



Talos the living robot and guardian of the island Crete, forged from bronze and fueled by ichor...defeated by the sorceress Medea...

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I. Basic information before operating the microscope

This document is for trained users, contact the CryoEM Core Director Joshua Strauss to schedule a training session before using the microscope.

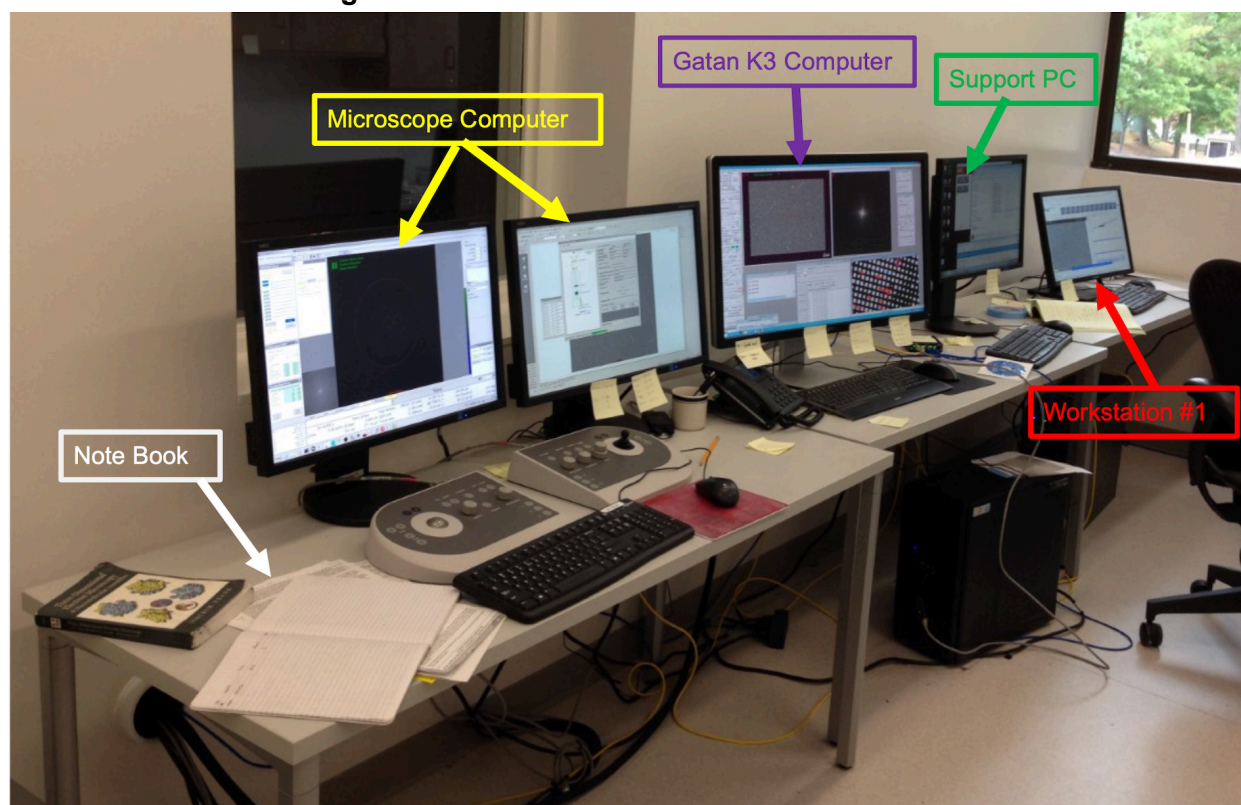
- Do not contact GTS or fix the problem without supervision.
- Do not make your own settings files or change the setting files without supervision.
- Do not download or install software on any of the cryoEM core computers unsupervised.
- Do not align the microscope or FEG.
- Do not transfer data from the microscope computer to a flash-drive.
- Do not transfer data from/to any of the computers in the microscope control room with a Flash-drive or external hard drive.
- If you noticed an error or if the alignments are not ideal or the microscope is not working contact Joshua Strauss ASPA. If you think that there is a problem with the microscope chances are you are correct.
- If you need help then ask.
- If you are uncertain about something then ask.
- If you have any questions please contact Joshua Strauss (Joshua.Strauss@med.unc.edu Glaxo room 008).

It is best to think of this document as a guide for using the Talos Arctica. The purpose of this document is to help guide you. If you treat this document as a to do list your ability to use the Talos Arctica will be limited by this document. Before you do anything with the microscope think about what you are trying to do.

Enter your Information into the Talos Arctica Notebook:

Check the **microscope notebook** and enter the following information: data, your name/initials, PI name, time started/ended, number of grids loaded, current, notes on the imaging session.

I. Before starting the session:

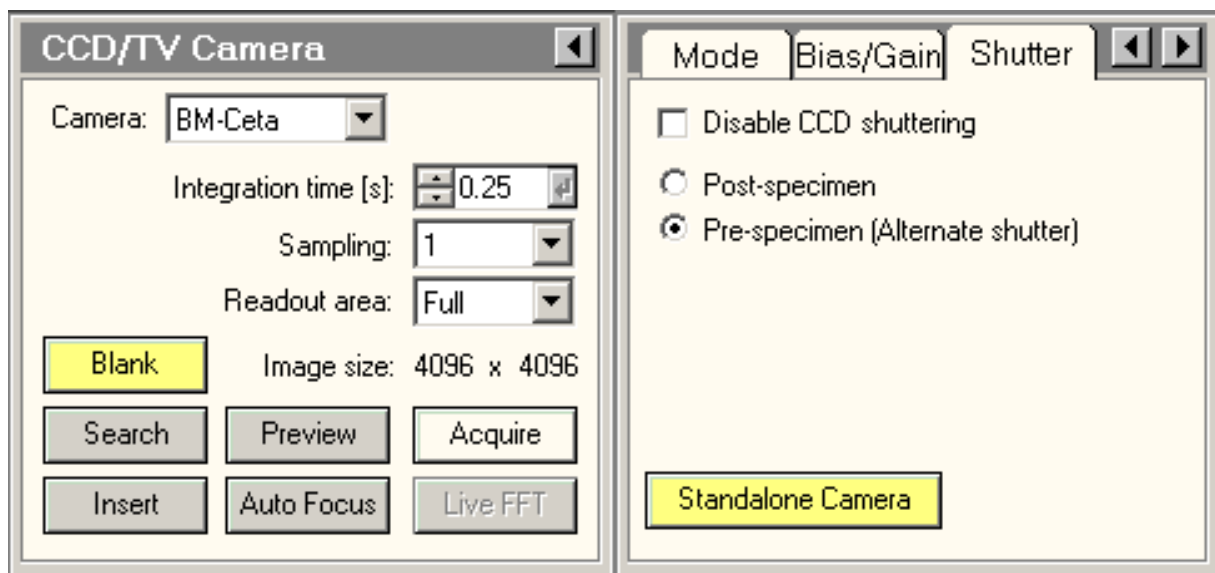


Enter your Information into the Talos Arctica Notebook:

Check the **microscope notebook** and enter the following information: data, your name/initials, PI name, time started/ended, number of grids loaded, current, notes on the imaging session.

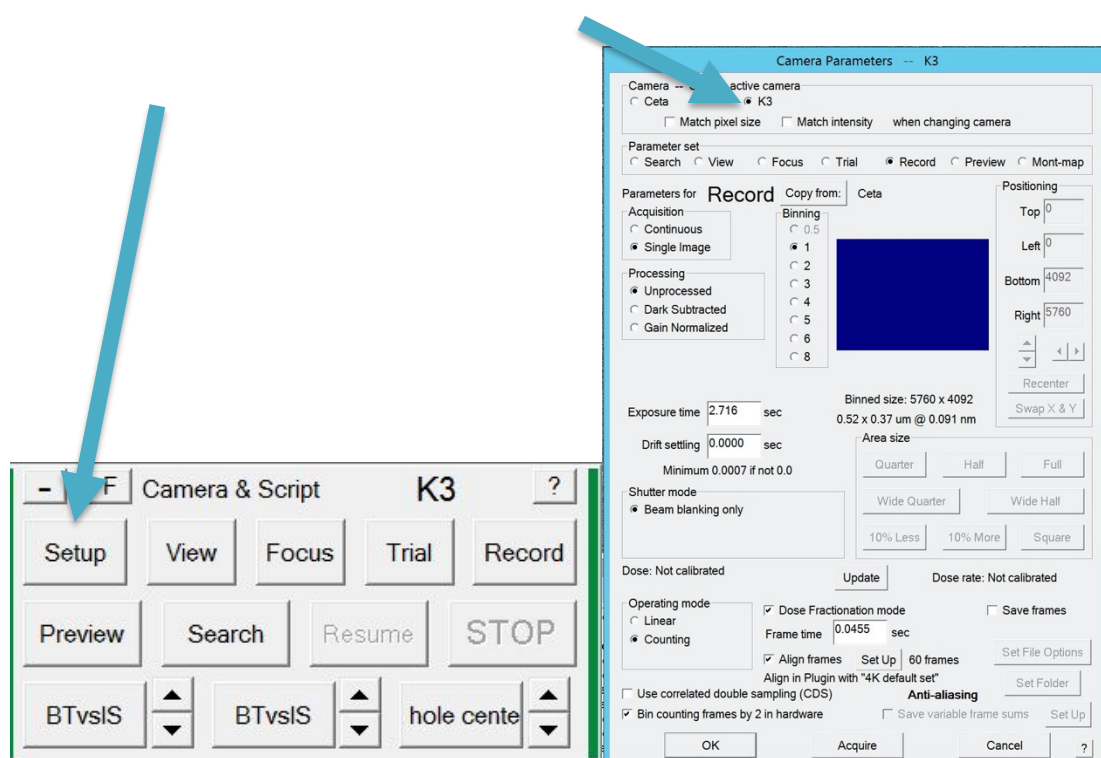
Check microscope computer:

- The Column Valves should be closed
- The FEG Gun should be on
- The Column and Autoloader vacuum and temperature should be green
- be closed, the FEG gun should be on, the Autoloader Vacuums
- SerialEM Plugin should be running
- Gatan Plugin should be running
- No error messages
- Select "**Standalone**" in the CCD/TV Camera Panel, also make sure that the "**pre-specimen** (Alternative Shutter) is checked in the Shutter tab.



Check the SerialEM / Gatan K3 Computer:

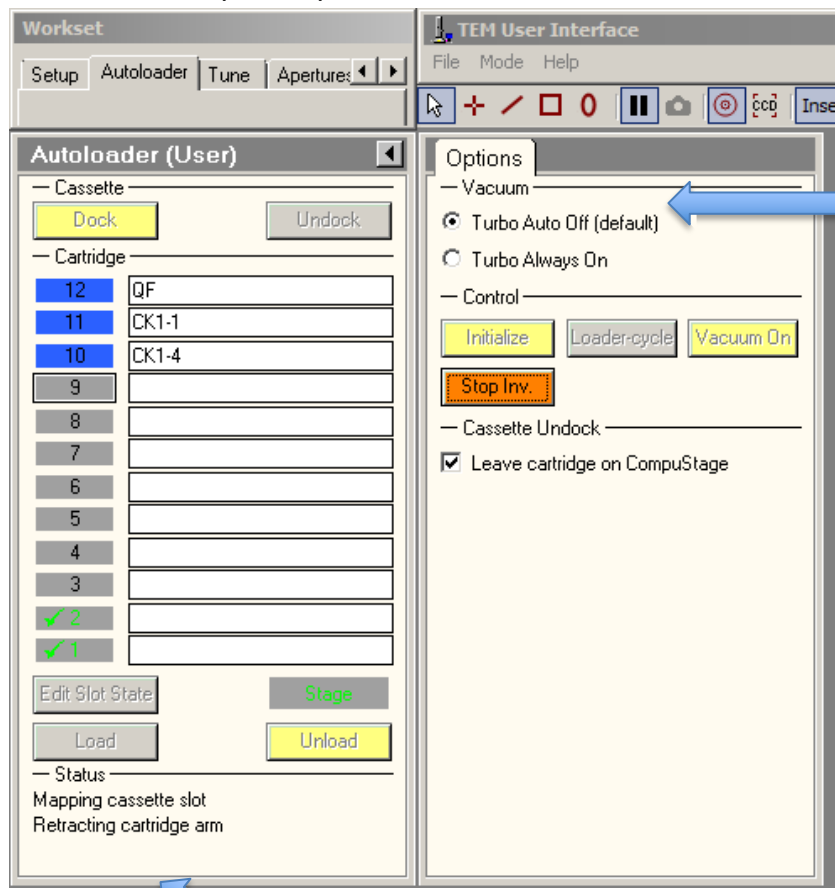
- Digital Micrograph should be open and running
- SerialEM should be open and running
- Load current SerialEM Settings: File→ open “20200628_LD_K3_45K.txt”
- Select the Gatan K3 Camera in SerialEM Camera Panel →Setup



III. Autoloader Inventory

At this point your cryo-grids have been clipped and loaded into the cassette and autoloader. Before starting the Autoloader Inventory, the vacuum and temperature of the autoloader should be green.

On the microscope computer find the “Autoloader” tab.



Should always have the Turbo pump off.

Normally the stage is **Initialized**. If not contact the Core Director.

“Loader-cycle”.....don’t click on this button!!!!

“Vacuum on”, this should be on.

Status-----

This panel will display what the autoloader is doing. Keep an eye on it during the session. If the autoloader is doing something ie. loading a cartridge don’t interrupt this process. Once it is finished the Status panel should be empty.

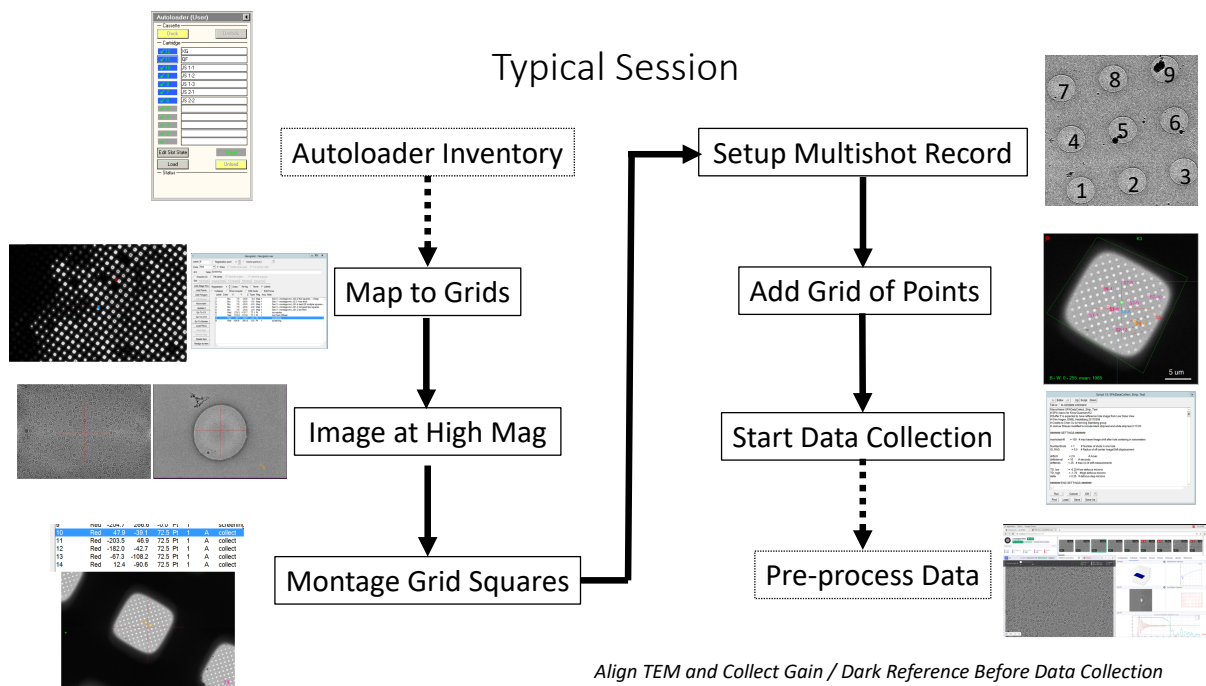
- The “**Turbo Auto Off (default)**” should be checked.
- Click on “**Edit Slot State**”, then click on the Cartridges changing the color to BLUE if the Cassette slot is occupied by a Cartridge. If it is empty click on it twice to make it dark grey. Light grey (not shown) if you don't know.
- Enter **label** for each of the Cartridges in the Autoloader.
- Click on “**Inventory**” in the Options tab.
- The microscope will check to see if there is a Cartridges in the Cassette.
- The image above was taken during the Inventory, once the inventory is completed there should be a green check mark next to each Cartridge. A Red X on the Cartridge indicates an Autoloader error.

If you get an Autoloader error during the Inventory do not attempt to fix this, immediately tell the Core Director, and enter this information in the notebook.

VI. Load Cross-Gradient TEM grid and perform direct alignments

- This will be performed each morning by a Core employee. If you believe that the alignment is off and are not comfortable adjusting it yourself, ask an employee to assist you.

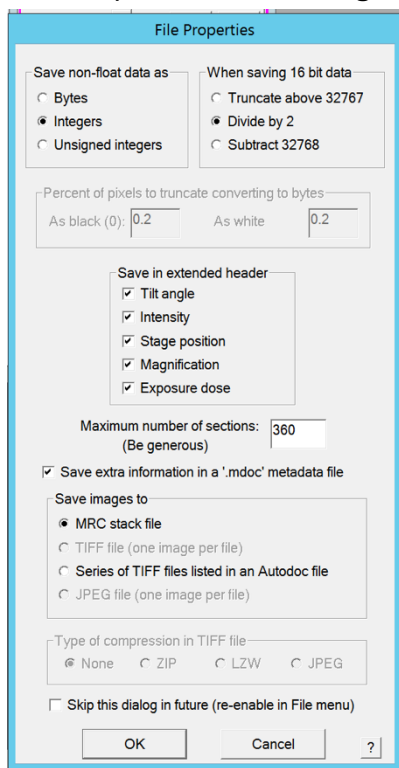
Typical session screening and data collection



V. Map to Grids

On the SerialEM Computer

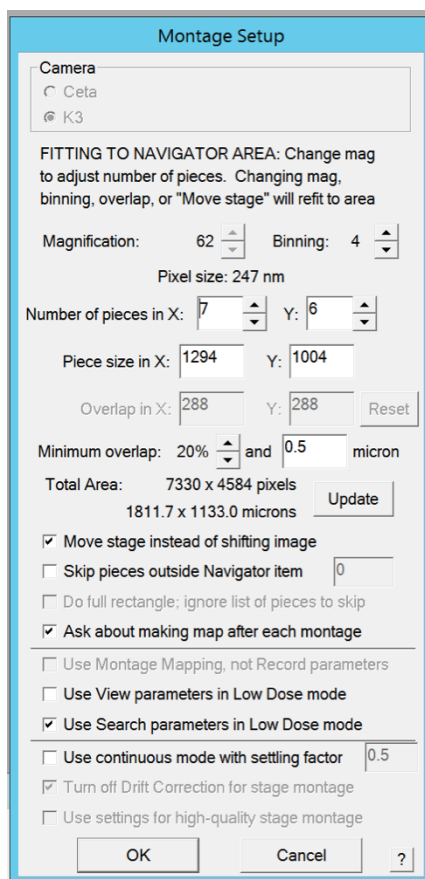
1. Open a new **Navigator** file
2. Setup **Full Grid Montage**, Navigator → Montage and Grids → Setup Full Montage



File Properties: normally don't need to change anything..

Click **"OK"**

Save the files as MRC stack in the directory where you are saving the data something like this
/k3data/20200606_JDS/Maptogrids/Fullgridmontage.mrc



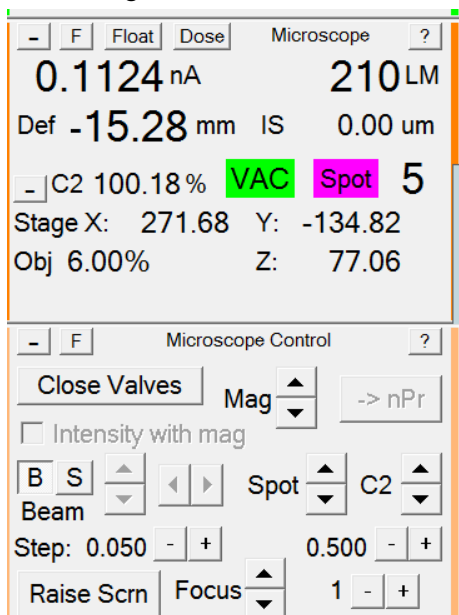
Montage Setup: Normally don't need to change anything.

Number of pieces in X and Y, usually set to 5 7. The more pieces the longer the montage takes.

Set Minimum overlap to 15-25% for **UltraAufoil TEM grids set this to 25%**.

Click "OK"

3. Change the Low-Dose Search Mode Magnification to 62X

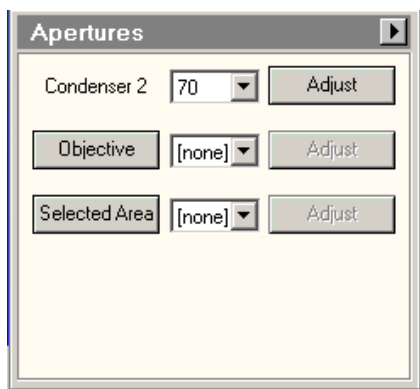


Microscope Control:

To change the magnification click on the Mag arrows.

The magnification is indicated in the Microscope panel, here in the image as LM 210.

4. On the microscope computer **Retract the Objective Aperture**
5. Insert and Center the **100 or 150 μ m Condenser 2 Aperture**

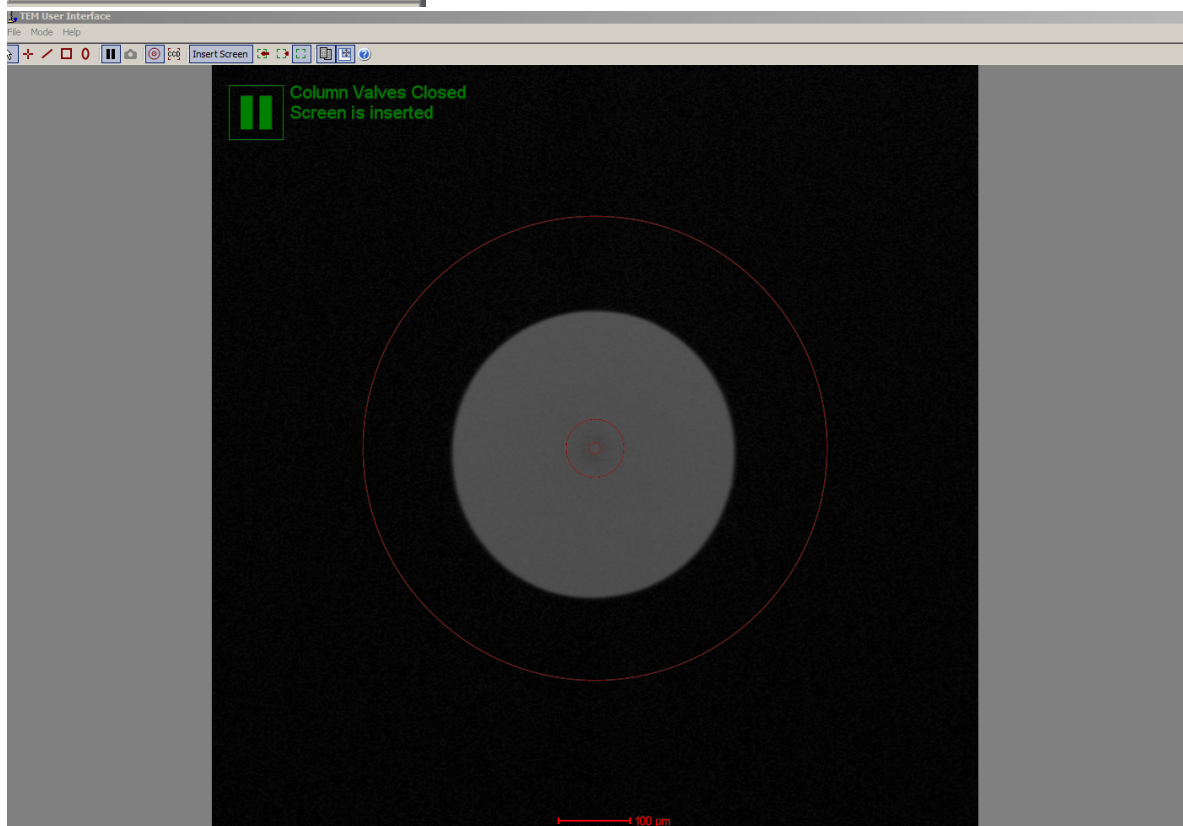


Apertures

To change apertures click on the pull-down tab.

To center the aperture click on “**Adjust**” and use the **Multifunction X Y knobs** on the Microscope Control Panels to move it.

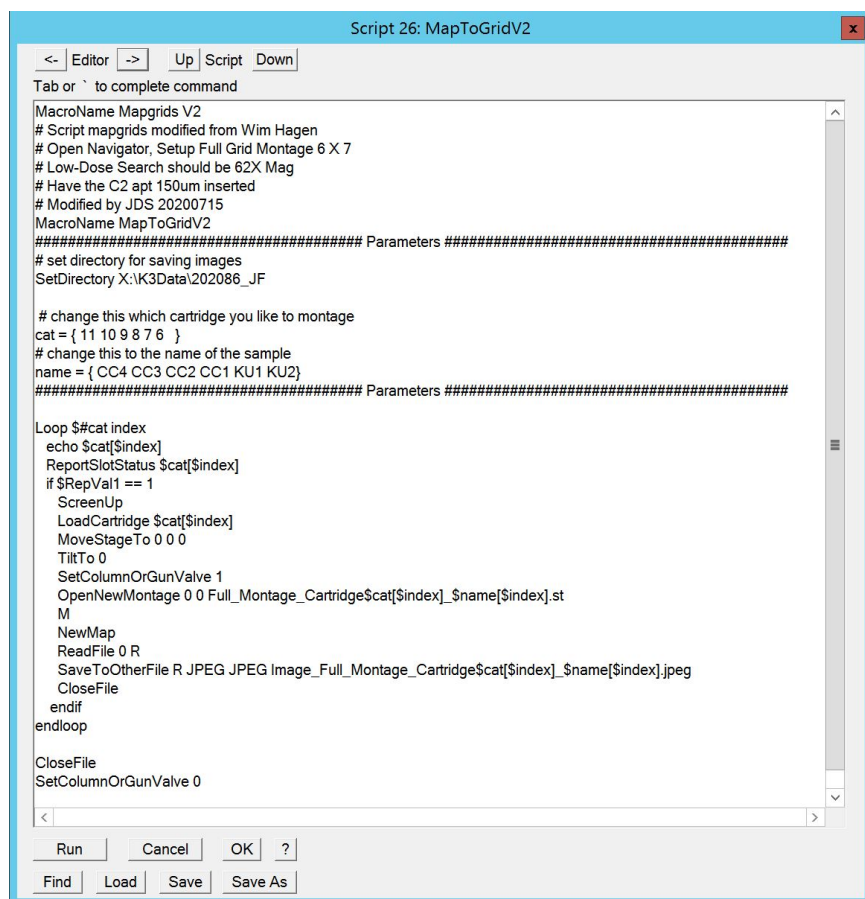
Lower the **FluCam** and to see if the beam and apertures are centered. See image below, note this is the 70 μm not the 100 or 150 but it is centered.



6. Open and **edit script 26 “MapToGridV2”**, Select the Script tab→ Edit

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```
Script 26: MapToGridV2
<- Editor -> Up Script Down
Tab or ` to complete command

MacroName Mapgrids V2
# Script mapgrids modified from Wim Hagen
# Open Navigator, Setup Full Grid Montage 6 X 7
# Low-Dose Search should be 62X Mag
# Have the C2 apt 150um inserted
# Modified by JDS 20200715
MacroName MapToGridV2
##### Parameters #####
# set directory for saving images
SetDirectory X:\K3Data\202086_JF

# change this which cartridge you like to montage
cat = { 11 10 9 8 7 6 }
# change this to the name of the sample
name = { CC4 CC3 CC2 CC1 KU1 KU2 }
##### Parameters #####

Loop $#cat index
  echo $cat[$index]
  ReportSlotStatus $cat[$index]
  if $RepVal1 == 1
    ScreenUp
    LoadCartridge $cat[$index]
    MoveStageTo 0 0 0
    TiltTo 0
    SetColumnOrGunValve 1
    OpenNewMontage 0 0 Full_Montage_Cartridge$cat[$index]_Name[$index].st
    M
    NewMap
    ReadFile 0 R
    SaveToOtherFile R JPEG JPEG Image_Full_Montage_Cartridge$cat[$index]_Name[$index].jpeg
    CloseFile
  endif
endloop

CloseFile
SetColumnOrGunValve 0

Run Cancel OK ?
Find Load Save Save As
```

You will need to change the **Directory** to the one you created to save your images.

Change the **Cartridge list** to reflect the autoloader grid slots you want to montage.

Change the **Name** of each grid, keeping the same order as the cartridge inventory order. This is how each montage file will be named so each grid is easier to keep track of.

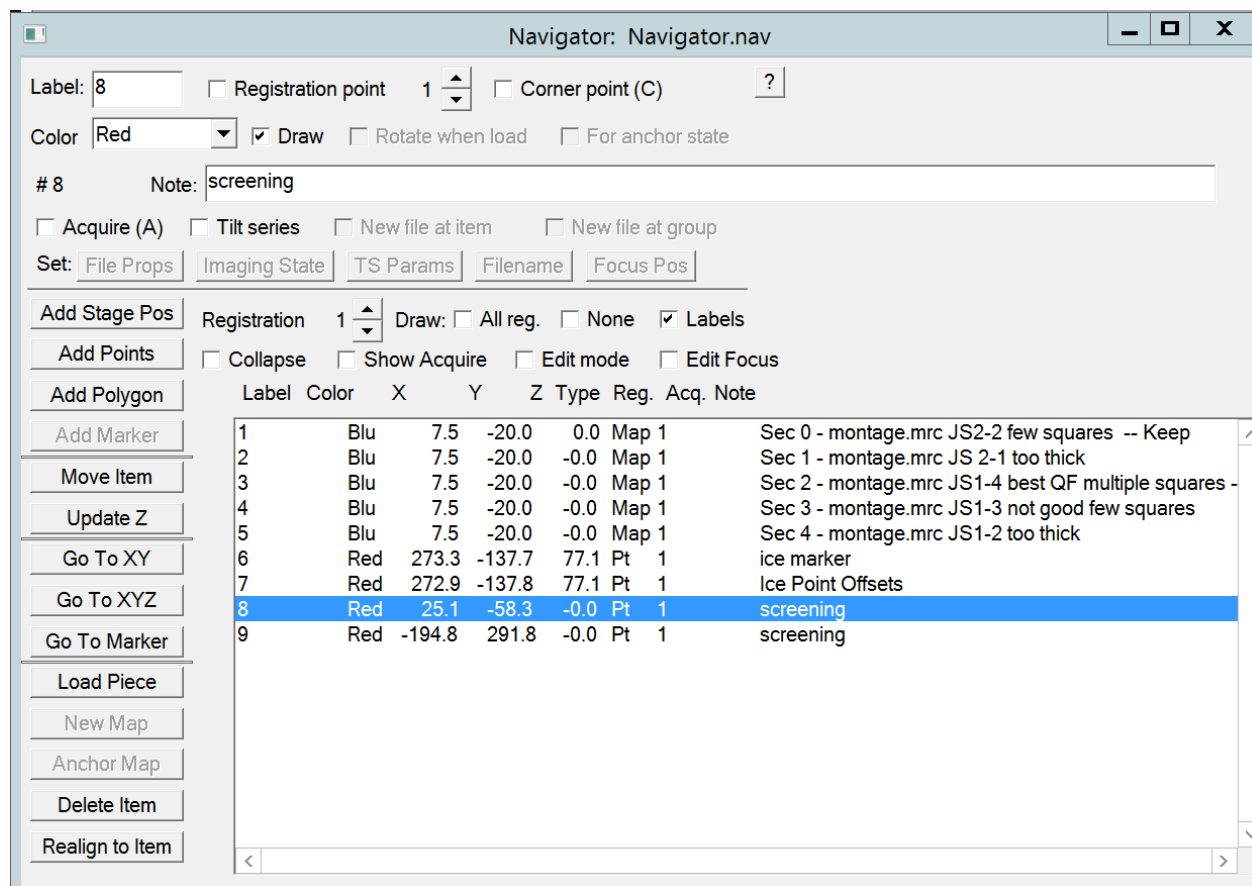
Nothing below **Loop \$#cat index** will typically need to be changed.

After changing the script click “**OK**”

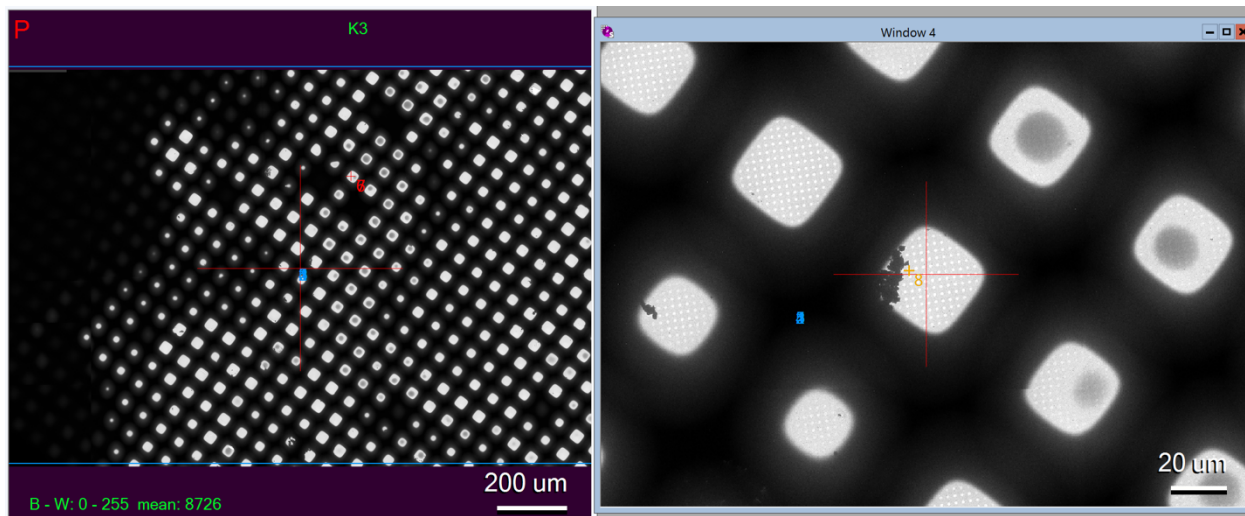
To Run the Scrip click “**Run**”

VI. Screening Cryogrids

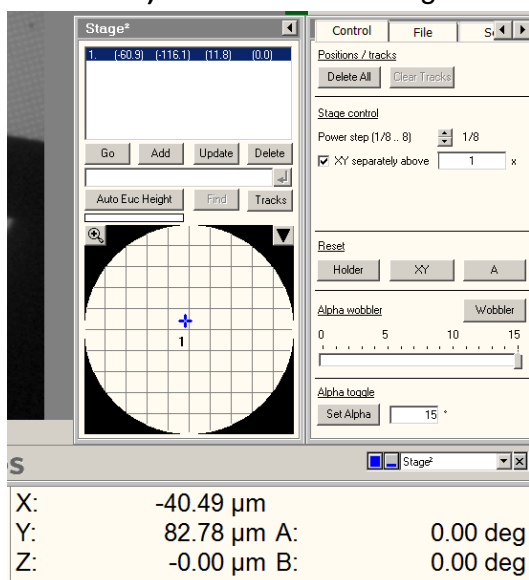
1. **Look at the montages (Maps) in the Navigator.** To open a Map **double click on it** in the Navigator, this will display it to the main window. You can open it in a separate window if you like in the Window tab. **If the grids do not contain thin vitreous ice or are too thick (completely black) don't spend time imaging these grids.** Pick the best one and take images at different magnification to determine if the grid can be used for high-resolution data collection. You can annotate each of the Navigator items like Maps or points in the Note tab.



This is an **example of a good cryo-grid** with multiple grid squares with thin vitreous ice.



7. Change the Low-Dose Search mode to 210X (as you did before).
8. Insert and center the 70 μm Condenser aperture.
9. On the Microscope Computer load one of the grids with the Autoloader Panel. **Close the Column Valves**, on the Stage² panel Click on “XY” or “Holder” to **Center the XY (or Holder)** then wait for the stage to stop moving.



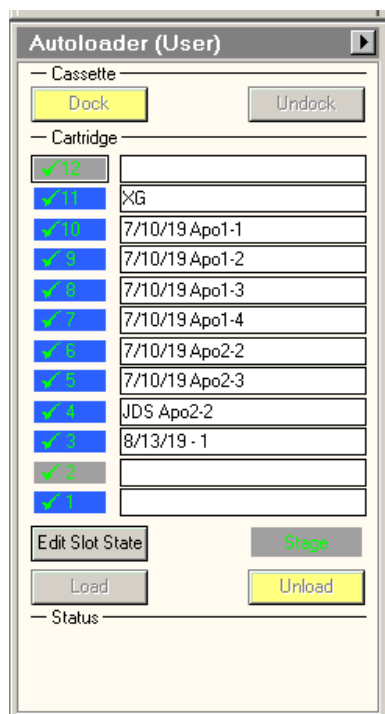
Holder – will reset X Y Z and Alpha to 0

XY – will reset X Y to 0

A – will reset Alpha to 0

When the Stage is moving or active the Reset icons will be grey and the text grey.

10. To load a cryogrid on The **Autoloader Panel** click on the Cartridge you want to load it should have a black outline over it then click “Load”.



Autoloader: Loading and Unloading Cartridges

To **select a Cartridge** to load click on it with the mouse, it will have a black outline on it, as show for Cartridge 12. Then click on **“Load”**

When the Cartridge in on the Stage it will be yellow.

You can put that Cartridge back into a Cassette by click on **“Unload”**

- Typically we collect low-dose images with the K3 and save them as jpg, but you can save the images as tif or mrc if you like.

Search 210 X LM

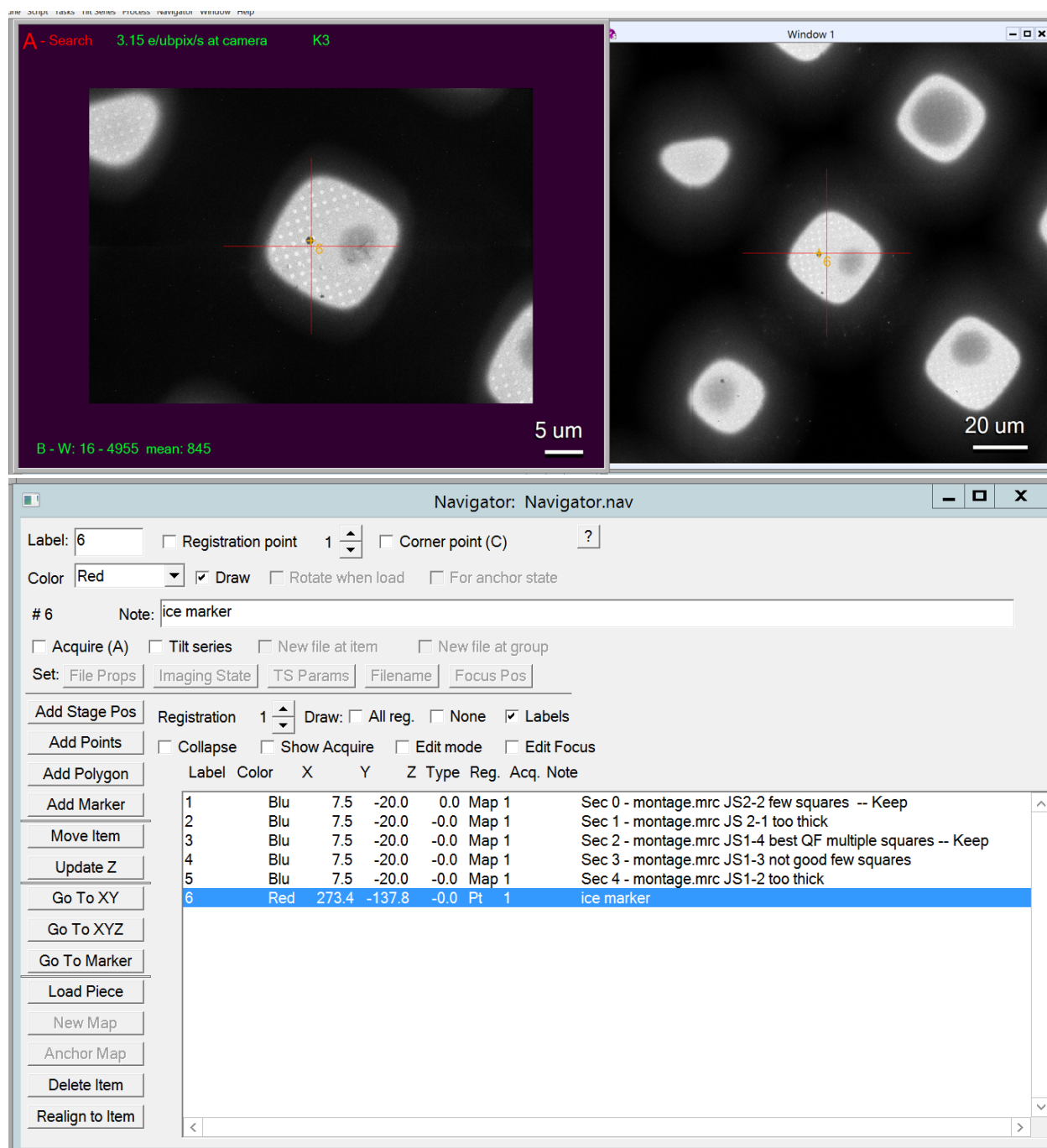
View: 5,300 X M

Record: 45,000 X M

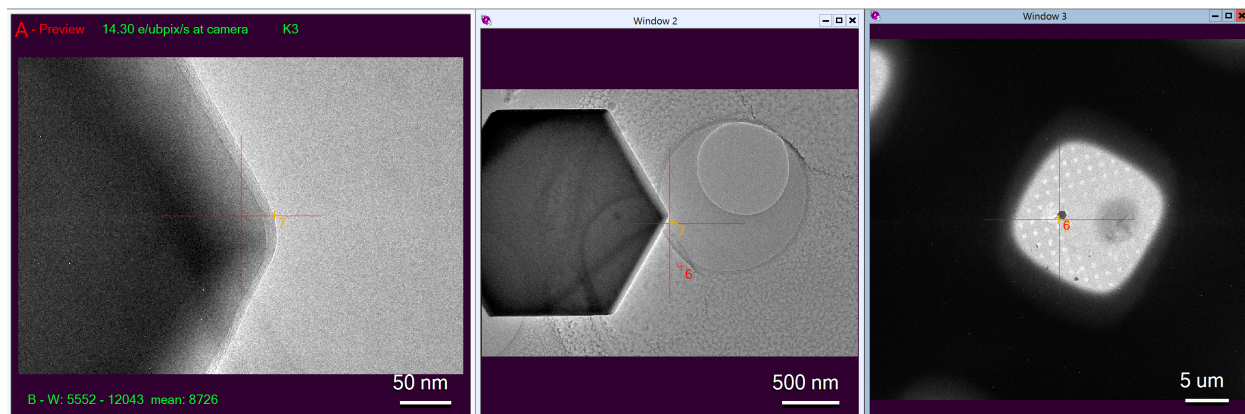
- Once a cryogrid is on the stage you can image it. Open the Full Grid Montage (or Map) of that cryo-grid. **Add a point to the center of a grid square you want to image.** In the Navigator you can annotate if the area is good or not, this is helpful for keeping track of where the good area are on the grid. Typically I'll pick 3 points on a grid to image.
- When you load, unload then load a cartridge it will not be at the same position on the stage.** So the Map will be displaced slightly. To correct for this use the **Shift to Marker**. Find a feature on the Full Grid Montage Map like a chunk of ice or dirt, to the Full Grid Montage Map put a point on it. Move the stage to that point. Take a **Search Image**, then mouse click to put a marker (green +) on the Search Image displayed in the window. Then on the Navigator tab → shift to marker. It should look like this....

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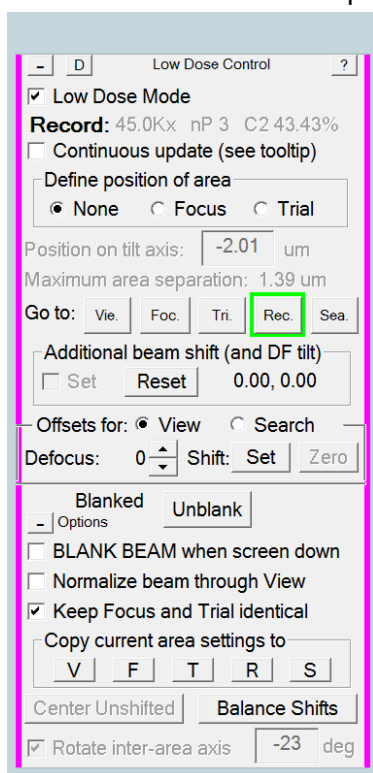
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- Adjust Offsets for View and Search** (optional, don't need to do this every time you use the microscope). Sometimes the Record, View and Search are off centered, to check for this find a chunk of ice and move to it in Record. Take Record, View and Search Image, the ice should be centered in each of the image.



9. If the images are not centered then you can apply a offset to the View and Search. This is done in the Low-Dose panel.



At this point the ice is centered in Record but not centered in View or Search or both.

Check “Offsets for ..View or Search depending on which one you want to change.

Take a View or Search image

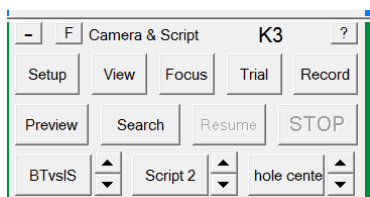
With the mouse drag that image so the ice is in the center. You might need to uncheck “Move stage for large image shifts” option in the Image Alignment and Focus Panel.

Click Shift “Set” to set the offset.

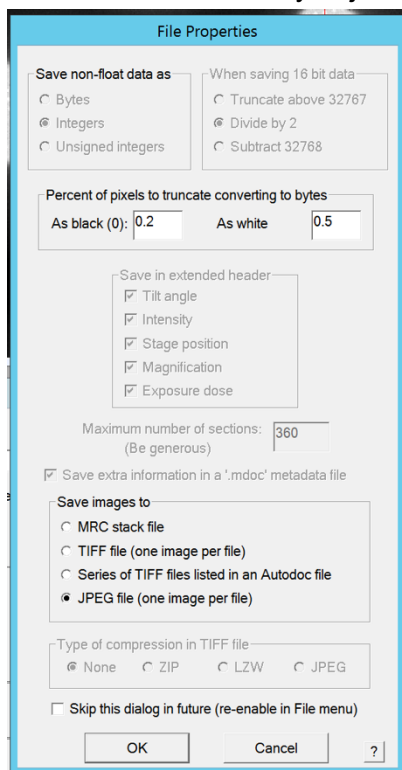
Do this for the View or Search.

10. To **Move to the point** click on it in the navigator file point, should be highlighted in blue, then click “Go to XY” or “Go to XYZ”
11. After moving to a point adjust the **eucentric height** using the Microscope Stage Wobbler or in SerialEM use Rough Eucentric, Task → Rough Eucentric. *Note the Rough Eccentric will not work if the Z height of the stage is very far (75-50 μm) from focus. If you change the Z height of the Stage you can save this to the Navigator point by clicking “Update Z”.*
12. To take a **Search image**, click on the “Search” icon in the “Camera and Script” panel.

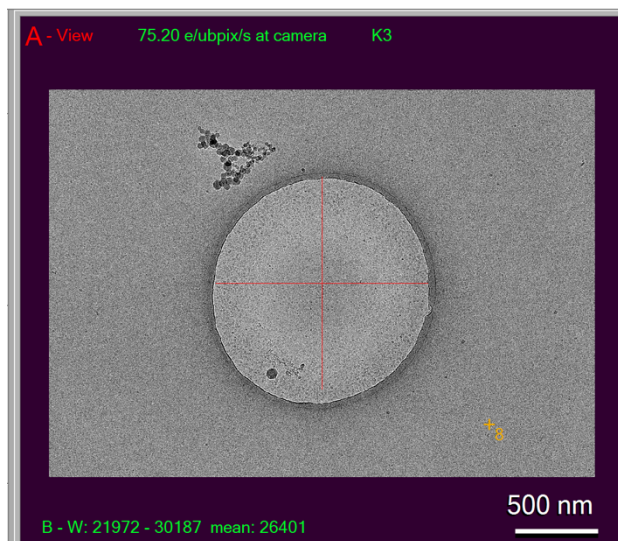
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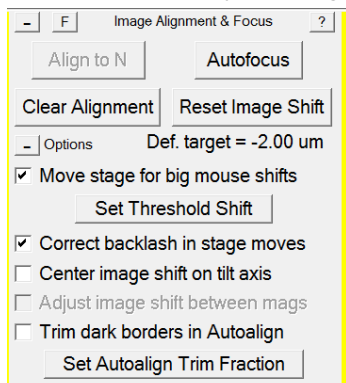
13. To save this (and other images) select File → save to other. Then click ok. Usually just save as a JPEG if you just want a picture for documentation (not image processing).



14. To take a **View image**, click on the “View” icon in the “Camera and Script” panel. *To increase the contrast for the View image you can change the “defocus offset” on the “Low Dose” Panel, typically 10-20 μm is fine.*
15. To take a **Record image**, set **Target Autofocus** to - 1 to -3 μm .
16. **Move the stage or image shift over a hole**, by clicking on the View Image in the window with the mouse and dragging it to the center (will either stage shift or image shift depending on distance). Should look like this.



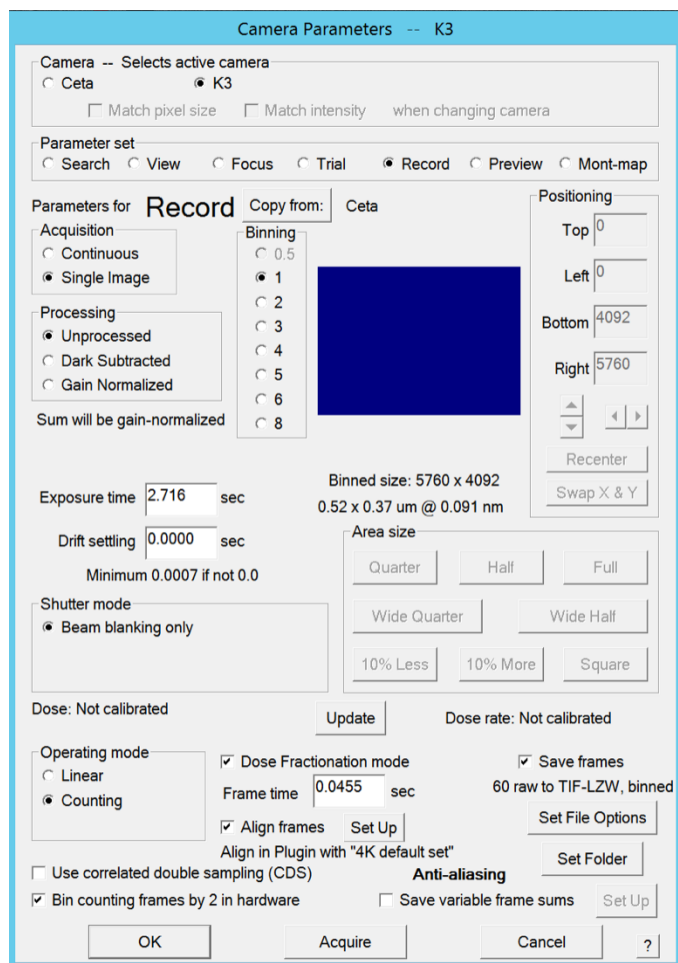
17. **Autofocus** by clicking on the “Autofocus” icon in the “Image Alignment and Focus” panel



Look at the SerialEM log, Window, and FFT of the image to judge if the autofocusing is working.

18. Take a record by clicking on the “Record” icon on the “Camera and Script” panel. *Note before collecting images check the **Camera Parameters**, can be selected by clicking on “Setup” icon in the “Camera and Scrip” panel.*

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Camera Parameters for Screening

Don't need to change the parameters but ...

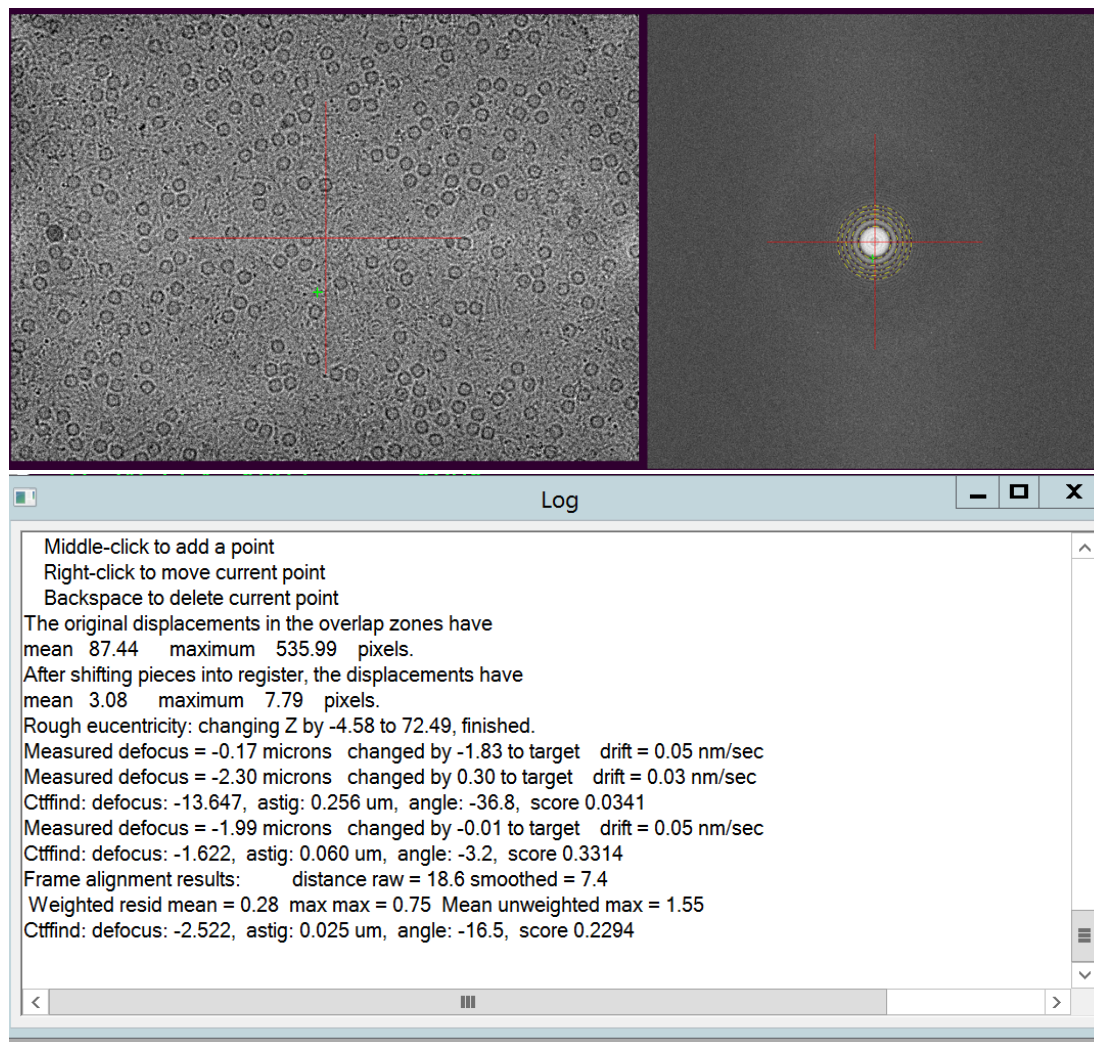
For screening / collecting a few images select the following:

Processing **"Gain Normalized"**

Uncheck **"Save frames"**

19. To take a record image click on the **"Record"** icon in the "Camera and Script" panel.

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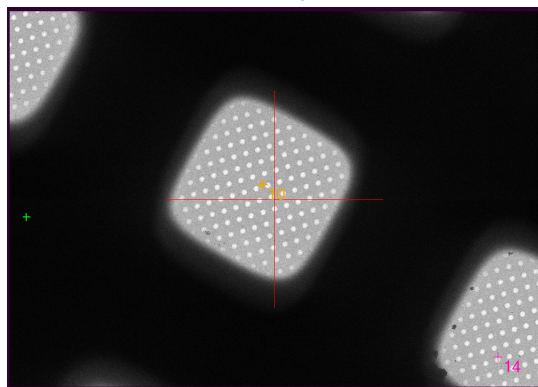


You can click on the FFT to activate **Ctffind** plugin....will print out info in the serialEM log

VII. Montage Grid Squares

At this point you have identified a cryo-grid for data collection, and it is on the stage.

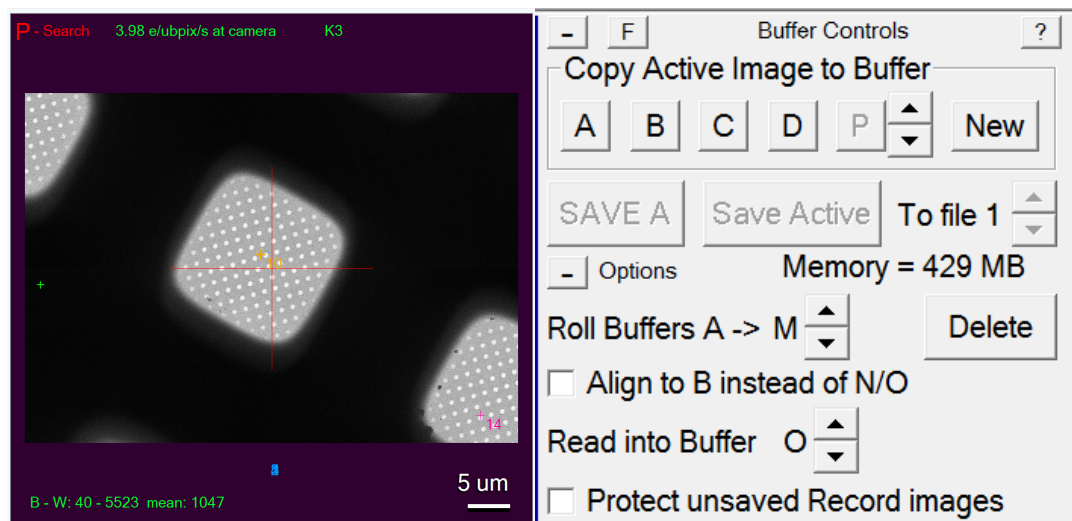
1. On the Full Grid Montage Map add points to the center of grids squares that you want to image. Try to estimate how many grids squares you will image, to do this count how many holes are on each grid square.



2. On the Navigator mark these points as **Acquire**) (check box), the points will have a "A"

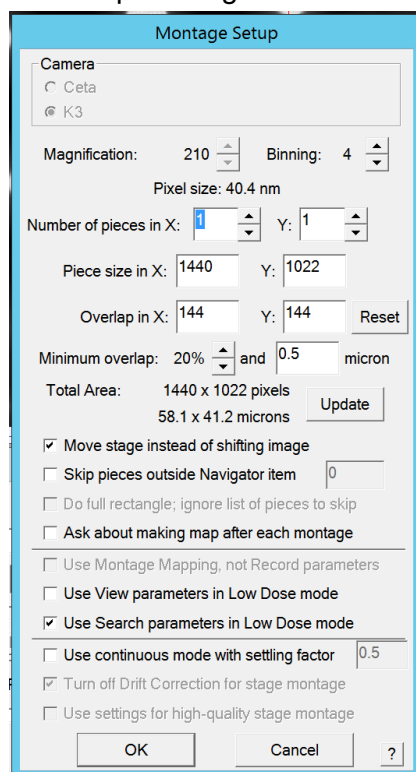
10	Red	47.9	-39.1	72.5	Pt	1	A	collect
11	Red	-203.5	46.9	72.5	Pt	1	A	collect
12	Red	-182.0	-42.7	72.5	Pt	1	A	collect
13	Red	-67.3	-108.2	72.5	Pt	1	A	collect
14	Red	12.4	-90.6	72.5	Pt	1	A	collect

Take an **Search image** of one grid square make sure it is centered, save it as a MRC, or copy it to **Buffer P** in the "Buffer Control" panel by clicking on the Icon "P" . The Buffer is displayed in the Window in red letter.



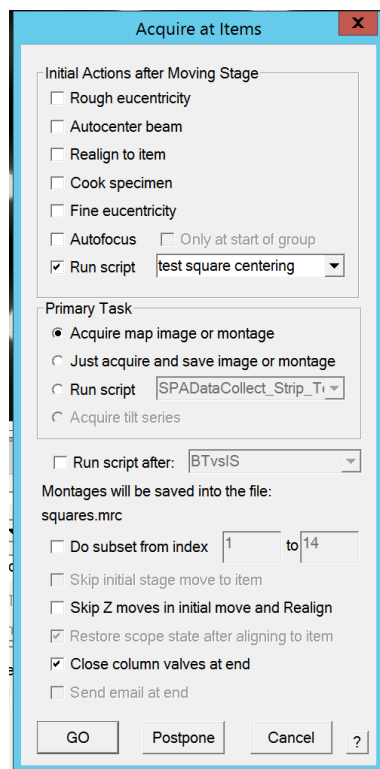
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3. Make sure Full Grid Montage is not open in SerialEM. If it is open: File→Close
4. Setup Montage to take one image at 210 X Search



5. Select the Navigator tab --> **Acquire at items**

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Make sure to select **“Run Script test square centering”**.

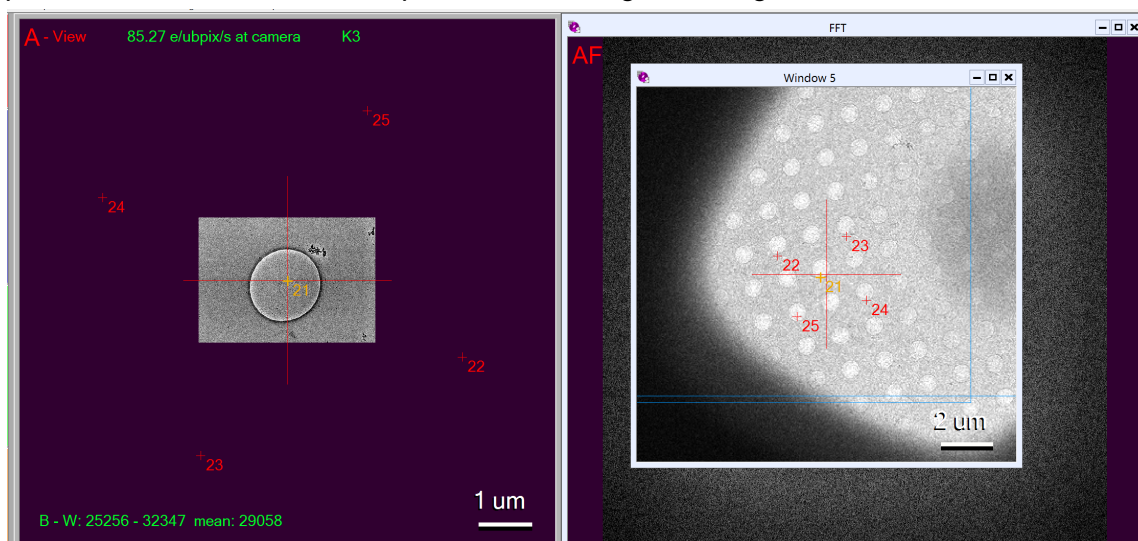
It takes about 1 min to collect a montage of each grid square.

VIII. Gain Reference for K3 in Linear and Counting Mode

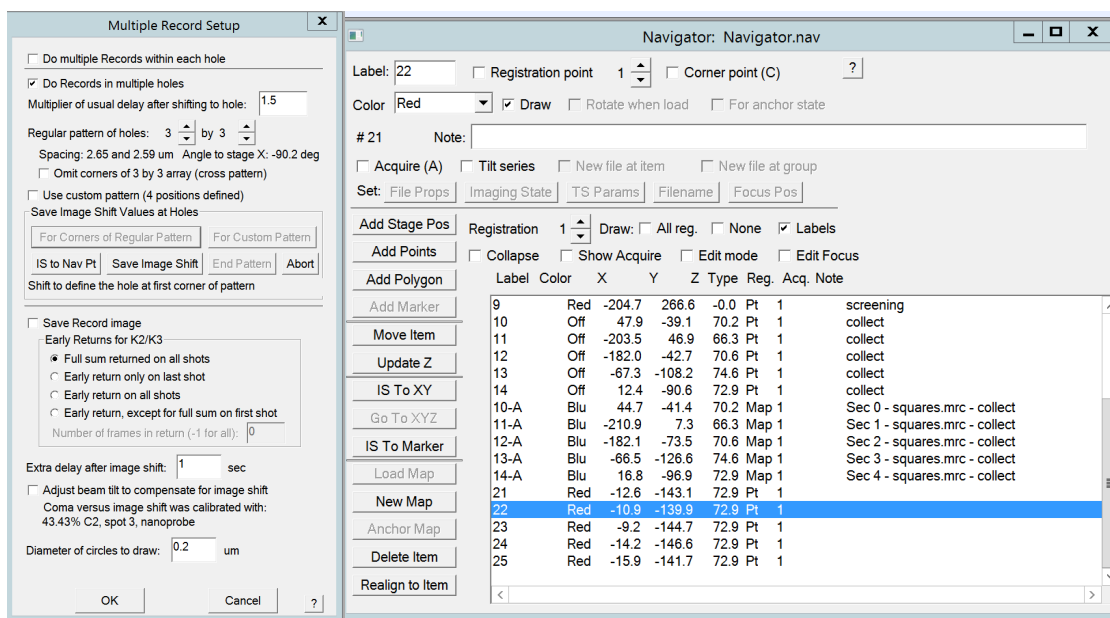
- IX. This will be done each morning by a Core employee. If the microscope parameters were changed and you wish to obtain a new gain reference before your data collection and are not comfortable doing so, you can ask a Core employee to assist you.

X. Setup Multishot Record

1. On one of the grid square montages **add 5 points**, a center point and 3 diagonal points.
2. To ensure that each point is in the center of a hole, take an image in View, and move the points to the center of their respective hole using the Navigator “Edit” mode.



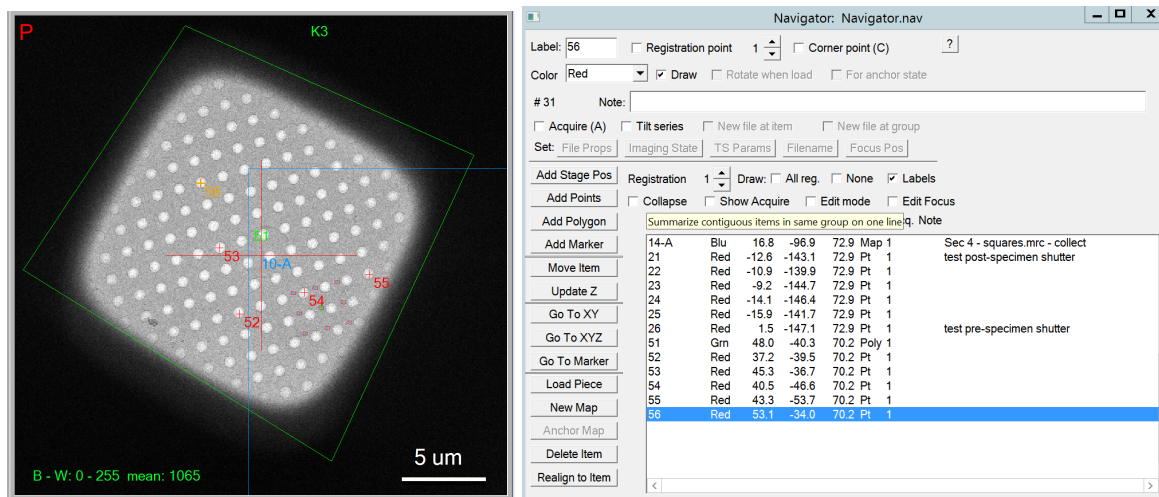
3. Once all the points are set, **Setup Multiple Record** in the Navigator → Montaging and Grids → Set Multishot Parameters



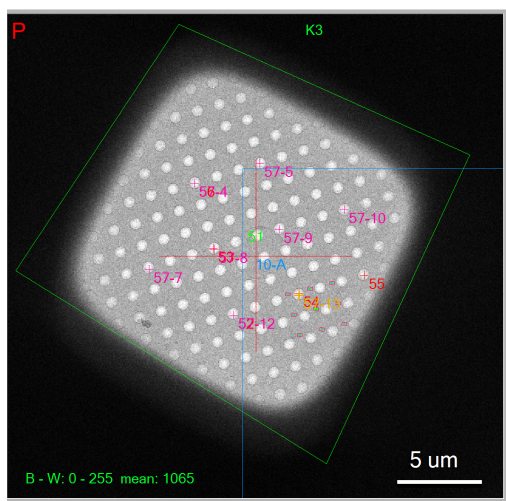
4. Return to the center point using **Go to XY**
5. Select **For Corners of Regular pattern** in the Multiple Record Setup window. In the Navigator select the **corner points** (highlighted in blue), then click “IS XY”, to image shift to that point. Each image shift should be ~3.5 μm, this will be reported in the “Microscope Control” panel. Then in the Multiple Record Setup click on “**Save Image Shift**”. Repeat this for the other 3 corner points. The corner points should be ordered CW or CC.
6. **Double check** by mouse clicking on a hole in a search image, it should show a diagram of where each of the record image will be acquired.

XI. Add Grid of Points

1. **Open a grid square montage** in the main window, on the Navigator double click on the map.
2. Add a **polygon** around the grid square, shown in green. To do this on the Navigator click **“Add Polygon”**. On the grid square montage add 5 points, spaced 4 holes apart, as shown in the image, to do this in the Navigator click **“Add Points”**. Add grid of points in the Navigator → Montage → add grid of points.



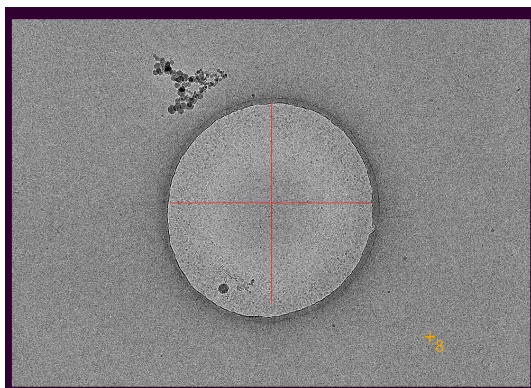
It should look like this



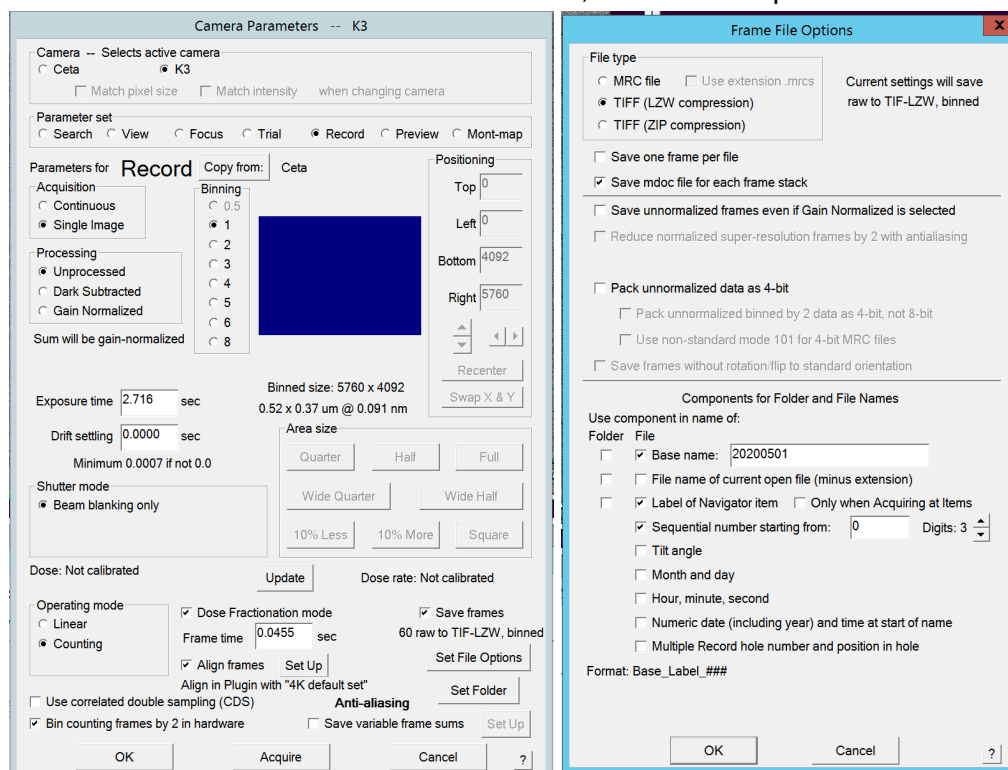
3. To add more point on the other grid square montages, open the grid square montage. Add a polygon around it. Add one point to the center. On the Navigator → montage → add grids like last one. This is add a grid of points within this polygon. Mark these points for data collection by click on the **“Aquire (A)”** in the Navigator. Make sure to save the Navigator file periodically.

XII. Data Collection

1. Acquire a **View Image of a Hole**, it should be centered.
2. Copy this image to **buffer P**, click on “P” in the Buffer Control panel. The script will use this image to move the stage/image shift to the acquired points over a hole.

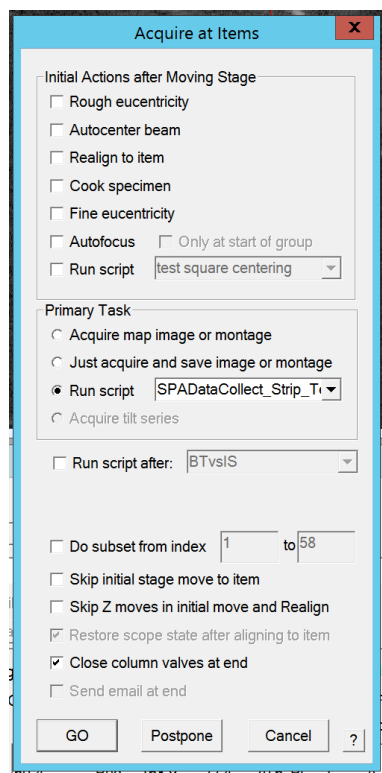


3. Check the **Camera Record Parameters**, click on “Setup” in the Camera and Script panel.



- Check the following options: “Unprocessed”, “Binning 1”, “Beam blanking only”, “Counting”, “Dose Fractional mode”, “Align Frames”, “Save frames”.
 - Click on “Set File Options”, to open the “Frame File Options” panel. Change the “Base name” to the date.
 - Click “Set Folder” to save the moves to the correct directory. Note, must save data to the (X):/k3data/ do not save the data to another hard drive ie D or E.
4. Check position of focus.
 5. On the Navigator → **Acquire points**

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6. Check “Run script” select script 19 . Note don’t usually need to modify this script, but feel free to look it over. Then click “Go” to start data collection.

XIII. Pre-process Data

Ask the Core Director to help setup CryoSPARC Live or Scipion

XIV. Saving data from a screening session

To save images and data (including serialEM log and navigator) follow the following naming conventions. Be sure to save the data on the K3 Server X: drive and not the C: drive!!!

Make directory in /K3data/
20191111_JS_TK/"date" "PI initials" "Users Initials"

For each grid make a new directory to save images and navigator
20191111_JS_TK_TK3_G1/

File naming...save as tif or mrc or jpg
20191111_JS_TK_TK3_G1_Montage.mrcFull grid montage
20191111_JS_TK_TK3_G1_Pt2_Search.mrcPt2 "navigator point 2"
20191111_JS_TK_TK3_G1_Pt2_View.mrc
20191111_JS_TK_TK3_G1_Pt2_Record.mrc

Make sure to save the montages and Navigator files, and serialEM log file.

If you collect data on a grid, take a screenshot of the Full Montage in SerialEM Window indicating which areas were collected on and save it as a png.