Low dose serial EM tomography setup for UTSW Krios

Main steps

- 1. Align scope
- 2. Set up LMM shot on Standard grid
- 3. Insert sample grid, take practice shot, set up and start LMM.st
- 4. Navigator shift to marker at 2250X
- 5. Set up and run several MMM.st with polygon Montage
- 6. Set up Serial EM low dose mode
- 7. Set view shift offset
- 8. Navigator shift to marker at 6700X (or desired view mag)
- 9. Make grid of points
- 10. Set file and folder options, edit and run script 10 SPA

Check health status of K3 camera, must be all green

After aligning scope, set up LMM shot and take a record

- -SS6-7
- -135X
- -100 micron C2 app.
- -no objective app.
- -C2 100% spread
- -record binning 4, counting mode, .2s-.3s exposure, NOT DOSE FRACTIONATED

Insert grid of interest in autoloader control panel by clicking "load"

Setup LMM.st

-Open navigator in serial EM

-Navigator -> montaging and grids -> setup full montage

-Set the number of tiles, mag, binning etc (135x 7X9).

- -Click OK, save all information in extended header (check all 5 boxes)
- -Select save as MRC stack file, click OK

-Save as LMM.st in KEEP folder

Montage Setup	
Camera BM-Ceta	
© K2-0001	File Properties
FITTING TO NAVIGATOR AREA: Change mag to adjust number of pieces. Changing mag, binning, overlap, or "Move stage" will refit to area Magnification: 46 + Binning: 2 +	Save data as When saving 16 bit data Bytes Truncate above 32767 Integers Divide by 2 Subtract 32768
Pixel size: 170 nm	Number of pixels to truncate converting to bytes
Number of pieces in X: Y: 10 +	As black (0): 40 As white (255): 40
Piece size in X: 1740 Y: 1878 Overlap in X: 574 Y: 574 Reset Minimum overlap: 30% → and 1 micron Total Area: 12234 x 13614 pixels 2081.7 x 2316.5 microns Update ✓ Move stage instead of shifting image	Save in extended header Tilt angle Intensity Stage position Magnification Exposure dose Maximum number of sections: (Be generous) 360
Skip pieces outside Navigator item	Save extra information in a '.mdoc' metadata file
 Do full rectangle; ignore list of pieces to skip Ask about making map after each montage Use View parameters in Low Dose mode Use continuous mode with settling factor Use continuous mode with settling factor Turn off Drift Correction for stage montage Use settings for high-quality stage montage 	MRC stack file TIFF file (one image per file) Series of TIFF files listed in an Autodoc file Type of compression in TIFF file None ZIP LZW
OK Cancel ?	OK Cancel ?

Note* you have now created directions to run LMM.st and should see the LMM.st file open at the top of the screen, but you need to click START in the montage control panel to run the LMM.st. uncheck the "treat as very sloppy montage" box and click START. approximate run time 15mins.

When LMM is finished SAVE NAVIGATOR as Nav1.nav

Do eucentricity

-Go to 2250X

-Condense beam (III area approximately 50µm) then center with beam shift

-Put in Obj app (100 microns)

-defocus for contrast in practice shot

-Tasks -> Eucentric Rough

-Update Z of the LMM and any points.

Find a feature and shift to marker

-Identify large recognizable feature in LMM

-In the navigator select Add Points, then add a point on the feature

-Select stop adding

-Highlight the point in the navigator and click 'Go to XY'

-Take a record image

-Right click and drag to move stage, taking record images until feature is found

* Note: If you do not find the feature, you can always click "go to XY" again, and go in another direction to search for the feature. If you still cannot find it, you click "go to XY", then you can put your screen down and move the stage with the joy stick. Remember, you should always try to avoid putting your screen down during data collection, as this will dose damage your sample.

-When the feature is in the record image, put green marker (left click) in the feature

-Select Navigator -> shift to marker, verify that the shift is a reasonable distance, and click OK

-This process can be repeated until LMM and 2250X are well correlated

Note* If the shift is not correct, you can always undo this by clicking Navigator -> undo last shift.

Setup and run several MMM.st

-Add Nav points in the centers of desirable squares

-Make all points "acquire" points by ticking the acquire box in the navigator

-Add a square polygon to the inside of desirable square

- Highlight the polygon in the navigator, click Navigator -> Montaging and Grids -> Set up polygon montage. the MMM.st file (3x4- 5x7) depending on mesh) bin 4, .4s exposure counting mode *** deselect "ask about making map after each montage" box.

-Click OK

-Save all info in extended header

-Click OK, save as MMM.st in KEEP folder

-Click Navigator -> acquire at points

-Tick the rough eucentricity box and select acquire map image, select "close column valves at the end".

-Click GO

Montage Setup	
Camera O BM-Ceta O K2-0001	Acquire at Items
Magnification: 940 - Binning: 1 -	Initial Actions after Moving Stage ✓ Rough eucentricity
Pixel size: 4.44 nm	Autocenter beam
Number of pieces in X: 5 Y: 5 •	Realign to item
Piece size in X: 3708 Y: 3838	
	I Fine eucentricity
Overlap in X: 1112 Y: 1150 Reset	🗖 Autofocus 🗖 Only at start of group
Minimum overlap: 30% — and 1 micron	Run script SPAK2
Total Area: 14092 x 14590 pixels	Primary Task
62.6 x 64.8 microns	Acquire map image or montage
Move stage instead of shifting image	O Just acquire and save image or montage
Skip pieces outside Navigator item	© Run script SPAK2
Do full rectangle; ignore list of pieces to skip	C Acquire tilt series
🔽 Ask about making map after each montage	Montages will be saved into the file:
After a montage, open message box asking if you want to	Grid_Training-T/May30_01.mrc
Use continuous mode with settling factor 0.5	Restore scope state after aligning to item
Turn off Drift Correction for stage montage	Close column valves at end
Use settings for high-quality stage montage	Send email at end
OK Cancel ?	GO Cancel ?

When LMMs are done SAVE THE NAVIGATOR

<u>Go to undesirable area (using LMM or preferably on a bad MMM) and turn on</u> <u>Low Dose Mode</u>

Set up Low Dose Mode in serial EM

-Remember to check "continuous update"

View: 4800X, .1s-.2s, bin 4, full, SS7, C2

Trial: 26,000X, .1s-.2s, bin 4, full, SS7, C2 (non-parallel beam is OK)

Focus: 26,000X, .1s-.2s, bin 2-4, wide half, SS7 (non-parallel beam is OK)

Preview: 26,000X, .05s, bin 4-8, full, SS6, (parallel beam)

Record: 26,000X, 2.5+s, SS5, bin .5-1 (super res. or counting), full, C2 41.2% (parallel beam) dose fractionation on, .05-.1s frames.

-Take a view and set the "define position" of area for Focus and Trial, can also check "Keep Focus and Trial Identical"

-Center all beams, starting with the preview/record beam, then trial/focus, then View. This can be done with Beam shift and X,Y MF knobs, go to bad area and put screen down to center beams.

Note* never offset the record/preview beam, only add offsets to the F/T/V beams.

Set View Shift Offset

-Center feature in Preview image

-Take View image

-Check continuous update

-Uncheck "move stage for big mouse shifts" in the Image alignment and focus control panel.

-right click and drag to center feature in view crosshair

- Under offsets for view click "set"

-Take preview to insure feature is centered, repeat if necessary

Calibrate/calculate electron dose in hole with 1s bin1 exposure at record beam settings

Click Calibration -> electron dose and follow prompts

Set file options in the setup window for the dose fractionated record beam.

-Save as normalized .MRC file

Note To save space, one can select LZW compressed TIFF, and "do not normalize images even if gain normalized is selected" file type in the setup. But in the file options select "Save unnormalized even if gain normalized is selected". Make sure to copy a copy of the gain reference (In the C -> Program Data -> Gatan -> Reference Images (all of them should be copied to your Images-Disc folder. This is not necessary for tomography data.



Edit the dose symmetric script, change tilt time to ~ 15 and click OK

Save the Navigator

Do Coma (objective out) and then objective Stig. (objective in) on bad carbon area

Set up Volta Phase Plate

-Go to an empty area

-insert VPP

-find blow up point either on screen in high contrast or on K3 with continuous preview

-Stigmate condenser 2 to make features round

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-repeat steps if desired
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-charge VPP ~ 180 seconds at SS7.

Note * 50 nano coulumbs of charge is necessary to achieve proper (90 degree) phase shift. To calculate, read the screen current with screen down in record conditions with VPP out. Then divide 50 by that number to get the number of seconds required for charging.

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-run script 2 Dose symmetric tomo
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-rinse repeat

Updated 4/22/19 D.S.

Acquire at Items	ĸ
Initial Actions after Moving Stage	
Rough eucentricity	
Autocenter beam	
Realign to item	
Cook specimen	
Fine eucentricity	
Autofocus Only at start of group	
Run script SPAK2	
· ,	
Primary Task	
 Acquire map image or montage 	
O Just acquire and save image or montage	
Run script SPAK2	
O Acquire tilt series	
Rectare coope state after aligning to item	
Resole scope state alter aligning to term	
Close column valves at end	
Send email at end	
GO Cancel 2	

Updated 4/22/19 D.S.