

## 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

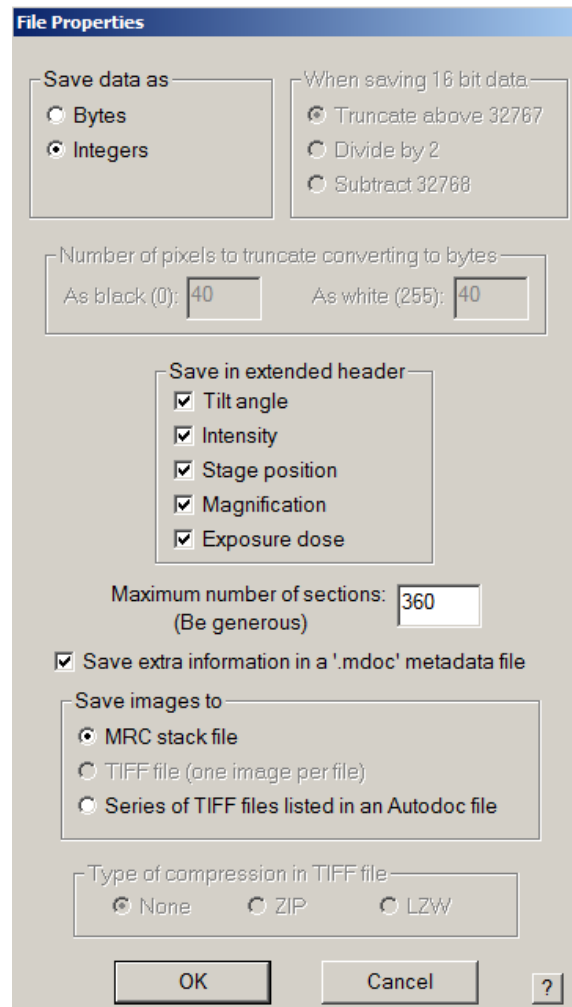
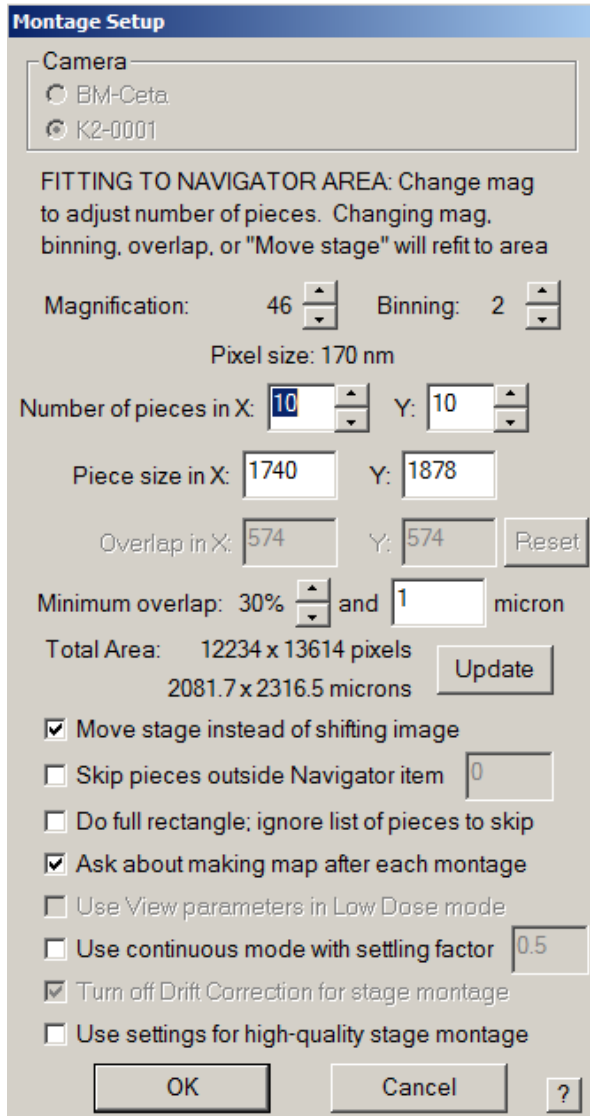
-record binning 4, counting mode, .2s-.3s exposure, NOT DOSE FRACTIONATED

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



**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 (Ill area approximately 50 $\mu$ m) 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

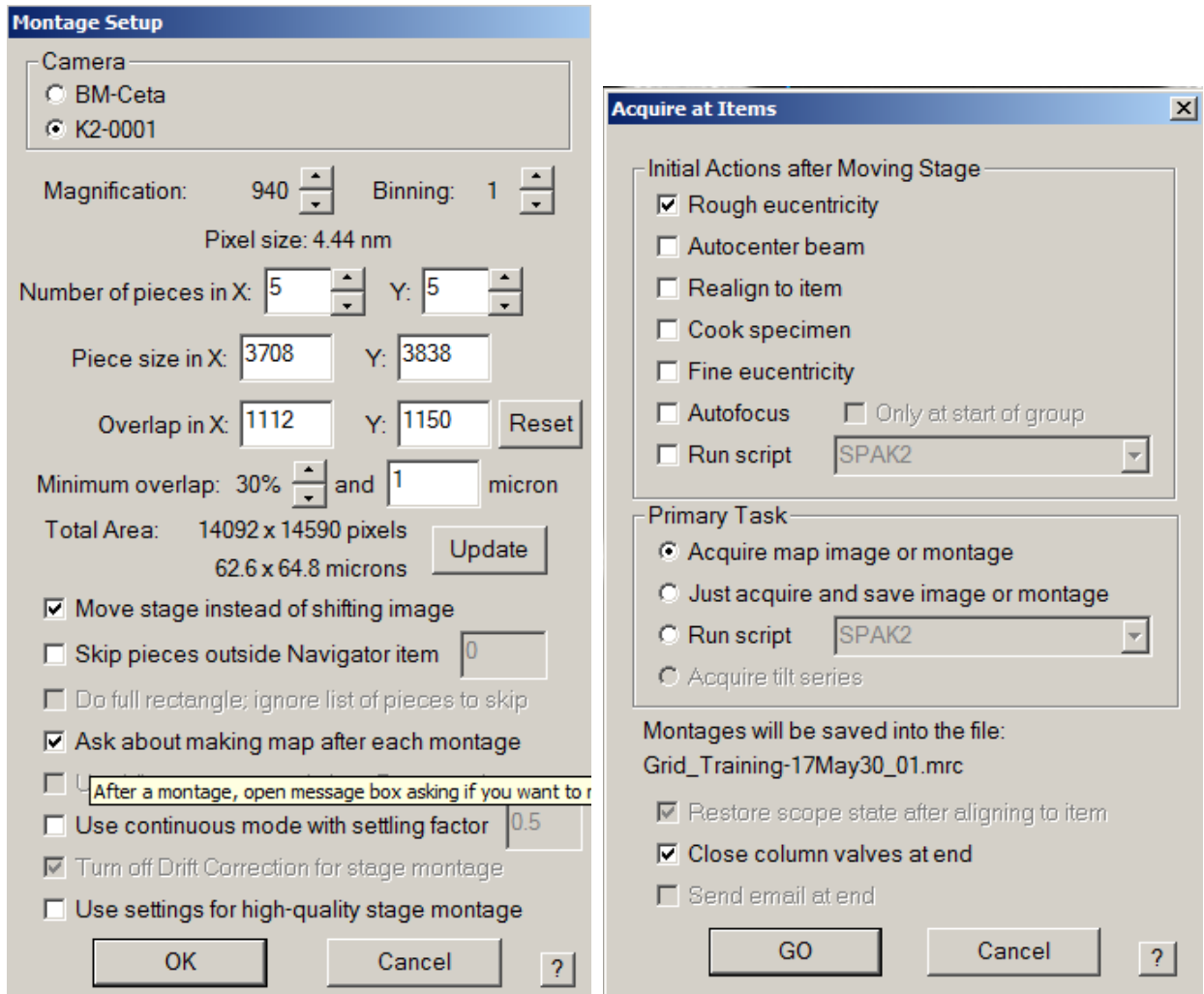
-Save all info in extended header

-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



**When LMMs are done SAVE THE NAVIGATOR**

**Go to undesirable area (using LMM or preferably on a bad MMM) and turn on Low Dose Mode**

**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**

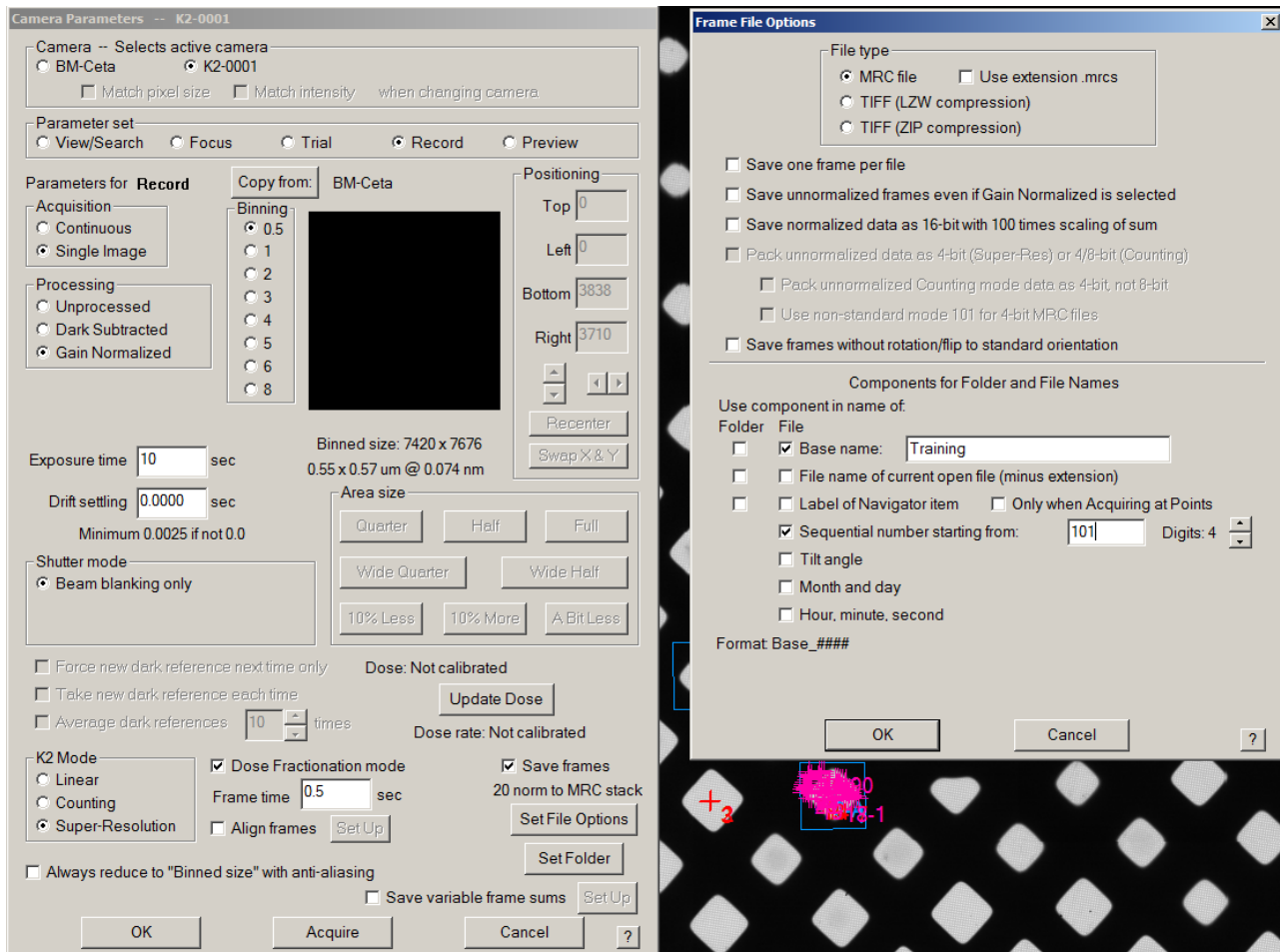
**Click Calibration -> electron dose and follow prompts**

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

Updated 4/22/19 D.S.

## -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**

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**-insert VPP**

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

**-Stigmat condenser 2 to make features round**

**-repeat steps if desired**

**-charge VPP ~ 180 seconds at SS7.**

**Note \* 50 nano coulombs 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.**

**-run script 2 Dose symmetric tomo**

**-rinse repeat**



**Acquire at Items** [X]

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 [v]

Primary Task

- Acquire map image or montage
- Just acquire and save image or montage
- Run script      SPAK2 [v]
- Acquire tilt series

Restore scope state after aligning to item

Close column valves at end

Send email at end

GO      Cancel      ?

Updated 4/22/19 D.S.