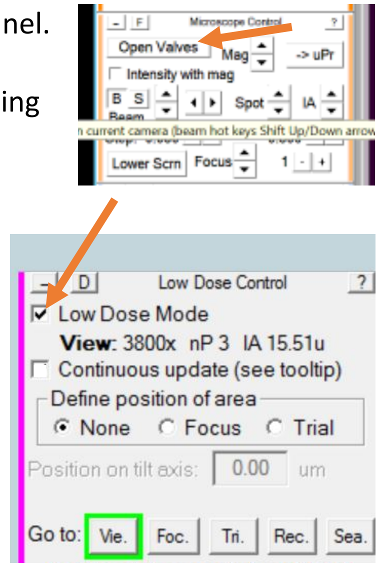
Titan Krios Remote User Guide

- Everything is slower remotely. Please plan your experiments and manage your time wisely.
- 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.
- 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**

Screening – Low Magnification (low-mag)

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) Use **Search** mode in the pink Low Dose Control panel for low-mag screening (accessing ice thickness).
5) Take a Search image.
   a. Right click and drag the mouse to move about your grid.
   b. Take a new Search image.
   c. Repeat a and b until to assess the ice and global grid quality.
   d. Repeat for each sample of interest.
   e. Ideally you should already have an idea of the particle behavior of this sample from preliminary screening. However, given the variability from grid to grid, some screening may be necessary for your particular sample. Your experience should tell you whether you need to screen first (See **Screening – Checking particle density**) or if you can immediately proceed to **Acquiring an LMM (low magnification montage).**
6) **Alternatively,** you may opt to collect LMMs of all your grids at the beginning of your session. Staff can help you with the setup of the 'Cars' script. Each LMM takes about 15-20 minutes.

Screening – Checking particle density

1) 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.*

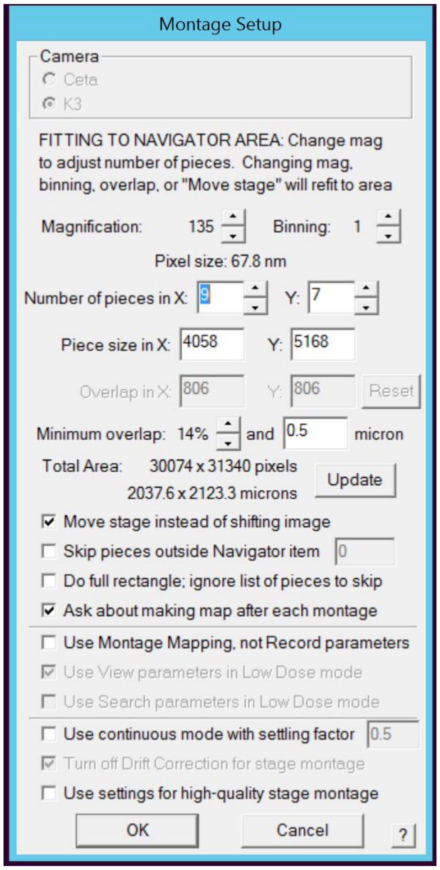
2) Take a View image. The microscope magnification will change to View mode (note the green box around **View** in the pink Low Dose Control panel).
3) Center the image on the square by using your mouse to right click/hold and drag to the center. Alternatively, you can place a green marker with a single left-click and click ‘Go to Marker’ in the Navigator window.
4) Adjust the Z-height automatically: Scripts panel > Z.
5) Take a View image by clicking ‘View’ in the dark green Camera & Script Controls panel.
6) Repeat steps 2 and 3 until you have centered the View image on a hole.
7) In the yellow Image Alignment & Focus panel, press ‘Autofocus’. Check the Log window to see when the target defocus is reached, press ‘Autofocus’ again as necessary.
    a. To change your target defocus (yellow panel, Def target = xxx): Focus/Tune menu > Set Target
    b. To check the focus position, in the pink Low Dose Control panel, under ‘Define position of area’, click the radio button next to ‘Focus’. Click on the View image to place the focus position on the carbon. You can manually adjust the focus position changing 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 finished.
8) In the dark green Camera & Script Controls panel, click ‘Setup’ and change the Record parameters to an appropriate length, e.g. 2-3 seconds and ensure that Dose fractionation is **not selected**.
9) Press Record.
    a. You may need to align the View and Record modes (see **Align View to Record** below)
10) 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 (e.g. GridXX_image_) 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. Alternatively, you can save a single image by choosing ‘Save to Other…”. *We recommend saving images as JPEG for easy viewing.*
11) Repeat in other grid areas as desired to determine if grid is suitable for data collection.
12) Repeat with other grids as necessary.

Screening Summary:
Go to location → Z → View → Center View → Autofocus → Record Image
Repeat as necessary to assess the sample in different ice thicknesses or with various defocus values.

Acquiring LMM (Atlas)

Use the Search mode settings (135x) for the LMM acquisition.

1) Open a new Navigator window: Navigator menu > Open
2) Set up LMM collection: Navigator menu > Montaging and grids > Setup Full montage
    a. In Montage Setup (shown here), make sure ‘Move stage instead of shifting image’ and ‘Ask about making a map after each montage’ are selected. Click OK.



○ Ceta
● K3

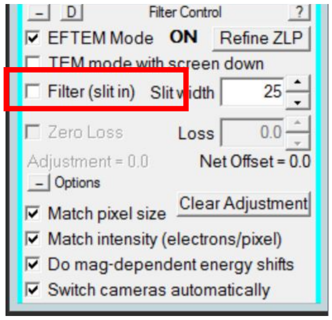
FITTING TO NAVIGATOR AREA: Change mag to adjust number of pieces. Changing mag, binning, overlap, or "Move stage" will refit to area

Magnification: 135
Binning: 1
Pixel size: 67.8 nm
Number of pieces in X: 9 Y: 7
Piece size in X: 4058 Y: 5168
Overlap in X: 806 Y: 806
Minimum overlap: 14% and 0.5 micron
Total Area: 30074 x 31340 pixels
2037.6 x 2123.3 microns

☑ Move stage instead of shifting image
☐ Skip pieces outside Navigator item 0
☐ Do full rectangle; ignore list of pieces to skip
☑ Ask about making map after each montage
☐ Use Montage Mapping, not Record parameters
☑ Use View parameters in Low Dose mode
☐ Use Search parameters in Low Dose mode
☐ Use continuous mode with settling factor 0.5
☑ Turn off Drift Correction for stage montage
☐ Use settings for high-quality stage montage

    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. Enter a file name as something useful such as SampleXX_LMM.mrc and it must be saved in your folder in the X Drive, but not in the DATA folder. ***Do not save in your SerialEM_users folder in the C drive.***
    d. Check that the energy filter (cyan Filter Control panel in SerialEM) is **out** as it will likely occlude your sample at this low magnification. Checked box means the filter is in, **unchecked box means the filter is out.**
    e. Go to the brown Montage Controls panel and click 'Start'.
        - LMM will take about 20 min to complete
        - Click 'Yes' to dialogue box

3) Save the Navigator with a meaningful name (Date.nav): Navigator menu > Save



Filter Control
☑ EFTEM Mode ON
☐ TEM mode with screen down
☐ Filter (slit in) Slit width 25
☐ Zero Loss Loss 0.0
Adjustment = 0.0 Net Offset = 0.0
Options
☑ Match pixel size
☑ Match intensity (electrons/pixel)
☑ Do mag-dependent energy shifts
☑ Switch cameras automatically

Aligning LMM to View

You may need assistance with this procedure – please ask a staff member for help.

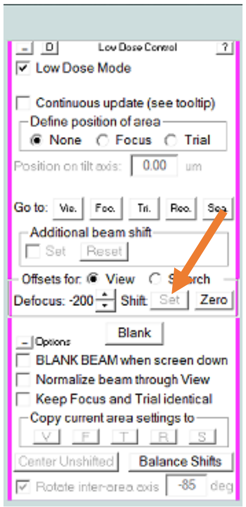
1) Find an easily recognizable feature in the LMM atlas.
    a. In the Navigator window, select 'Add Points'.
    b. Click on a feature in the LMM.
    c. Click 'Stop Adding' when the point is selected.
    d. In the Navigator window, click 'Go to XY'.
    e. After the stage stops moving, click 'Run' on the 'MoveStage XX XX' one-line script.
    f. Click 'View' – move to an actual square if you are on a grid bar.
    g. Click 'Z' in the Scripts panel.
    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. 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.

Align View to Record

If this alignment is performed during screening, still double check view/record before data collection begins.

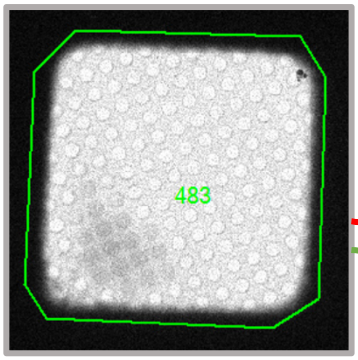
1) In a View image, find an easily recognizable feature in a square you don't plan to collect images on that will be useful at high mag (i.e. not so large that it fills the entire screen at your acquisition magnification).
2) Hold down right mouse button and drag until feature is centered **OR** place a green marker in the center of the feature and click 'Go to Marker' in the Navigator window.
3) Take a Record image.

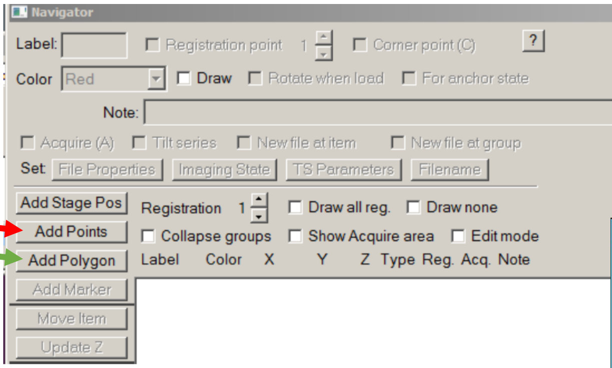
4) Hold down right mouse button and drag **OR** repeatedly place a green marker in the center of the feature and click ‘Go to Marker’ in the Navigator window until the feature is centered.
5) Take another Record image to ensure the feature is centered.
6) Take a View image.
7) **In the yellow Image Alignment & Focus panel, turn ‘Move stage for big mouse shifts’ off (unchecked).**
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 View 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 (checked).**
11) Repeat 3-10 until Record and View are well aligned.


Low Dose Control
☑ Low Dose Mode
☐ Continuous update (see tooltip)
Define position of area: ● None ○ Focus ○ Trial
Position on tilt axis: 0.00 um
Additional beam shift: ☐ Set
Offsets for: ● View ○ Search
Defocus: -200
☐ BLANK BEAM when screen down
☐ Normalize beam through View
☐ Keep Focus and Trial identical
☑ Rotate inter-area axis -85 deg

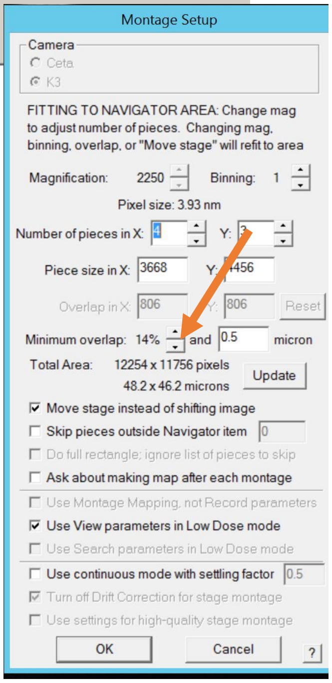
Acquiring MMMs

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



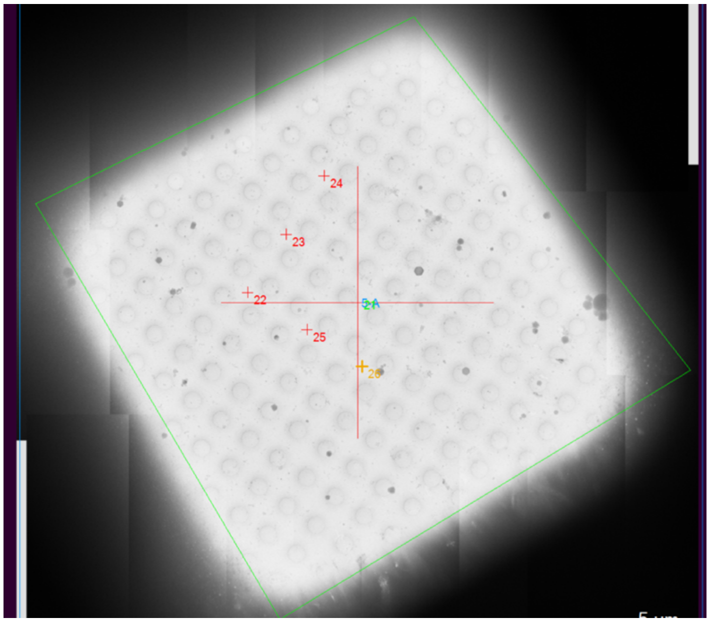


4) The ‘Montage Setup’ window will appear with the settings automatically based on the polygon created:
    a. Make sure ‘Use View parameters in Low Dose mode’ is selected
    b. Adjust the ‘Minimum Overlap’ to optimize the ‘Number of pieces in X:’, and then click OK.
    c. The next window is File Properties. Save data as Integers. You may click all options for saving if desired, and then click OK.
    d. 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).


Montage Setup
Camera: ○ Ceta ● K3
FITTING TO NAVIGATOR AREA: Change mag to adjust number of pieces. Changing mag, binning, overlap, or "Move stage" will refit to area
Magnification: 2250 Binning: 1
Pixel size: 3.93 nm
Number of pieces in X: 4 Y: 3
Piece size in X: 3668 Y: 4456
Overlap in X: 806 Y: 806
Minimum overlap: 14% and 0.5 micron
Total Area: 12254 x 11756 pixels
48.2 x 46.2 microns
☑ Move stage instead of shifting image
☐ Skip pieces outside Navigator item 0
☐ Do full rectangle; ignore list of pieces to skip
☐ Ask about making map after each montage
☐ Use Montage Mapping, not Record parameters
☑ Use View parameters in Low Dose mode
☐ Use Search parameters in Low Dose mode
☐ Use continuous mode with settling factor 0.5
☑ Turn off Drift Correction for stage montage
☐ Use settings for high-quality stage 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 in the Navigator window.
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. **Watch the first one to make sure your MMMs are stitching well!**
    f. Note that this will take some time – plan appropriately.
8) **The onsite staff member will perform alignments, usually after the MMMs finish**
9) **Decide your intended collection scheme (4 or 9 holes per stage movement, 2-5 shots per hole).**
10) ***Save the Navigator when MMMs finish.***

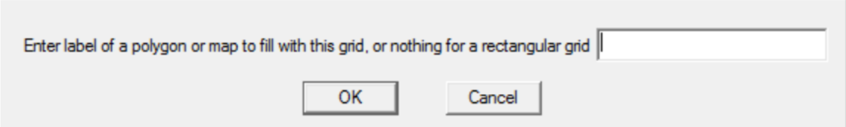
Selecting Targets on MMMs



Take your time and make sure the targets selected are well-centered. The example shown here is for picking targets with 4 holes per stage movement. Optimal target selection allows you the option of using ‘Copy A P’ instead of using a template.

1) In the Navigator window, load the MMM of a 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 again in the Navigator window).
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) When using a Multiple hole setup, each “point” is composed of 4 or 9 sample holes.
5) Add the specific targets
    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 below). To add these 5 points, accurately center the mouse by zooming in (scroll wheel) followed by clicking on the 5 desired points.
    c. Click ‘Stop Adding’ when the appropriate 5 points have been chosen. **The Navigator numbers of these 5 points must be consecutive.**
    d. To select all holes in the polygon, go to Navigator tab > 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’.



Enter label of a polygon or map to fill with this grid, or nothing for a rectangular grid

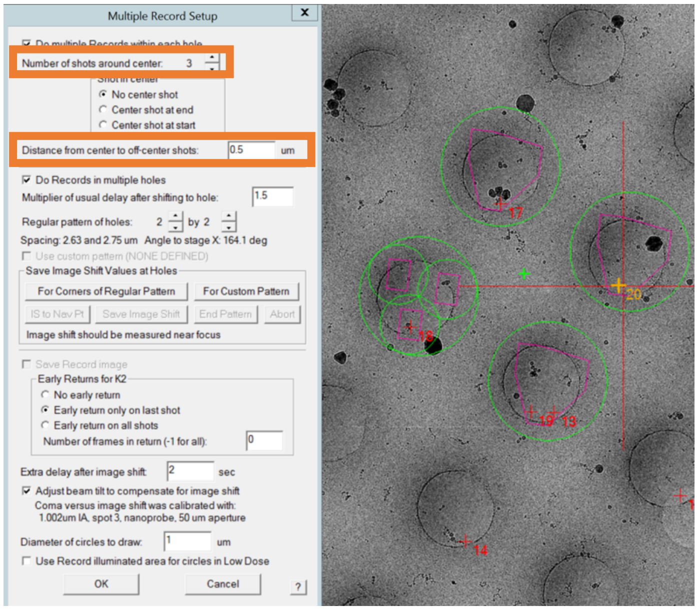
   f. Click ‘Yes’ to turn on Acquire at all points and ‘Yes’ to keep the groups of points.
   g. Check that the acquisition points are roughly in the center of the groups of four holes. If not, delete the points, keeping the polygon, and draw the group of 5 points in a different orientation.

6) 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.
7) Save the Navigator.
8) To increase the efficiency of data collection time, select data collection targets on 2-3 MMM maps, save the Navigator, and begin data acquisition. The remaining targets can be selected in DUMMY SerialEM while the main SerialEM interface is collecting data (see below).

Setting up Multihole/Multishot parameters

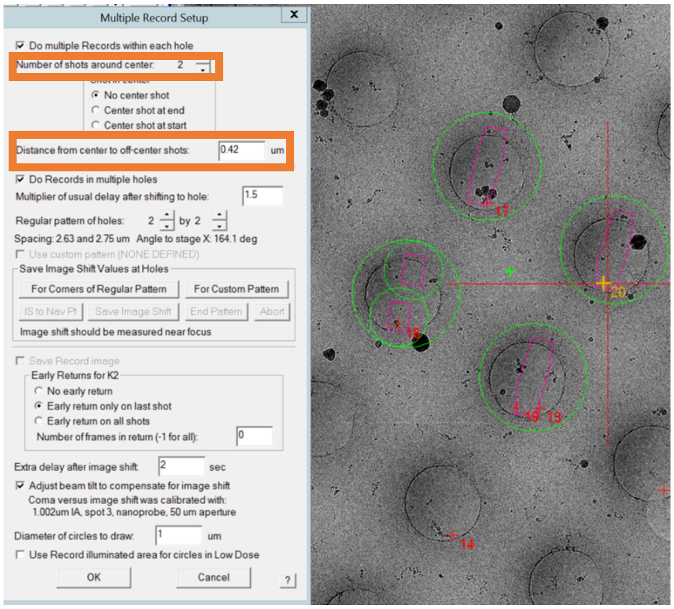
Following microscope alignments, staff will set up the multiple shot/image shift corrections during your first few sessions, but **as you advance, you will need to learn how to set up your own parameters**. The batch-to-batch variability in grid production means the number of shots per holes needs to be optimized for each sample.

1) Navigator menu > Montaging & Grids > Set Multi-shot Parameters
2) With the Multiple Record Setup window open, a left-click on an image will display the beam diameter (green circle) and the size of the detector (pink rectangle). You can adjust the number of shots per hole and how far the shots are spaced form one another (orange boxes). You do not want beam overlap over the detector area. An example with 4 shots per stage position and 3 vs 2 shots per hole is shown here.



Multiple Record Setup

☑ Do multiple Records within each hole
Number of shots around center: 3
Shot in center: ● No center shot ○ Center shot at end ○ Center shot at start
Distance from center to off-center shots: 0.5 um
☑ Do Records in multiple holes
Multiplier of usual delay after shifting to hole: 1.5
Regular pattern of holes: 2 by 2
Spacing: 2.63 and 2.75 um Angle to stage X: 164.1 deg
☐ Use custom pattern (NONE DEFINED)
Save Image Shift Values at Holes
Image shift should be measured near focus
☐ Save Record image
Early Returns for K2: ○ No early return ● Early return only on last shot ○ Early return on all shots
Number of frames in return (-1 for all): 0
Extra delay after image shift: 2 sec
☑ Adjust beam tilt to compensate for image shift
Coma versus image shift was calibrated with: 1.002um IA, spot 3, nanoprobe, 50 um aperture
Diameter of circles to draw: 1 um
☐ Use Record illuminated area for circles in Low Dose



Multiple Record Setup

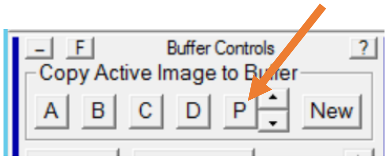
☑ Do multiple Records within each hole
Number of shots around center: 2
Shot in center: ● No center shot ○ Center shot at end ○ Center shot at start
Distance from center to off-center shots: 0.42 um
☑ Do Records in multiple holes
Multiplier of usual delay after shifting to hole: 1.5
Regular pattern of holes: 2 by 2
Spacing: 2.63 and 2.75 um Angle to stage X: 164.1 deg
☐ Use custom pattern (NONE DEFINED)
Save Image Shift Values at Holes
Image shift should be measured near focus
☐ Save Record image
Early Returns for K2: ○ No early return ● Early return only on last shot ○ Early return on all shots
Number of frames in return (-1 for all): 0
Extra delay after image shift: 2 sec
☑ Adjust beam tilt to compensate for image shift
Coma versus image shift was calibrated with: 1.002um IA, spot 3, nanoprobe, 50 um aperture
Diameter of circles to draw: 1 um
☐ Use Record illuminated area for circles in Low Dose

Creating a Template

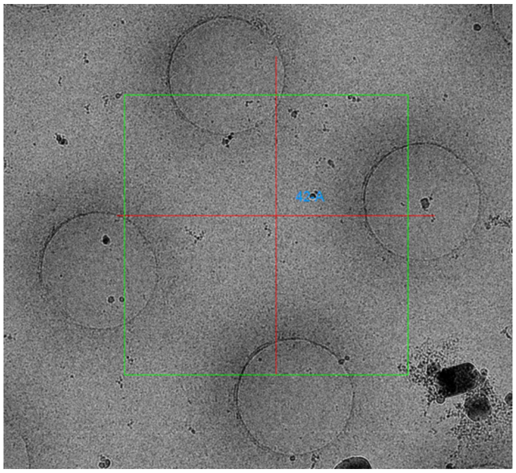
Many data collections will utilize ‘Copy A P’ which will align to positions based on where targets are selected on the MMMs. This scheme works well if your targets are well-centered and is ideal for samples that have ice contamination or have a non-homogenous appearance such as variable ice thickness. The **alternative method** to ‘Copy A P’ is to create a template which will be used as a reference at each new position.

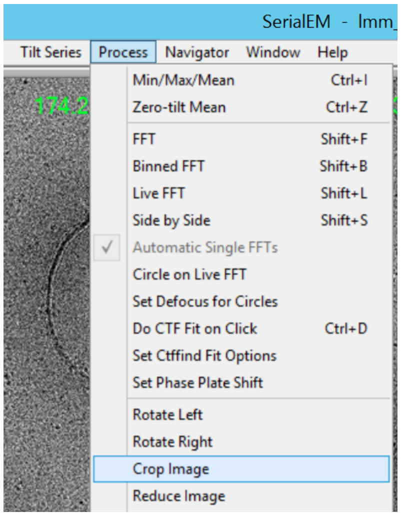
***Please note that templates must be adjusted periodically.**

1) Select an MMM in the Navigator window and click ‘Go to XYZ’ (uncheck ‘Collapse’ to use this option). Take a View image at this new location.
2) Right-click, hold and drag a group of four holes to the center cross. Take a new View image.
3) Press Ctrl+Shift and left-click on the mouse to drag and draw a green box in SerialEM.
4) Symmetry of the template is important to ensure proper alignment. Once you’re satisfied with the box, go to Process menu > Crop Image. Then in the dark blue Buffer Controls panel, click the ‘P’ button to copy the image to Buffer P.
5) The optimal template depends on the sample. If you notice that the script does not appear to be aligning to the template properly, you should Pause the data collection, take a View image and create a new template (steps 3-4).

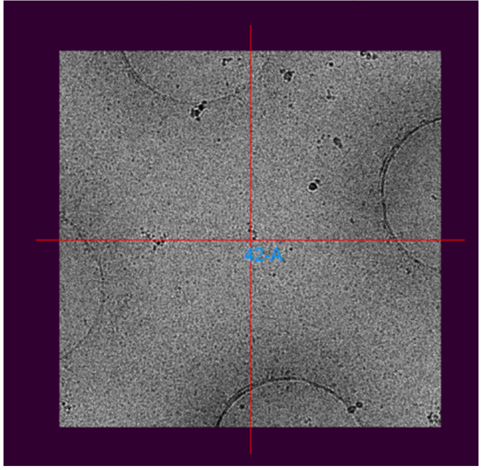


Draw a box





Cropped image



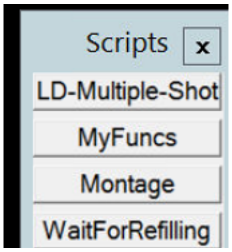
Before Beginning Automated Data Collection

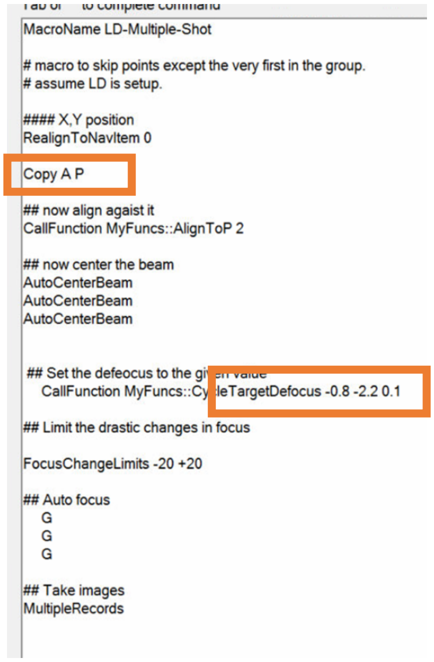
Now you are ready to begin automated data collection but check the following items before starting. Please ask staff if you need assistance.

1) **Is the Autoloader turbo set to auto off?** Leaving the setting to “Turbo Always On” will cause drift in your images.
2) **Objective aperture (100 µM) inserted and centered**.
3) **Check the Focus and Trial position.** It’s in the pink Low Dose Control panel under ‘Define position of area’. Focus and Trial should have the same ‘Position on tilt axis XX µm’ value.
4) **Make sure the column valves are open**.
5) **Insert and center the energy filter (refer to ZLP centering sheet).**
6) **Determine your dose rate.**
7) **Make sure you have selected save dose fractionated images in your Record settings.** Click Setup on the dark green Camera & Script control panel to adjust the ‘Record’ collection parameters.
8) **Check that your DATA folder is empty.** Delete the previous gain reference and defect file if present.

Check Script and Start Data Collection

1) **Press Ctrl and left-click on LD-Multiple-Shot** in the Scripts panel to edit the data collection script.
2) Inspect the script and change values as desired (image on page 8 of guide):
    a. Defocus values: -0.8 -2.2 0.1 (data collection will begin at -0.8µm defocus and increase stepwise by 0.1µm)
    b. **If you are using a template**, comment out ‘Copy A P’ → #Copy A P
    c. Click OK
3) Save your navigator. Go to Navigator menu > Save
4) Save your settings. Go to Settings menu > Save As…
5) Start the collection. 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: LD-Multiple-Shot’.
    c. Select ‘Close column valves at end’.
    d. Press ‘GO’.





```
Tab or    to complete command
MacroName LD-Multiple-Shot

# macro to skip points except the very first in the group.
# assume LD is setup.

#### X,Y position
RealignToNavItem 0

Copy A P

## now align agaist it
CallFunction MyFuncs::AlignToP 2

## now center the beam
AutoCenterBeam
AutoCenterBeam
AutoCenterBeam


 ## Set the defeocus to the gi en value
   CallFunction MyFuncs::Cy leTargetDefocus -0.8 -2.2 0.1

## Limit the drastic changes in focus

FocusChangeLimits -20 +20

## Auto focus
   G
   G
   G

## Take images
MultipleRecords
```

***If you are using a template, watch to ensure your template is aligning correctly**. If it is not, Pause the collection and define a new template (see the section **Creating a Template**). Once data acquisition appears to be proceeding efficiently select the remaining targets using the SerialEM Dummy.

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.