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Low dose serial EM setup for UTSW Talos Arctica

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 280-500X**
5. **Set up and run several MMM.st**
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. **Setup Multishot/Multihole parameters**
10. **Make grid of points**
11. **Set file and folder options, edit and run script 10 SPA**

Check health status of K2 camera, must be all green

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

-SS5-8

-46X

-150 micron C2 app.

-No objective app

-C2 100% spread

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

Insert grid of interest in autoloader control panel by clicking “load”

Setup LMM.st 5X7 or 5X8

-Open navigator in serial EM

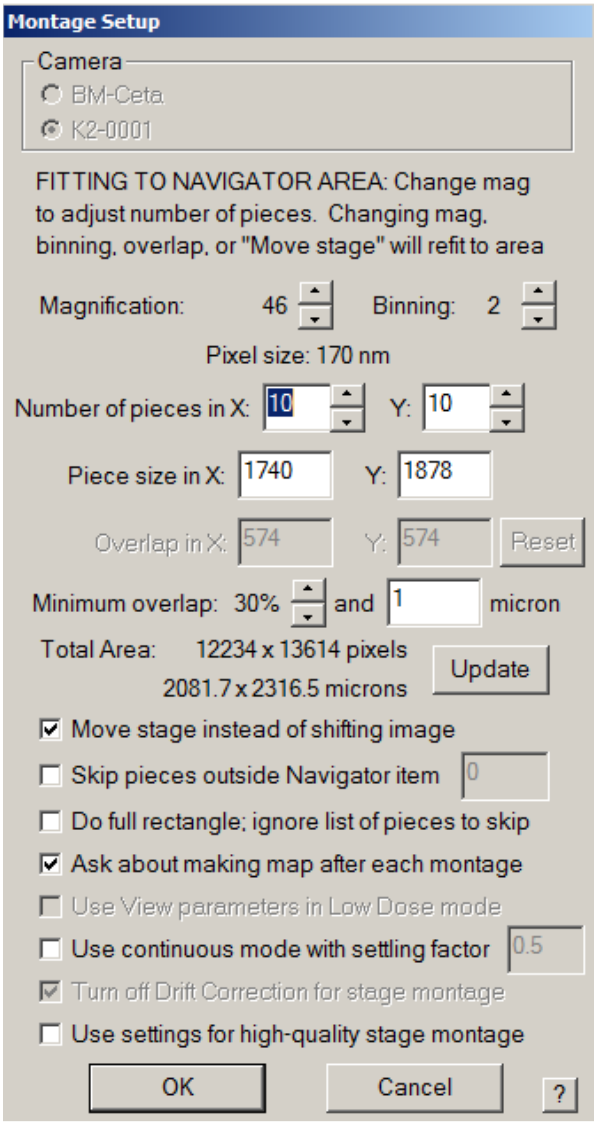
-Navigator -> montaging and grids -> setup full montage

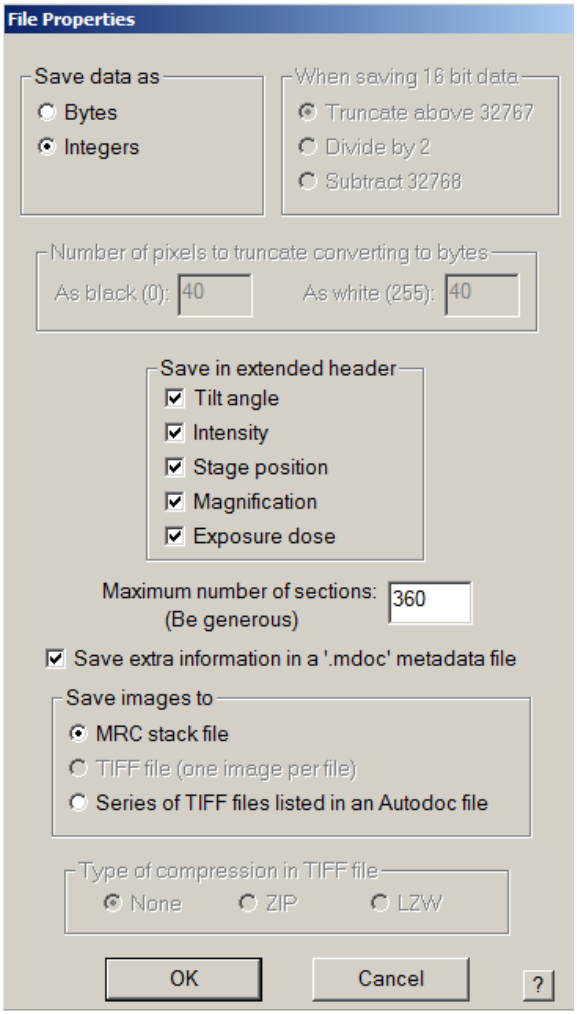
-Set the number of tiles, mag, binning etc.

-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 left 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 12-15 mins.

When LMM is finished SAVE NAVIGATOR as Nav1.nav

Do eucentricity

-Insert 50 micron C2 aperture

-Go to 280-500X (depends on the mesh size)

-Condense beam (C2 approximately 50-70%), then center with beam shift

-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 280X 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

-Go to first point and tick the "new file at item" box

-Set up the MMM.st file (1X1) bin 1, .3s 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

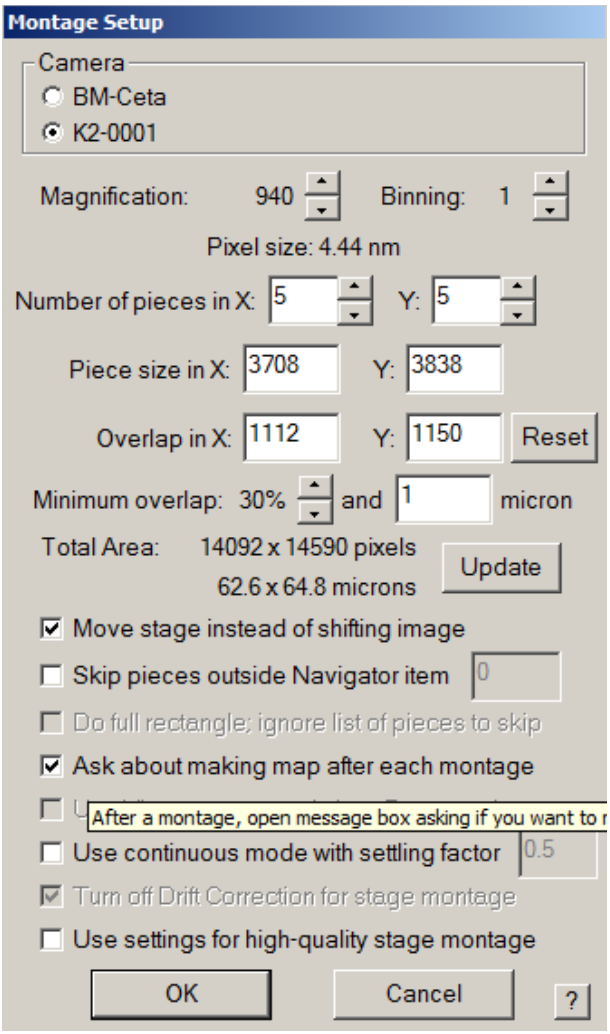
-Select each acquire point in the navigator and check the "new file at item" box

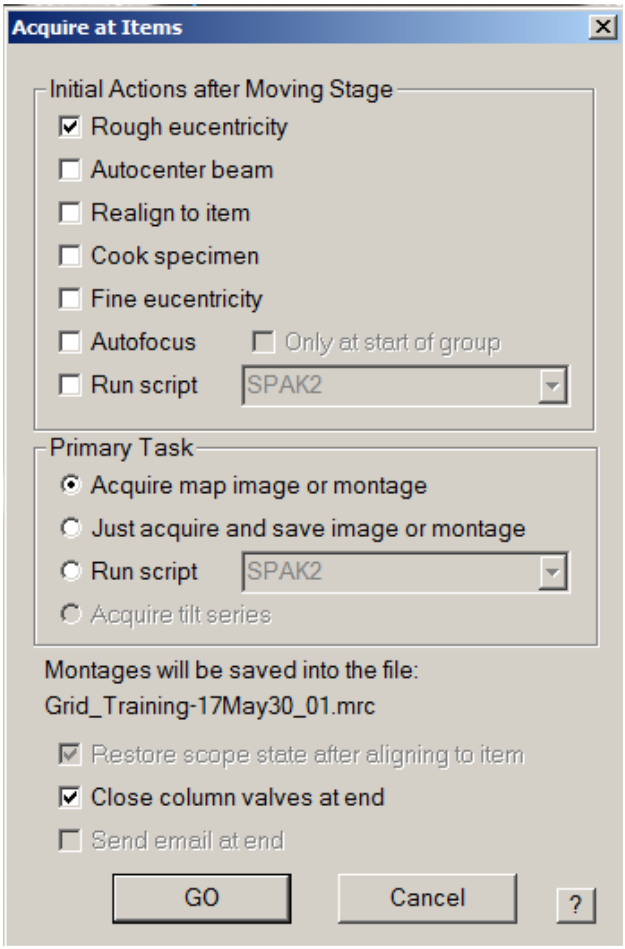
-Click Navigator -> acquire at points

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

-Click GO

Updated 11/08/19 D.S.





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: 6700X, .1s-3s, bin 4, full, SS5 np C2 50%-55%

Trial: 45,000X, .1s-3s, bin 4, full, SS5 np

Focus: 45,000X, .1s-3s, bin 2-4, wide half, SS5 np

Preview: 45,000X, .2s, bin 4-8, full, SS5, C2 41.2% (parallel beam) np

Record: 45,000X, 2+ s, bin .5X or 1X (super res. or counting), full, C2 41.2% (parallel beam), SS5 np, dose fractionation on, .05s frames.

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

-Insert objective aperture and center if necessary

-Center all beams, starting with the preview/record beam, then trial/focus, then View. Go to bad area or bad map 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

Do the Navigator-> shift to marker procedure on a MMM, finding a feature as described above.

Setup multishot/multihole collection parameters

(9 holes)-Add a nav point in the center of a group of 9 holes (for best results, the edge of the hole can be used for exact template centering) and go to it

Note* make sure it is a flat area of the quantifoil film. You can check to see if it is flat by tilting to 60 degrees at low mag.

-Tune the Coma and Objective Astigmatisim of the Record beam (use continuous preview, or the automated functions in serialEM that use CTF under the focus/tune tab)

-Run the Coma Vs. Image shift calibration under Calibration -> focus Tuning -> Coma vs. Image shift.

Note* This calibration needs to be done at your exact record conditions and alignment. If anything other than beam shift has been changed since the last session, this needs to be recalibrated.

- Make sure record beam is at -1-2 microns defocus. Go to 0 defocus offset with the view beam, (usually -20 microns is used for view at 6700x or 8500x)

-Uncheck the "move stage for big mouse shifts box", make sure image shift is zeroed and right click and drag to add points to the neighboring holes. Either four corners of your pattern, or all points in the pattern i.e. "for corners of a regular pattern" or "Custom pattern".

-Add points to LMM at four corners.

(Points could also be added using the view beam with image shift, (right click and drag).

Note* having features in your template setup area is helpful. If there are no features, you can always burn in a feature with the beam to use as a waypoint. Do this with the screen down by condensing your view beam.

-Click Navigator-> montaging and grids-> set multi-shot parameters

-select "do records in multiple holes"

-select "Use record illuminated area for circles in low dose"

-Select regular pattern of holes, 3X3

Note* If you cannot see the circles of your pattern click the center of your screen. Note, the holes may not match up between the view mag and MMM mag, we will test for accuracy later so don't worry about this now.

-Select the first of the four (or nine) nav points in the navigator window.

-Click "for corners of a regular pattern" or "Custom pattern (for all 9 holes)" button in the multishot setup menu

- Make sure you "Click IS to NAV pt" and the second point will turn yellow

-Take a view shot (6700X) and center the hole by right clicking and dragging to image shift. Then retake the view to ensure that the hole is centered

Note* To improve accuracy of the image shift, one can go through a second round of template setup, centering template on the edge of the holes, taking a preview image after shifting the view beam to insure that the edge of the hole is in view. If additional image shift needs to be added, add it here before saving IS. This has not been necessary for single shots/hole.

-Click "OK" to exit the setup window

-Return view defocus offset to -20 (or whatever is desired).

Note* If your rotation center/coma free alignment are done accurately, there should be almost no movement between 0 and -20 defocus offset with the view beam.

-Save NAV.

-Recheck "Move stage for big mouse shifts"

-Test template by running the macro on your center point, change this point to be an acquire point, Navigator -> acquire at points.

- You must make sure that Multiplerecords is present and not commented out in the SPA macro for multishot to work. This script is number 13 on the Arctica SPAK3 Multiplerecords

Add grid of points

-Add 3 corner and two end points of rectangle on MMM

-Navigator-> add grid of points, then select option to turn Acquire on for all points. One can add the center of a 4 or 9 hole pattern , but it may be easier to add 9 hole patterned point centers manually if there are few holes available/gridsquare.

-Repeat for each MMM

X o o o o

o o o o o

o o o o o

o o o o o

X o o o o

X X o o X

Test V align to P

-Go to clean hole that will not be collected

-Center view image

-Save to P buffer

-Make image not centered

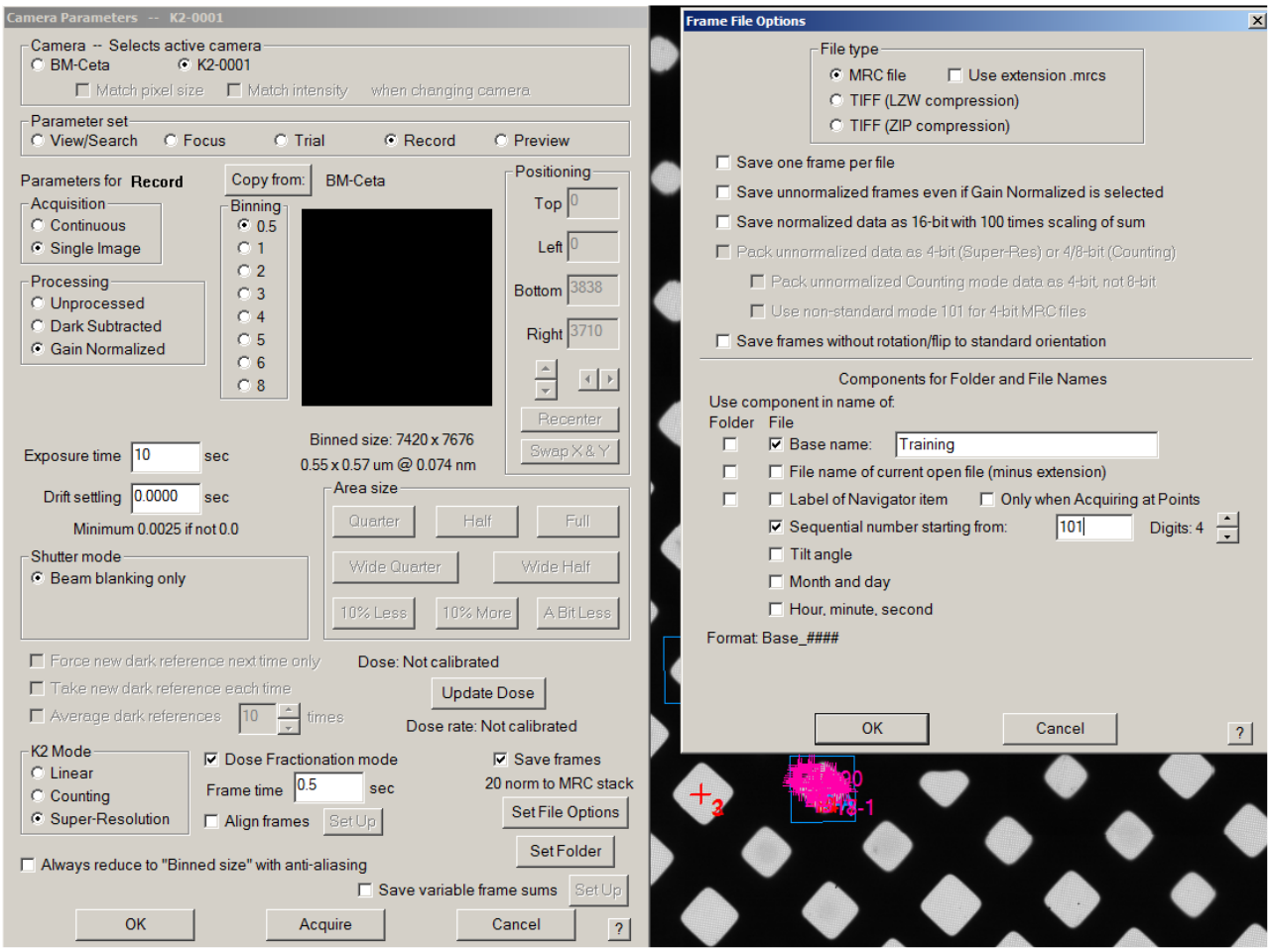
-Run script 2 V align to P. This should center the hole (for a four hole pattern use the center of 4 holes as the P buffer template)

Calibrate/calculate electron dose in Hole in DM with 1s bin1 exposure at record C2 and mag (or use the chart provided)

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

Updated 11/08/19 D.S.

-Save as MRC (or .MRCS) in Images-Disc folder



<u>Edit the SPA script, adding your X:/…Images-Disc/ file path and click OK</u>

<u>Save the Navigator</u>

<u>Setup on-the-fly parameters</u>

-Copy the "para.txt" file from the desktop to the Images-disk folder that the data will be collected in.

-Edit the pixel size (this is your super res. Pixel size in nm) and the dose (this is your dose/frame in e/Å2)

-Copy your counting or super res. Gain ref DM4. file into the same folder.

Note* If you do not wish to use the on-the-fly processing, write the word "Cancel" in your para.txt file and place that file in the Images-Disc/basename folder.

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Navigator -> acquire at points

-Deselect eucentric rough

-Under Primary Task select run script

-Select Close column valves at the end

-GO

-Wait for first few images to ensure all is well.

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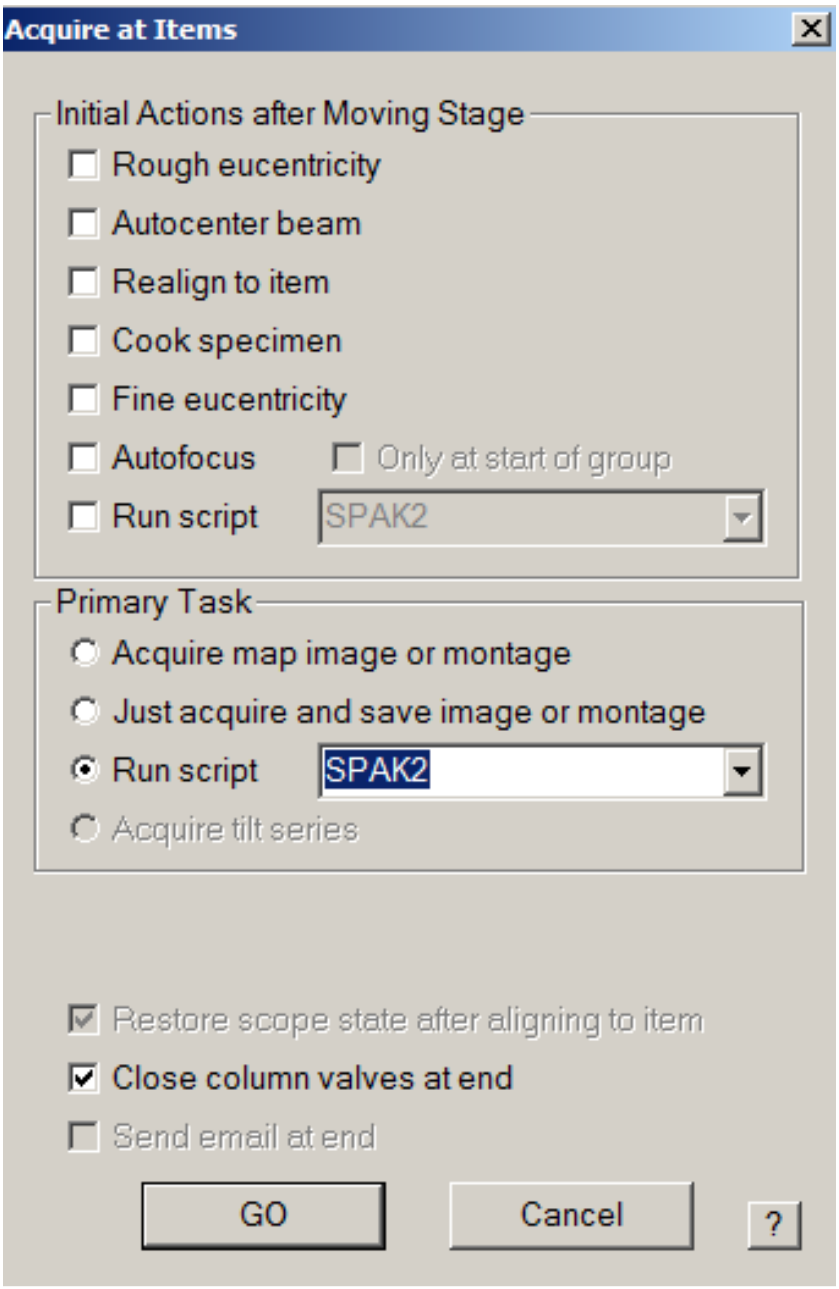

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