile	at group	
Set File Props	Imaging State TS Params Filename	Focus	Pos	
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Add Points	Collapse Show Acquire Edit	mode	🗖 Edit I	Focus
Add Polygon	Label Color X Y Z Ty	pe Reg	i, Acq. N	lote

 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		_		$\times$
Label:	🗖 Registration point 🛛 1 💻 🗖 Corne	ər point (C	3	?
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Set File Props	Imaging State TS Params Filename	Focus	Pos	
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Add Points	🗆 Collapse 🗖 Show Acquire 🗖 Ed	it mode	🔲 Edit P	ocus
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

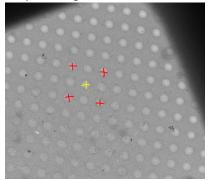
# Align Record Mag to View Mag, View Shift Offset

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 ...

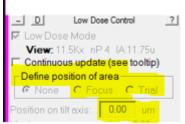
_ F Image Alignment & Focus ?	_ D Low Dose Control ?
Align to G To Marker Clear	✓ Low Dose Mode     View: 11.5Kx nP.4 IA.11.75u     Continuous update (see tooltip)
Reset Image Shift Autofocus	Cefine position of area
_ Options Def. target = -1.50 um	Position on tilt axis: 0.00 um Maximum area separation: -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
<ul> <li>Correct backlash in stage moves</li> <li>Center image shift on tilt axis</li> <li>Adjust image shift between mags</li> </ul>	Defocus: -20 <u></u>
Trim dark borders in Autoalign	Copy current area settings to
Set Autoalign Trim Fraction	Balance Shifts

#### Multi-shot Parameters Setup

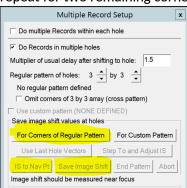
 On NAV open MMM> create 9 hole pattern highlighted below> make center point (yellow) by Add Points> Stop Adding > add 4 corner points (red) by Add Points> make 4 corners points> Stop Adding



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

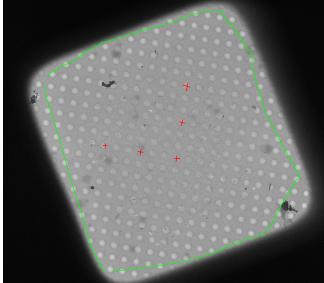


- Take VIEW pic > drag to carbon area > take RECORD pic to confirm strong thon rings on FFT > if strong rings present move on to do 3 cals under SerialEM top ribbon> Focus and Tuning >
  - 0 Correct Astigmatism by CTF
  - 0 Correct Coma Free Alignment by CTF
  - 0 Coma vs Image Shift settings for depend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



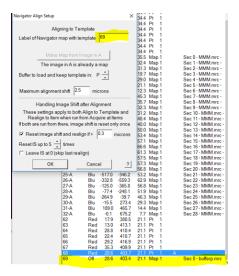
- On Navigator ribbon > Montaging and Grids > Add Grid of Points > input Polygon number (green number on MM > on keyboard hit Enter, then Enter again> acquire points will populate inside polygon in magenta color> repeat steps for about 20-30 MMMs> save NAV
- **Option 2 for selecting acquire points**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



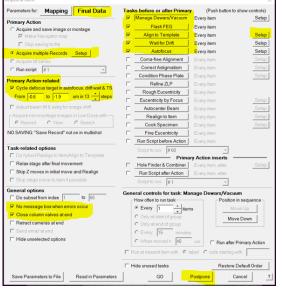
on Nav re open BufferP/View Aligned Hole > on Low Dose Panel find Define Position of Area >



 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

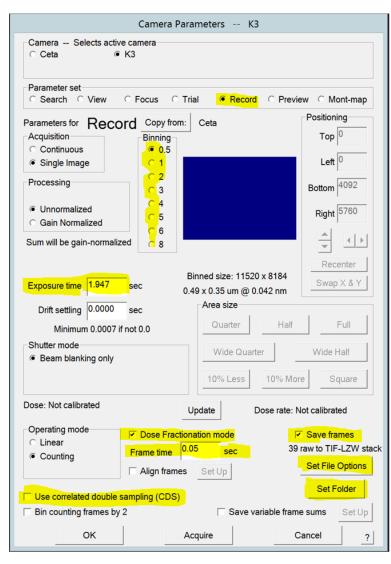
Created by Jose Adrian Martinez 8.2.23

## Final Data Camera Setup

• Go to the Camera Tile > click Setup

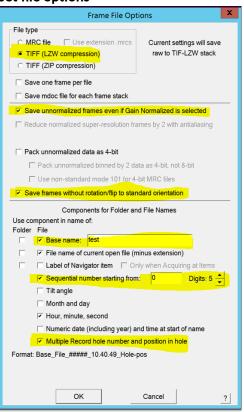


• Go over highlighted areas below



• 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