

TEM - TFS Titan Krios G3i - 3: Set up data acquisition with EPU

This guide from the Center for Microscopy and Image Analysis, UZH, Zurich, shows how to set up data acquisition with EPU at the Thermo Titan Krios G3i.

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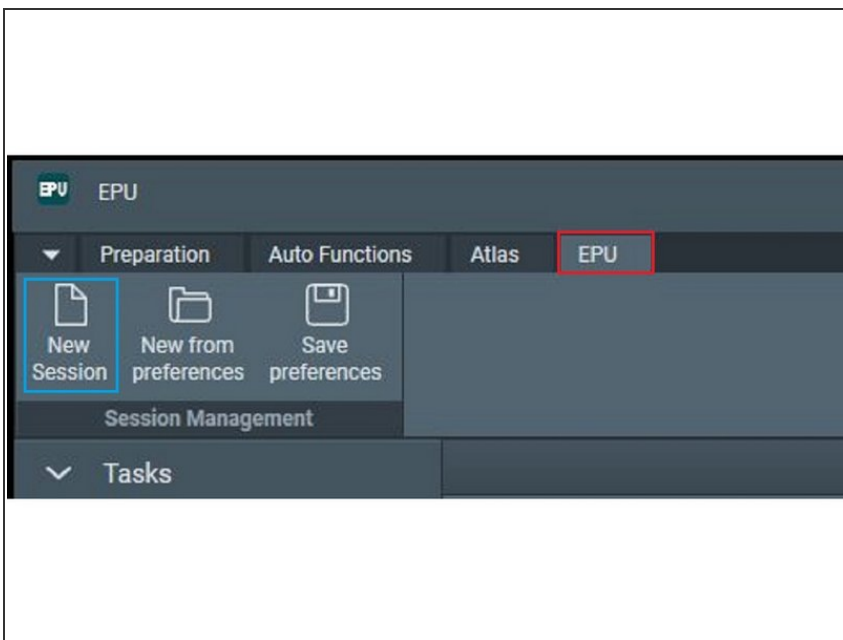


INTRODUCTION

This guide from the Center for Microscopy and Image Analysis, UZH, Zurich, Switzerland shows how to set up data acquisition with EPU 2.7 at the transmission electron microscope Titan Krios G3i.

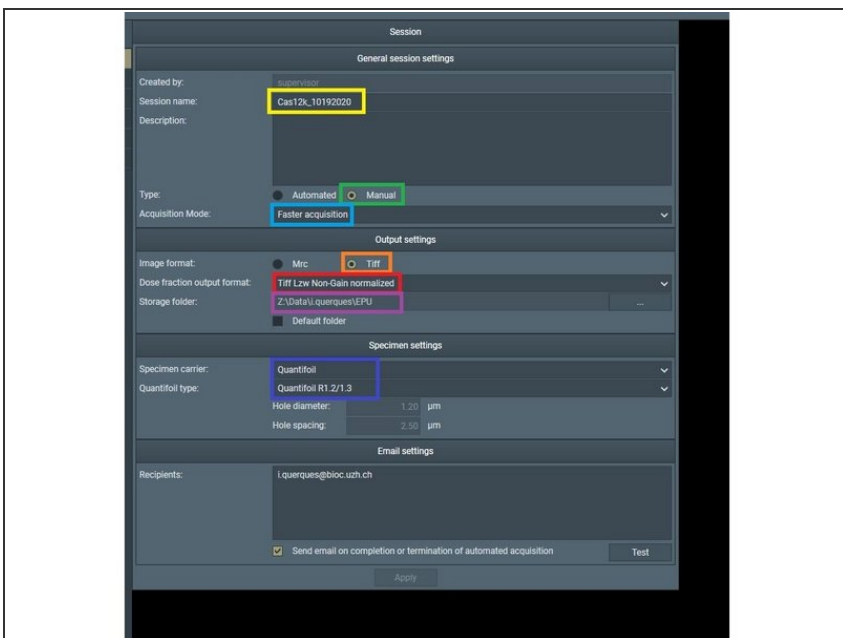
Please find more detailed information about the system [here](#).

Step 1 — Creat a new session



- Create a new Session. Epu ->New Session.

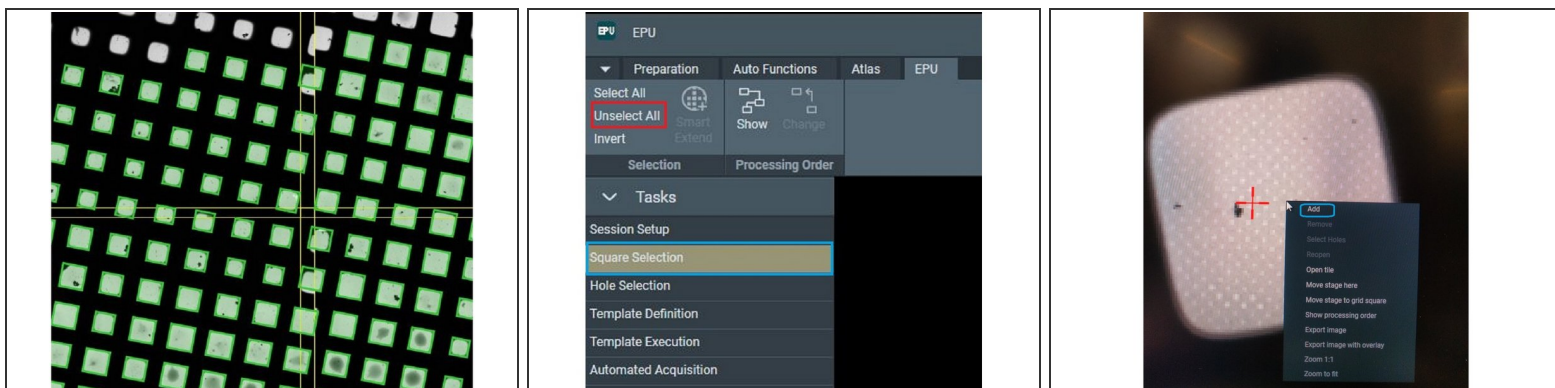
Step 2 — Set up you session settings



- Name your session.
 - The session can be defined as either "Manual" to be able to decide on which holes the program will acquire data.
 - Or "Automated". In which case manual selection of holes will not be possible.
- "Faster acquisition" will use beam and image shift to speed up the data acquisition (to acquire ~300 images per hour).

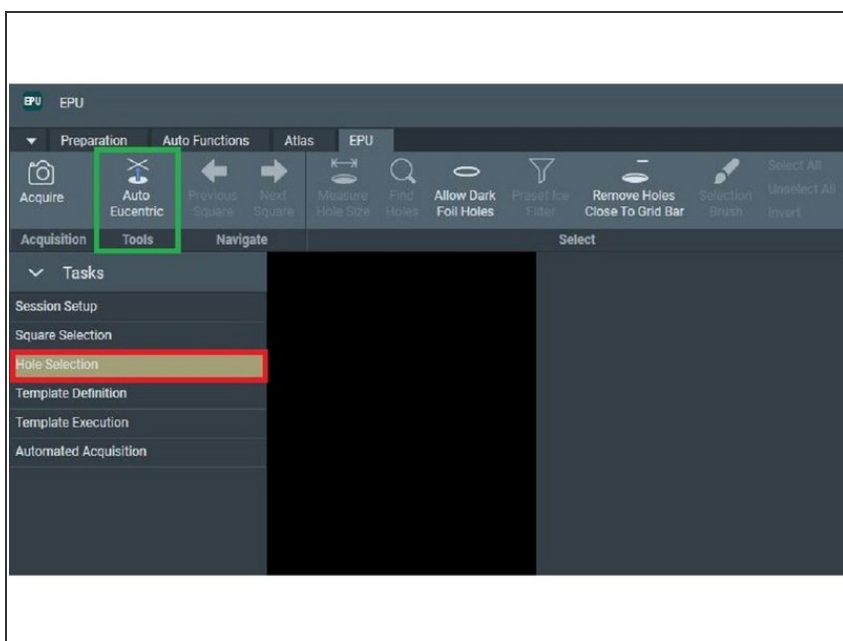
- Select .tiff to save space. This is the image format of the images that EPU takes to find back your positions (not your movies).
- Here you select the format of your movies. Movies are compressed and not gain normalized. "Lzw Non-gain normalized" is the standard format used in the community to save space.
- Here is the storage folder where EPU saves the grid squares/foil holes and low resolution previews of the data acquisition. It is better to save those files into the K2 computer, as it has more storage space.
- Select your grid type.

Step 3 — Select the grid squares for data acquisition



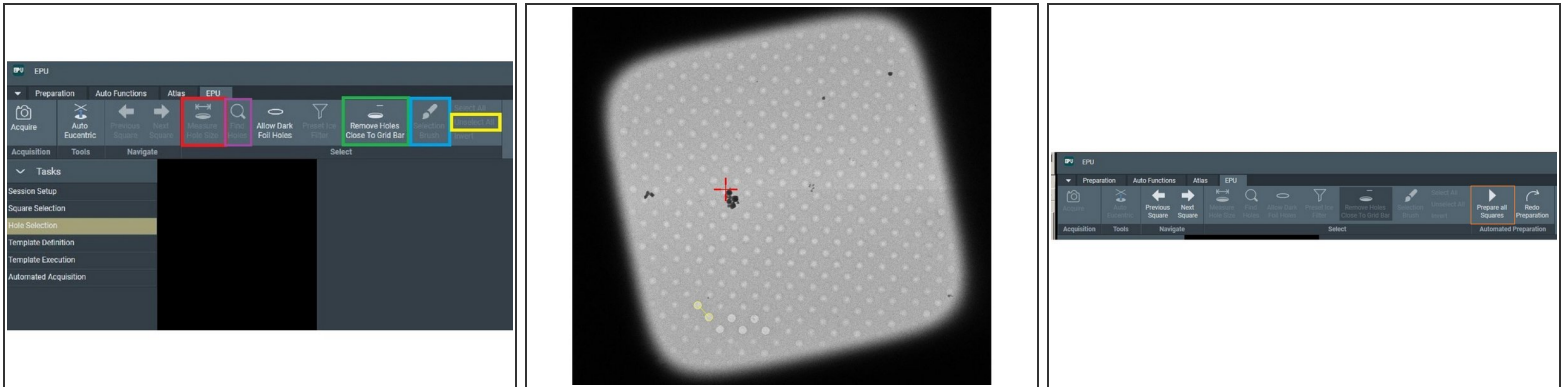
- Navigate to EPU -> "Square Selection" to choose the grid squares for data acquisition.
- ❗ The program finds the grid squares automatically. By default all the grid squares will be selected.
- Unselect all to clear up the selection.
- Right mouse click to select the grid squares you would like to acquire and select the "Add" option.
- Add all the grid squares you want to collect data on.

Step 4 — Set up for hole selection



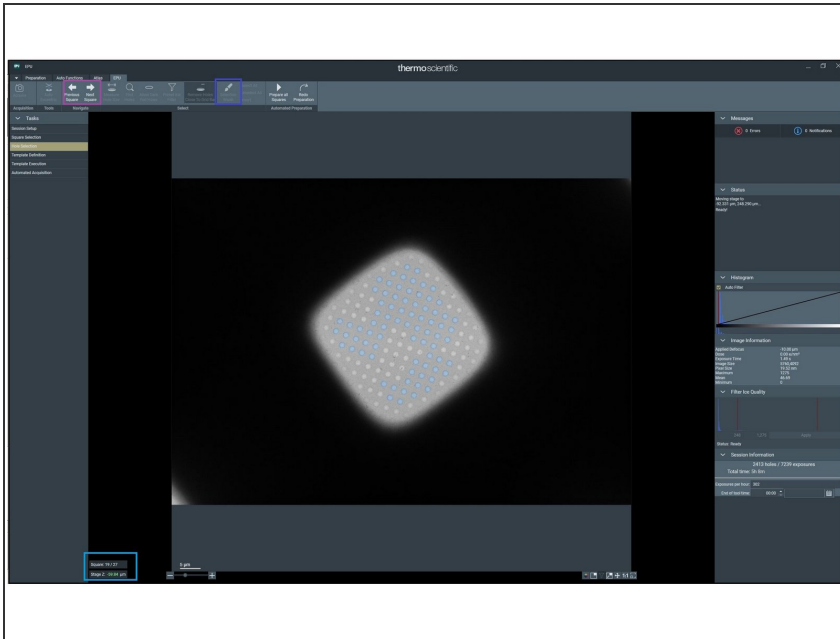
- Go to the "Hole Selection" tab.
- Select Auto Eucentric.
- ❗ The Auto Eucentric button will perform Eucentricity by Beam Tilt using the Eucentric Height presets. After the Eucentric height is determined, it will store the Z value and it will acquire a Grid Square image automatically.

Step 5 — Find and select the holes



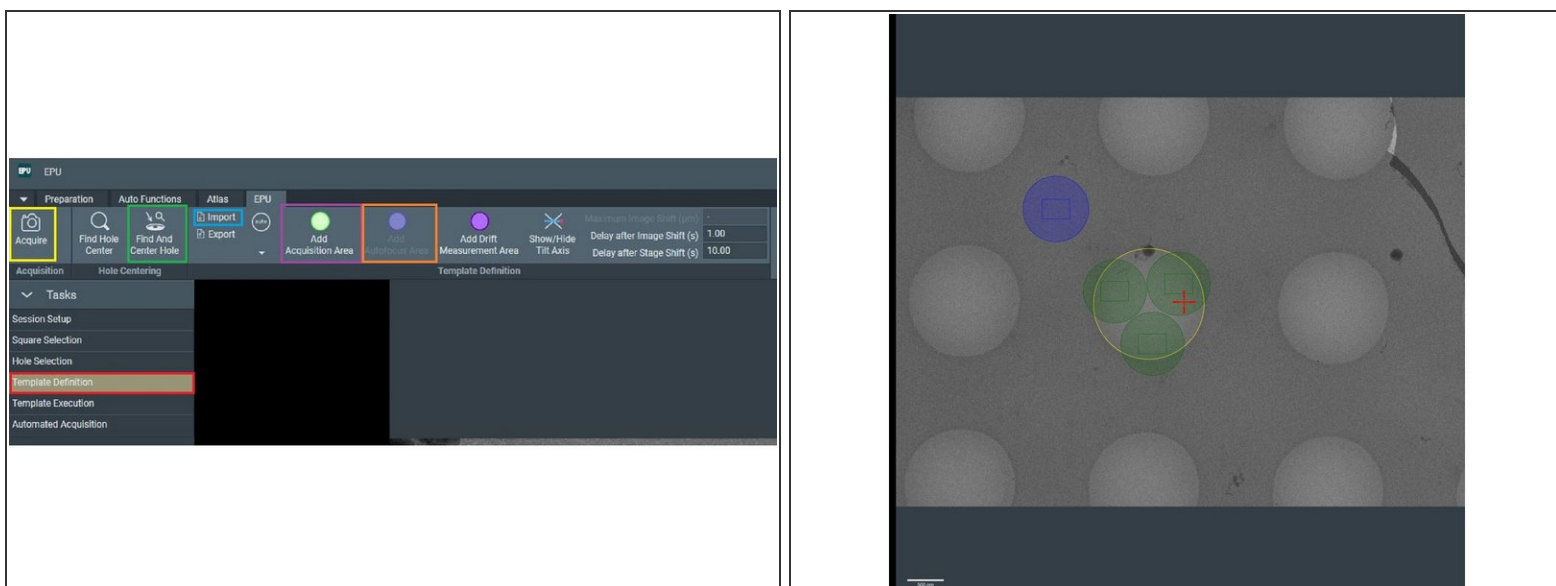
- Select "Measure Hole Size" to measure the size of the holes from your grid square image.
- Measure the hole diameter and spacing from your grids' square image.
- To find all the holes present in your image, select "Find Holes".
- To remove the holes close to the grid squares, you can select the "Remove Holes Close to Grid bar" option.
- If you are unhappy with the selection, and you wish to measure the holes again, unselect the holes with the "Unselect All" option.
- To modify the found selection, you can use the selection brush.
- To prepare all the others grid Squares, you can use the "Prepare All Squares" function.

Step 6 — Clean the Hole Selection in all selected grid squares



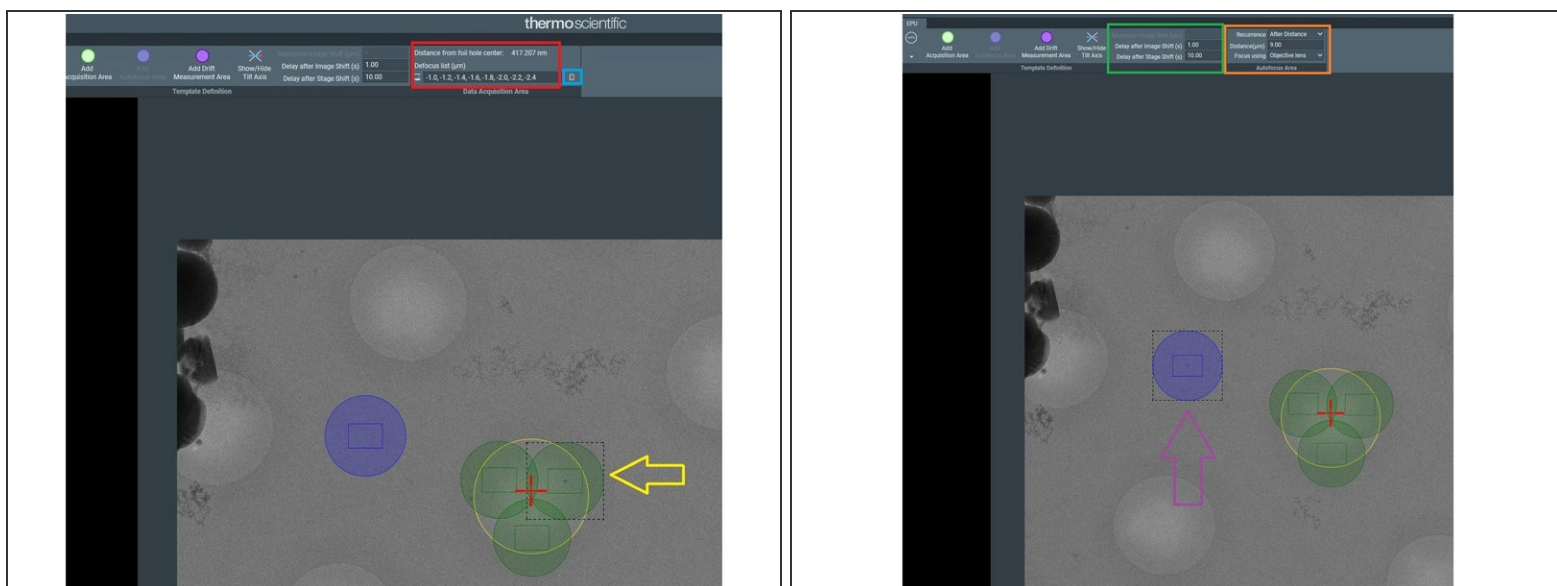
- i** You need to observe which holes were selected in all the grid squares, and clean from all of them.
- The grid square assignment is shown in the lower left of the EPU window.
- You can navigate between the grid squares with the "Previous Square" and "Next Squares option".
- Clean with the selection brush all of the added grid squares.

Step 7 — Define your template: Data Acquisition and Focus Position



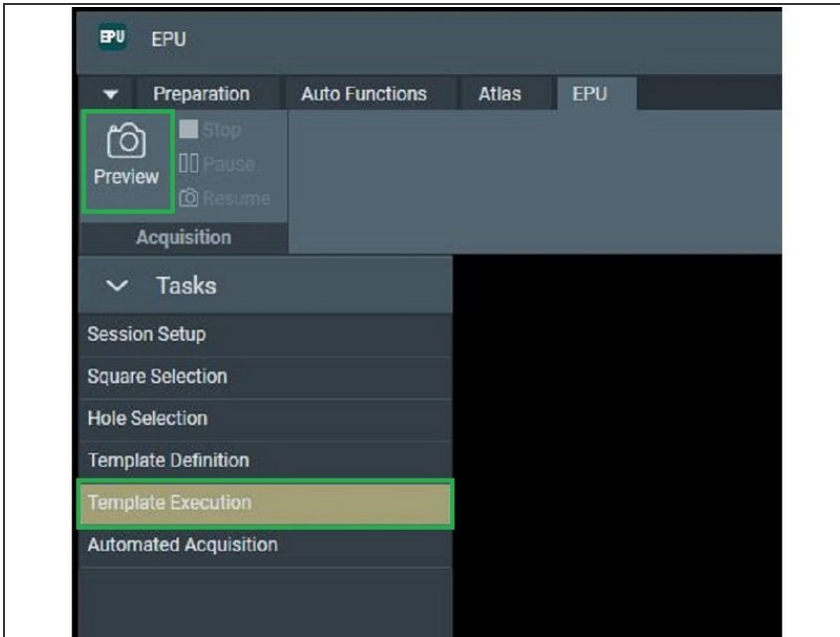
- Click on the "Template Definition" button.
- "Acquire" an image with the Eucentric height presets to be able to define the template.
- ❗ The "Acquire" button is active only when at least 1 hole is selected on the current grid square.
- Check that the hole measuring from the step before it's correct, with the "Find and Center Hole".
- If you already have a set template that you wish to use, you can import it here.
- If you don't have a template and you need to prepare one, select "Add Acquisition Area", and then click on the image to add the acquisition area .
- To add the focusing Area, select the "Add Autofocus Area" and add it by clicking on the Foil Hole Image.
- ❗ Add as many Acquisition Area as they fit in the hole. Be sure to irradiate the Carbon a bit, so that you don't have charging during Acquisition. For the autofocus to be efficient, the Autofocus area should be on carbon.

Step 8 — Define your template: defocus list and other parameters



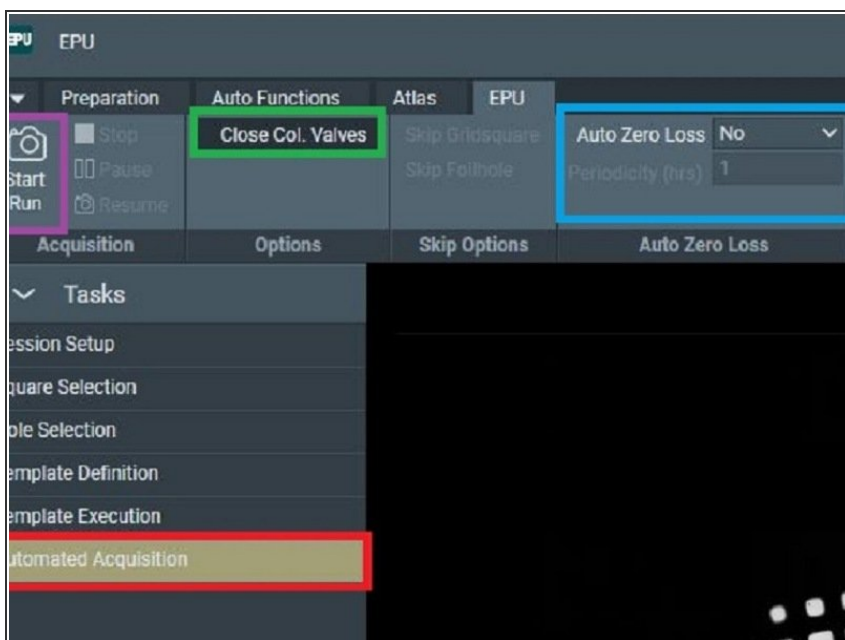
- i To Define the defocus values to acquire your data, you need to provide a defocus list.
 - To be able to define the defocus list, select one of your acquisition areas by clicking on it.
 - Define your defocus list. The defocus value should be defined in um and separated by a comma. An example of the format is: "-1.0, -1.2, -1.4".
 - To apply the defocus list you have just defined to all the acquisition areas, click on the small page icon close to the defocus list.
 - To visualize the method selected for autofocus, activate the autofocus by clicking on the autofocus area.
 - Autofocus should be performed with the "Objective lens". You can select also how often autofocus would be performed during data acquisition. "After Distance" 9um usually works.
 - You can also select how long the program should wait to acquire an image after Image Shift and after Stage Shift. 1 sec "Delay after Image Shift" and 10 sec "Delay after Stage Shift" usually works.

Step 9 — Test the template



- In the "Template Execution" tab, select "Preview".
- ❗ The program will execute the template in the closest Foil Hole. It will do an autofocus and acquire the defined positions.
- ⚠ Observe the Template Execution. Make sure the autofocus is on carbon, and that it acquires data on the defined position of the Hole (e.g., not on carbon).
- ⚠ Check that there is no error message. If there is any issue now, there will also be one during data acquisition.

Step 10 — Start data acquisition



- Activated the "Automated Acquisition" tab.
 - Select "Close Col. Valves" so that the column valves close automatically when the data acquisition is done.
 - If you want, you can select the energy filter to be centered during data acquisition. In that case change the default value "No" to "Yes".
- ⚠ Before Starting your run, make sure you did the direct alignments and inserted the objective aperture ([instructions here](#)).
- Start your run.
 - Sit back and let the microscope work for you. Things in general work pretty smoothly, but we still recommend to check the data acquisition every once in a while.