

Latitude Data Collection Set-Up Procedure

Note: This software is sensitive and certain functions can cause the software to crash.

It is important to do the steps in this protocol in order. Read each step through to the end before performing them. When unsure of a step in this protocol, close column valves to protect grid. Then seek help. Report all warning messages and all problems to SMIF Staff **IMMEDIATELY.**

Technique Notes:

1. Zooming in and out in an image can be done with the mouse wheel.
2. In order to pan within an image in the software, hold the spacebar, then left click and drag. If it does not work at first, then make sure that the image being used has been selected.
3. In order to delete selections, select the object (template, square etc.) and when the corners have lit up as selected, click the delete button on the keyboard.

Krios Set-Up

1. Perform Direct Alignments (See Krios Direct Alignments Procedure)
2. In the Krios software, go to the EFTEM tab and press the EFTEM button (button will turn yellow indicating the Krios is in EFTEM mode).
3. In the Krios software, go to the Vacuum tab and check to make sure column valves are open (Col Valves button should be gray).
4. Press R1 to retract the Flu screen.

Latitude Setup:

1. Go to the Latitude Software monitor.
2. Under the Latitude S panel, press the New button.
3. Check (✓) *Based on Prior Selection.*
4. Open a previous folder and use those settings. It is recommended to select a previous data folder from your lab, in which settings were used that apply to your current sample.
5. The software will open a new folder to store the new data collection files. This folder will be numerically named. It can be renamed to follow the format of previous collections from your lab.
6. The software may indicate that there is an issue and give an error notification saying folder cannot be found. If this happens, repeat the above process but select the renamed folder instead of renaming the new one that comes up.

Image Alignment:

Note: This is an important process. If not completed correctly, the beam will not shift to the correct locations during data collection. It is worth taking extra time to do this section precisely.

Before starting the Mag alignment, check to see if beam is centered in all mags.

1. In the State Setup Atlas panel click **Goto**, lower the screen to check if beam is centered. The green (gif) reference circle should be covered and beam should be centered. If not use the roller ball to center the beam. Then click on scope on the Atlas (latitude) panel. Confirm by taking a capture and seeing if all corners of image are covered by the beam. Repeat this for Grid, Hole, Focus and Data. For Data mag if you need to center the beam use beam shift under direct alignment. Now proceed to Mag alignment.

Latitude Data Collection Set-Up Procedure

2. Select the **State Setup – Atlas** menu box.
 - a. Press the Goto button. This sets the Krios to the Atlas magnification and spot size.
 - b. Press the Capture button to acquire an image.
 - c. Right click on the image and a function box will appear.
 - d. Select the Centering cursor; it looks like a gray circle. **Be careful with this function.** Anytime you click inside the image, the software will reposition center. This shift will not be visible until the next image is collected.
 - e. Find a grid square with a piece of dirt or ice.
 - f. Using the Centering cursor, click on this feature to center the Atlas image on this grid square. Choose a grid square with poor quality, but it must not be broken or cracked.
 - g. Press the Capture button to acquire another Atlas image.
 - h. Check the image to see if the grid square with the particle is in the center of the screen.
 - i. Go back to State Setup-Atlas panel and press the Next button. The software will goto the next magnification in State Setup.
3. **State Set-Up Grid** menu box
 - a. Press the Goto button. This sets the Krios to the **Grid** magnification and spot size.
 - b. Press the Capture button to acquire an image.
 - c. Use the Centering cursor to center the image on the piece of dirt/ice from the first image.
 - d. Go back to the State Setup panel and press the Next button. The software will goto the next magnification in State Setup.
4. **State Set-Up Hole** menu box
 - a. Press the Goto button. This sets the Krios to the Hole magnification and spot size.
 - b. Press the Capture button to acquire an image. The image will flip, so keep that in mind when centering the feature.
 - c. Use the Centering cursor to center the image on the piece of dirt/ice from the first image.
 - d. If unsure if the image is centered where you want it or if the Centering cursor was accidentally clicked in the wrong place, capture the image again.
 - e. Go back to the State Setup panel and press the Next button. The software will goto the next magnification in State Setup.
5. **State Set-Up Focus** menu box
 - a. Press the Goto button. This sets the Krios to the Focus magnification and spot size.
 - b. Press the Capture button to acquire an image.
 - c. Use the Centering cursor to center the image on the piece of dirt/ice from the first image.
 - d. Go back to the State Setup panel and press the Next button. The software will goto the next magnification in State Setup.
6. **State Set-Up Data** menu box
 - a. Press the Goto button. This sets the Krios to the Data magnification and spot size.
 - b. Press the Capture button to acquire an image.
 - c. Verify that edges of the beam are not present in the image. If beam edges are present:
 - Go to the Krios software and select Beam Shift under Direct Alignments
 - Press R1 to insert the Flu screen
 - Use the Multifunction X and Y knobs to shift the beam
 - Click done at the bottom of the Direct Alignments panel

Latitude Data Collection Set-Up Procedure

- Press R1 to extract the Flu screen
- In the Latitude Data menu box, press Capture to acquire another image and verify that beam edges are no longer present.
- Use centering tool to center the piece of dirt or ice.

7. Perform Fine Alignment

- Open the Fine Alignment Panel and press the Capture button.
- Images will appear from Atlas to Data magnifications.
- Once the 5 images have been collected, right click on one of the images and select the black arrow cursor. This is very important – accidentally using the centering cursor will move the stage and require the fine alignments to be repeated.
- Inside each image, move the red cross so it is centered over the same edge location of a piece of dirt/ice. Zooming in on the low magnification images can help ensure the same location is selected. This is aligning the images to the same point in the software.
- Once the red crosses are all aligned well, press the Calculate button.
- Press the Capture button again to verify that the alignments calculated properly.
- If the red crosses are not in the same location in each image, then re-center the crosses and Calculate again. Click **Next** when satisfied with alignment.

Collect an Atlas:

- Open the Latitude Experiment panel.
- The Atlas size should be set to 5 by 7. If it is not, change the values.
- Center the stage in the Krios software.
 - Go to the Stage+ tab.
 - In the second to left panel press the XY button. This will move the stage to the x=0 and y=0 position.
- Go back to the Latitude software and press the Capture button.
- This will collect the atlas image in sets of 5 by 7 grid squares, and will take 10-15 mins to complete.

Note: The Schedule button in a panel of the workspace schedules only tasks in that window. The Schedule button in the Latitude Summary task bar schedules all tasks in the software

Calculating the dose:

- Once Atlas is complete, select a broken square (no carbon/sample). Hit schedule under that tile window. See the cursor move to that position on the Stage window. Click stop on latitude summary panel. This will take the stage over vacuum. Click the square and delete the yellow box.
- Confirm if you are over vacuum by capturing under the Data Setup panel. If you see pure noise, you can proceed or center so that you see pure noise in case you see some feature.
- If Gain has not been performed, do gain references (see procedure) before you calculate dose.
- Verify that the measured dose rate is in the 20 e/pix/sec and total dose is in the 50-60 e/A2 range.
 - The dose can be read at the bottom of the Latitude software screen, or by clicking the “More” button in the Set-Up Data menu box

Latitude Data Collection Set-Up Procedure

- If dose is not in this range, adjust the illuminated area (intensity knob) and/or spot size on the Krios, then click [Scope] in the State Set-Up Data menu box to update the beam conditions from the microscope.
- Capture an image and recheck the dose. Repeat these steps as needed until dose is in the correct range

Select Grid Squares:

1. Once the Atlas is complete, select the squares you want to use by clicking in the center of each square.
2. It is okay to select squares on the edges of the atlas; the software will take a full picture of that square. However, do not select squares that are on the edge of the grid, this causes problems with data collection.
3. It is important that the center of the square is selected. If it is not, problems can occur in data collection.
4. If the box around the grid square is not centered, click on the edges and adjust box.
5. Select one optimal square and click schedule under the window panel which shows the square. Wait till it gives you a grid image of that square.
6. **Make hole reference**
 - Open State Setup- Hole panel. Press the GoTo button and then press the Capture button.
 - When the image appears, center this image to get the best image of full holes/ mostly visible holes. (Right click on the image and a function box will appear. Select the Centering cursor, it looks like a gray circle. Click in the image were you would like the center to be.)
 - Press the Capture button again to verify change in center is correct.
 - Continue to adjust the image until it is centered as desired.
 - When satisfied with the hole positioning in the image, press the Update button in same tab and minimize image.
7. **Create a hole template**
 - Select [add position] to the grid square image in the working panel and click in the center of a hole so that the selection circle is centered, then press the schedule Button.
 - In the hole image, shift click to select a focus point located between holes, and then regular click a single hole.
 - Press the Save as Template button.
8. **Select holes to image**
 - In the same grid square delete the selection you had made.
 - Now select Autofind under the Grid image. Adjust the parameters. The maximum you can go in image shift is about 12um. Click on Find, it should center the right angle cursor. If not manually center the angle edge in the center of a hole. Click Mark to see the red circles, if they are well centered over holes click Add to get the yellow targets.
 - Insert the Objective aperture; In the Krios software, go to the Tune tab. Under the Apertures panel, select 100 for the Objective aperture, if not already selected.
 - Click on the [Objective} button on the left side of the Apertures panel. The button will turn yellow, and in the "Enable" pull out menu to the right of the Apertures panel it should indicate that the Objective is inserted.

Latitude Data Collection Set-Up Procedure

- Schedule the targets from the schedule tab under the grid panel. Check the checkbox for [Auto navigate to current task and acquired image] under the Tasks portion of the Latitude Task bar and view the data collection to ensure that the images being collected are satisfactory. You should not see excessive amounts of hole edges, or the presence of the beam edge. Once you have confirmed go to selecting more squares.
9. 50 to 60 good squares should be enough for overnight run and should give about 5000+ images. Press the schedule button in the Latitude Summary task bar to begin square collection in the software. Boxes around the squares will change colors
 - a. Yellow indicates the item has been selected
 - b. Green indicates the item has been scheduled for acquisition
 - c. Blue indicates the item has been acquired
 - d. Red selection indicates that the acquisition of the selection failed (this should not be an issue if only a few fail).
 10. Processing of these grid/Squares will take some time to complete depending on the number of squares chosen.
 11. Meanwhile as the grid squares are processed you can go ahead and add targets without scheduling the targets.
 12. Once all grid squares are processed press schedule in the Latitude summary task bar to begin image collection. Keep scheduling more targets.

Set-Up Data Pipeline (to store data on server for processing)

1. Once you are satisfied that the data being collected is satisfactory, go to the support computer screen (farthest screen to the right and near the wall) and open Google Chrome.
2. Click on the Krios Data Pre-Processing bookmark to create a project for data collection and compiling. This will open a link to the Krios data pre-processing form
 - a. Select the detector being used
 - b. Enter values for pixel size and dose. These can be found in the State Set-Up Data box by clicking the More button.
 - c. Enter an estimated particle radius for the sample you are imaging.
 - d. Select the Principal Investigator for this project.
 - e. In the Session Name field, enter the exact file name being used to store the data on the Gatan computer. You can find the file name in the Capture Data (X:)/Data folder within the folder assigned to your PI on the K3/Latitude computer. MAKE SURE TO ENTER THE FILENAME VERBATIM.
3. Once the pre-processing form has been completed, click Submit

Verify Data is Being Saved and Transferred:

1. On the K3/Latitude computer go to the Capture Data (X:)/Data folder and then select the appropriate PI folder.

Latitude Data Collection Set-Up Procedure

2. Select the folder that was created for this data collection set.
3. Select [DataImages] folder
 - a. Images should appear in this folder as data is being collected. The image files should start automatically being removed from this folder as the data is transferred to the server for processing.
 - b. If images are not going into this folder then stop collection and locate where the data is being written
4. Select [xxxxx] folder
 - a. Movie files should appear in this folder as data is being collected. The movie files should start automatically being removed from this folder as the data is transferred to the server for processing.
 - b. If movie files are not going into this folder then stop collection and locate where the data is being written