

# Talos Arctica Remote User Guide

- Everything is slower remotely. Please plan your experiments and manage your time wisely.
  - **Looking at each individual grid is unlikely especially if you plan to setup data collection.**
  - **If the Talos schedule permits, we will automatically run Cars to collect all the LMMs for you. Please let us know via email if you want to run Cars otherwise.**
- Do not hesitate to ask a staff member for help. Many things are difficult to do remotely.
- You will need a two-button mouse to operate SerialEM efficiently.
- Having a plan and good communication will facilitate a smooth data collection or screening.
- Staff will email you a photo of your grid inventory, TeamViewer login credentials, and a Zoom invitation.
- You can right-click on the Desktop and select Screen Resolution to make SerialEM easier to see on your computer. 2560X1440 works on a laptop. You will need to adjust the positions of the SerialEM panels if you change the screen resolution.

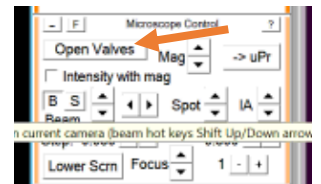
**\*Left mouse drag moves the image in SerialEM**

**\*Right mouse drag moves the stage in SerialEM**

## After LMMs are complete

*Spend some time examining your LMMs so you can prioritize the grids for screening.*

- 1) You can switch the grids using the 'Five One-Line Scripts' panel. One line should say 'LoadCartridge X'. Change to the cartridge number you want to load and click 'Run' at the end of the line.
- 2) You can open and close the column valves in the 'Microscope Control' panel.
- 3) Leave Low Dose Mode selected at all times (in SerialEM).
- 4) Find a good area in Search mode or LMM, center the stage position in SerialEM using 'Go to Marker' or for a point, 'Go to XY'. After the stage stops moving, click 'Run' on the one-line script 'Move Stage XX XX'. This will roughly adjust the stage position to align Search and View. Do not change the numbers after 'MoveStage' unless instructed by staff. *Skip the 'MoveStage' script if you have performed **Aligning LMM to View** (see below) already.*



## Aligning LMM to View

*\*You can screen samples without aligning LMM to View - skip this step during screening to increase your speed. You must align the LMM to View for data collection.*

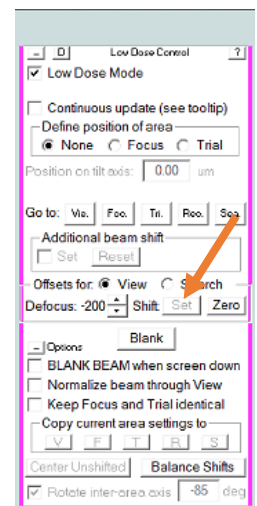
- 1) Find an easily recognizable feature in the LMM atlas.
  - a. In the Navigator window, select 'Add Points'.
  - b. Click on a feature in the LMM.
  - c. Click 'Stop Adding' when the point is selected.
  - d. In the Navigator window, click 'Go to XY'.
  - e. After the stage stops moving, click 'Run' on the 'MoveStage XX XX' one-line script.

- f. Click 'View' – move to an actual square if you are on a grid bar.
- g. In the Scripts panel > Z.
- h. When Z finishes, proceed to step 2.
- 2) In 'Camera & Script Controls' (dark green panel), click 'View' to take a view image.
  - a. If the feature you selected is not visible in the View image. Moving around by right clicking and dragging your mouse.
  - b. Retake View image. Repeat until you find the feature.
  - c. If unable to find in a reasonable amount of time, ask a staff member to help.
- 3) In the View image, put green marker (left click) on the same feature you previously selected in the LMM atlas.
- 4) In Navigator menu > click 'Shift to Marker'.
- 5) Add a new point on LMM, click 'Go to XY', and acquire a View to check if the LMM and View are now properly aligned. Confirm alignment is good by selecting another feature.
- 6) Save the Navigator: Navigator menu > Save.

### Aligning View to Record

*If this alignment is performed during screening, still double check view/record alignment before proceeding.*

- 1) In a View image, find an easily recognizable feature in a square you don't plan to collect images on that will be useful at high mag (i.e. not so large that it fills the entire screen at your acquisition magnification). Alternatively, you can burn an area in record mode to use as your feature.
- 2) Hold down right mouse button and drag until feature is centered **OR** place a green marker in the center of the feature and click 'Go to Marker' in the Navigator window.
- 3) Take a Record image.
- 4) Hold down right mouse button and drag until feature is centered.
- 5) Take another Record image to ensure the feature is centered.
- 6) Take a View image.
- 7) **In the yellow Image Alignment & Focus panel, turn 'Move stage for big mouse shifts' off.**
- 8) Hold down the right mouse to drag the feature to the center of the cross.
- 9) On the pink Low Dose Control panel, click the 'Set' button next to 'Shift:' under 'Offsets for View'. A new image will automatically be taken. If the feature is not centered in the new image, hold down the right mouse button to drag the feature to the center of the cross and press 'Set' again. Repeat until feature is centered.
- 10) **In the yellow Image Alignment & Focus panel, turn 'Move stage for big mouse shifts' on.**
- 11) Repeat 3-10 until Record and View are well aligned.



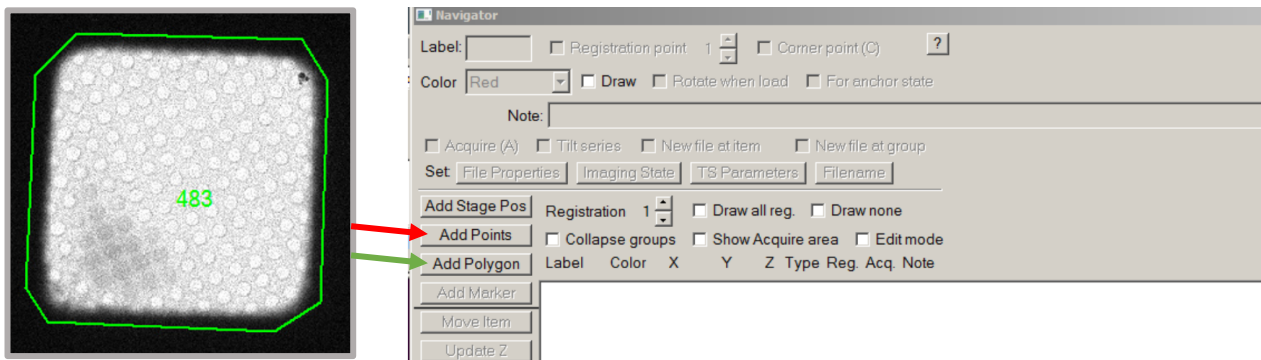
### Screening in SerialEM post LMM

- 1) In the LMM map, find a square of interest: In the Navigator window, select 'Add Points' and then click on the square. Click 'Stop Adding' when finished.
- 2) In the Navigator window, Click 'Go to XY'. The stage will move to the highlighted point.

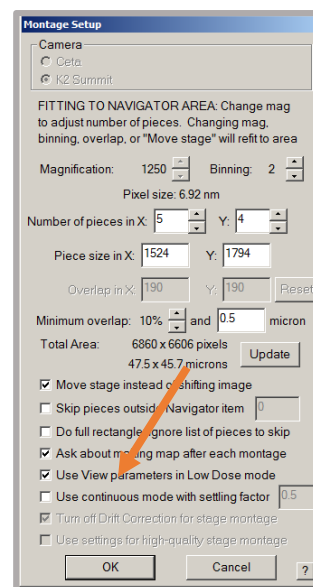
- 3) After the stage stops moving, click 'Run' on the one-line script 'Move Stage XX XX'. This will roughly adjust the stage position to align Search and View. Do not change the numbers after 'MoveStage' unless instructed by staff. *Skip the 'MoveStage' script if you have performed **Aligning LMM to View** already.*
- 4) Take a View image by clicking 'View' in the dark green Camera & Script Controls panel.
- 5) Adjust the Z-height automatically: Scripts panel > Z.
- 6) Take a View image.
- 7) Center the image on the hole by using your mouse to right click/hold and drag to the center. Alternatively, you can place a green marker with a single left-click and click 'Go to Marker' in the Navigator window.
- 8) Set your target defocus (yellow panel, Def target = xxx): Focus/Tune menu > Set Target
- 9) **Once per grid:** Define the focus position: in the pink Low Dose Control panel, under 'Define position of area', click the radio button next to 'Focus'. Assure that the focus position is on carbon by adjusting the 'Position on tilt axis XXX μm' and 'Rotate inter-area axis XXX deg', both in the pink Low Dose Control panel. Return to normal image collection by clicking the radio button next to 'None' when done.
- 10) In the yellow Image Alignment & Focus panel, press 'Autofocus'. Repeat if target defocus is not reached, as indicated in the Log window.
- 11) **In the dark green Camera & Script Controls panel, click 'Setup' change the Record Exposure time to an appropriate length for taking an image, e.g. 4-5 seconds.**
- 12) In the dark green Camera & Script panel, press Record to take an image.
  - a. You may need to align the View and Record modes (see **Aligning View to Record**)
- 13) To save an image: File menu > Open New... In the File Properties window that pops up, choose 'Integers', 'Series of TIFF files listed in an Autodoc file', and 'JPEG' and click OK. Give the file a useful name and click OK. When you want to save an image, click 'Save Active' in the dark blue Buffer Controls panel. Each image will be saved with the name and number as the filename. *These images will be quite lossy due to the compression but do allow for quick viewing while screening.* If you want to save an image of a different magnification after the 'Save Active' is set up: File menu > Save single... and then save the current image.
- 14) Repeat in other grid areas as desired to determine if grid is suitable for data collection.

## Acquiring MMMs

- 1) Return to LMM atlas (double click in Navigator window).
- 2) In the Navigator window, select 'Add Polygon' (green arrow):
  - a. Draw a polygon around a square of interest on the LMM atlas
  - b. Click 'Stop Adding' when finished



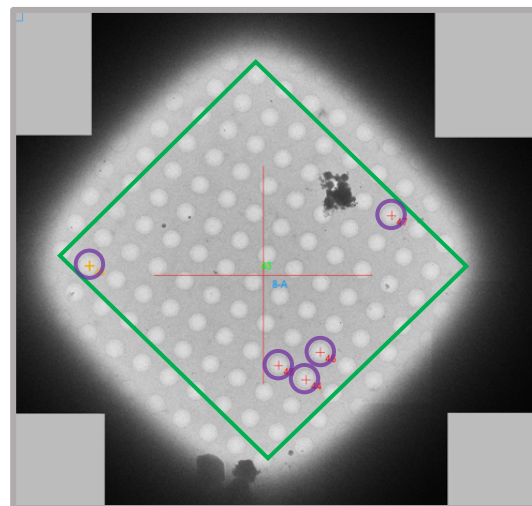
- 3) Go to Navigator menu > Montaging and grids > Setup Polygon Montage
- 4) In the 'Montage Setup' window, select 'Use View parameters in Low Dose mode'. Confirm the magnification is now 1250x in the top of the Montage Setup box. The Number of pieces in X/Y will automatically be determined by the polygon created.



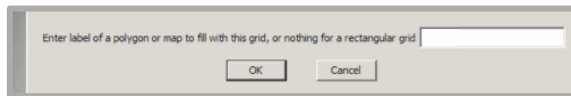
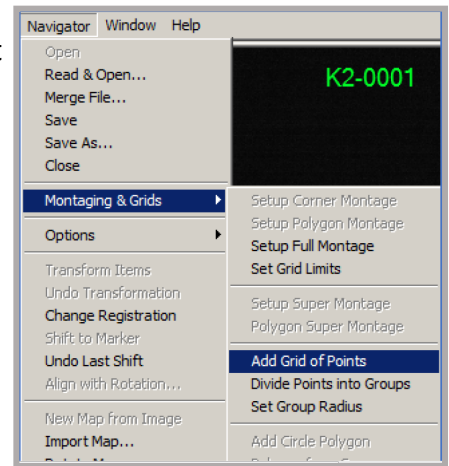
- a. Click OK.
  - b. The next window is File Properties. Save data as Integers. You may click all options for saving if desired, and then click OK.
  - c. Save file as something useful such as SampleXX\_MMM.mrc and it must be saved in your folder in the X drive, but not in the DATA folder.
- 5) In the Navigator window, select 'Add Points' (red arrow):
  - a. In the LMM, click the **center** of other squares where you want to acquire an MMM (medium mag montage).
  - b. Avoid choosing broken squares.
  - c. Click 'Stop Adding' when finished.
- 6) Make all points and the polygon acquisition points by checking 'Acquire' for each item.
- 7) With the polygon selected in the Navigator window, start the MMM acquisition. Go to Navigator menu > Acquire at Items
  - a. Under Initial Actions after Moving Stage, select 'Run script: waitforRefilling'
  - b. Under Primary Task, select 'Run script: Montage'
  - c. Select 'Close column valves at end'.
  - d. Press 'GO'
  - e. This takes a while!!!
- 8) Let the onsite staff member know what time your MMMs are supposed to finish.
- 9) Save the Navigator when MMM collection finishes.

### Selecting Targets on MMMs

- 1) In the Navigator window, load MMM of square by double clicking on the map.
- 2) If image did not stitch well, check/uncheck 'Treat as very sloppy montage' (brown Montage Controls panel) and reload the MMM map (double click in the Navigator window again).
- 3) In the Navigator window, click 'Add Polygon':
  - a. Draw a box (or appropriate shape) around the holes desired for data collection by clicking different points around the MMM map.
  - b. To complete the polygon, click 'Stop Adding'.
- 4) Add the specific targets in the holes:
  - a. In the Navigator window, click 'Add Points'.



- b. Define the position of the lattice by specifying a group of 5 points, three at a corner of a rectangle and two more at adjacent corners (see image). To add these 5 points, accurately center the mouse by zooming in (scroll wheel) followed by clicking on the 5 desired holes.
- c. Click 'Stop Adding' when the appropriate 5 holes have been chosen.
- d. To select all holes in the polygon, go to Navigator menu > Montaging and grids > Add Grid of Points
- e. In the prompt that pops up, add the label (number) that corresponds to the **polygon** you just made above and click 'OK'.



- f. Click 'Yes' to turn on Acquire at all points and 'Yes' to keep the groups of points, aiming for groups of about 15-25 targets.
    - If the group is too large or small, click 'No' and go to Navigator menu > Montaging and grids > Set Group Radius. In the prompt that pops up, adjust the radius appropriately and start the process from step d.
  - g. Check that the acquisition points are roughly in the center of the holes. If not, delete the points, keeping the polygon, and draw the group of 5 points in a different orientation.
- 5) To curate point selection, press 'Ctrl' (on the keyboard) and left-click to choose the point to delete, then in the Navigator window, click 'Delete Item'. Alternatively, check 'Edit mode' in the Navigator and use the 'backspace' key to delete points or the scroll wheel button to add points.
  - 6) Save the Navigator and repeat for all other squares.

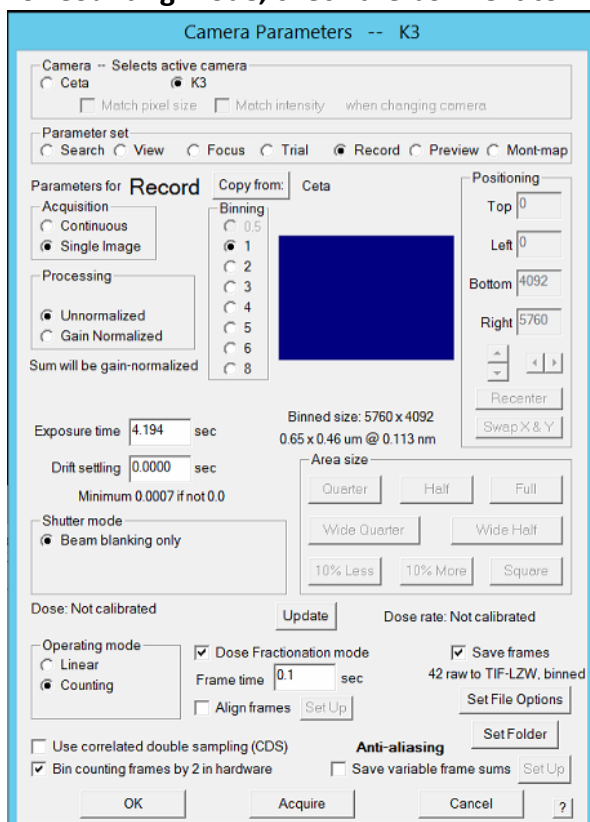
### Determine Dose and Adjust Camera Settings for Data Acquisition

- 1) You need to decide the total dose and number of frames you want for each movie. This will depend on the current dose of the beam settings. You want approximately  $1 \text{ e}/\text{\AA}^2$  per frame. For example, if for a one second exposure I have  $5 \text{ e}/\text{\AA}^2$  and each fraction is 0.25, I have  $1.25 \text{ e}/\text{\AA}^2$  per frame.
- 2) **Determine your dose rate.**
  - Find a broken square in your LMM, left click on the broken area and click 'Go to Marker' in the Navigator window. Take View image to assure you are over a broken area.
  - With no carbon/ice blocking the beam, go to the Record preset in the pink Low Dose Control panel. Make sure the screen is retracted.
  - In Digital Micrograph (GMS), unblank the beam by clicking 'Blank' in the microscope graphic on the left
  - Take a single capture (counted mode, 1 sec, 1x binning). At the bottom of the screen if you click on the image, it will display the electrons per pixel per second ( $\text{e}^-/\text{pix}/\text{s}$ ) and electrons per square angstrom ( $\text{e}^-/\text{\AA}^2$ ). For the best K3 performance, a dose rate of  $15 \text{ e}^-/\text{pix}/\text{s}$  is desirable. Above  $20 \text{ e}^-/\text{pix}/\text{s}$ , the dose is too high to count single electron events, and below  $10 \text{ e}^-/\text{pix}/\text{s}$ , you risk not having enough contrast in your images. Aim for  $13 \text{ e}^-/\text{pix}/\text{s}$ .
  - Record these values on a new Data Collection Parameters sheet.
- 3) In the dark green Camera & Script Controls panel, click the 'Setup' button and **adjust the settings**

for **Record** for data collection. *Record* should be the only mode that needs to be adjusted:

Low Dose Mode	Bin	Processing	Area Size	Exposure Time (sec)	K3 Mode	Dose Fractionation Mode
Record	0.5 or 1	Unnormalized	Full	Set value based on desired dose	Super-Resolution or Counting	Yes and save frames (Set up your folder for data collection)

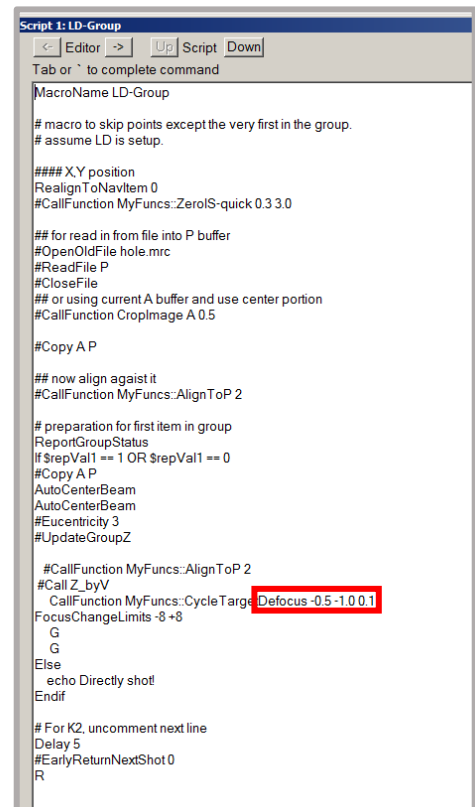
- 4) Using the dose rate information, decide what total dose will be applied to each movie by setting the desired Exposure Time (s).
- 5) Click the check box next to 'Dose Fractionation mode'. New options will become available to set the frame time, file name, and path for data collection. Make sure 'Save Frames' is checked. The number of frames is listed below the check box. To change the number of frames, adjust the 'Frame time' to the left.
- 6) Click 'Set File Options'. In the Frame File Options window, choose TIFF (LZW compression) and set your file name under 'Base name:'. Also check 'Sequential number starting from:' and set the starting point to 1 with 4 digits. Click OK.
- 7) Click 'Set Folder'. In the window that pops up, set file path to X:\yourname\DATA. The data placed in this folder is used in the OTF script.
- 8) **For Counting mode, check the box next to 'Bin counting frames by 2 in hardware'**



## Before Beginning Automated Data Collection

Now you are ready to begin automated data collection, but check these items before starting:

- 1) **Is the Autoloader turbo set to auto off?** Leaving the setting to “Turbo Always On” will cause drift in your images.
- 2) Condenser aperture is 50  $\mu\text{M}$
- 3) **Objective aperture (70  $\mu\text{M}$ ) inserted and centered.**
- 9) **Check beam for astigmatism.**
  - Is the beam round? Are the FFT rings round?
  - Make sure to select “Rec.” for “Go to area/show when screen is down” and continuous update are selected during this process.
  - Return to desired C2 value.
  - Uncheck continuous update.
- 4) **Make sure the column valves are open.**
- 5) **Define your focus area.**
  - See #7 in **Screening in SerialEM post LMM**
- 6) **Check the Script and set your target defocus range**
  - Under Script menu > Edit 1 is the LD-Group script. Inspect the script and change values as desired:
    - Defocus values, e.g. -0.5 -1.0 0.1 thus by group, data collection will begin at -0.5 $\mu\text{m}$  defocused and increase stepwise by 0.1 $\mu\text{m}$
    - Click OK
- 7) **Check that the folder X:\yourname\DATA is empty.** Delete any files if present.



```
Script 1: LD-Group
Editor > Up Script Down
Tab or ^ to complete command
MacroName LD-Group
# macro to skip points except the very first in the group.
# assume LD is setup.
#### X,Y position
RealignToNavItem 0
#CallFunction MyFuncs::ZeroS-quick 0.3 3.0
## for read in from file into P buffer
#OpenOldFile hole.mrc
#ReadFile P
#CloseFile
## or using current A buffer and use center portion
#CallFunction CropImage A 0.5
#Copy A P
## now align against it
#CallFunction MyFuncs::AlignToP 2
# preparation for first item in group
ReportGroupStatus
If $repVal1 == 1 OR $repVal1 == 0
#Copy A P
AutoCenterBeam
AutoCenterBeam
#Eucentricity 3
#UpdateGroupZ
#CallFunction MyFuncs::AlignToP 2
#Call Z_byV
CallFunction MyFuncs::CycleTarget Defocus -0.5 -1.0 0.1
FocusChangeLimits -8 +8
G
G
Else
echo Directly shot!
Endif
# For K2, uncomment next line
Delay 5
#EarlyReturnNextShot 0
R
```

## Start Data Collection

- 1) Navigator menu > Save
- 2) Navigator menu > Acquire at Items
  - a. Under Initial Actions after Moving Stage, select ‘Run script: waitForRefilling’
  - b. Under Primary Task, select ‘Run script: LD-Group’
  - c. Select ‘Close column valves at end’
  - d. Press ‘GO’
- 3) Watch the preview of your images in SerialEM to make sure you can see particles and they look appropriately defocused.

## OTF processing

Wait for the data collection run for 5 minutes, and then start the OTF processing to ensure it is working properly during normal business hours. If you experience any issues, let Shaun Rawson know immediately so he can fix technical issues. **Follow the separate instructions for remote OTF processing that were emailed to you. Currently, all data must be rsync'd (no HDD transfer). Please consult Shaun if you have any questions.**

## SerialEM Dummy/Merging Navigator Files

- 1) In your X drive folder, duplicate your Navigator and rename the copy: nav-DUMMY.nav.
- 2) Open the DUMMY SerialEM folder, right-click on the Dummy SerialEM shortcut and select 'Run as administrator'.
- 3) In DUMMY SerialEM, open your Dummy navigator file: Navigator menu > Read & Open.
  - a. Select the nav-Dummy.nav file.
- 4) Select additional targets (Follow the instructions in **Selecting Targets on MMMs**).
- 5) **Save the Dummy Navigator when finished:** Navigator menu > Save.
- 6) Minimize the DUMMY SerialEM.
- 7) In your active SerialEM session (where you are currently collecting data)
  - a. Press 'Pause' in the dark green Camera & Script controls window.
  - b. Allow SerialEM to finish the current item. Wait for 'PAUSE NAV ACQUIRE' on bottom panel.
  - c. Once data collection has paused, save the navigator: Navigator menu > Save.
  - d. Merge the Dummy Navigator file into the active Navigator: Navigator menu > Merge File > select your nav-Dummy.nav file.
  - e. After merging, save the active Navigator: Navigator menu > Save.**
  - f. Press 'Resume' in the dark green Camera & Script controls window.
  - g. After a few moments your navigator window will update to reflect the additional points added from merging the dummy file and the new completion time with this addition.