Krios Direct Alignments Procedure

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

Initial Microscope setup

1. Under the Vacuum tab check the pressure readings
    a. Gun should be ~1
    b. Liner should be ~16
    c. Octagon should be ~1
    d. Autoloader should be ~16

    If the pressure readings do not match the above values, contact a SMIF Staff person.
2. Under the Setup tab, check to make sure column valves are closed (Col Valves Closed button should be yellow).
3. Under the Autoloader tab, go to the temperature control panel to check the temperature readings.
    a. Temperatures for Docker, Holder, Cassette gripper, Autoloader dewar, and Column dewar should all be below -180^{o}C. If they are not, contact a SMIF Staff person.
4. Go to EFTEM tab. The EFTEM button should be gray. If it is yellow, press it to exit EFTEM mode.
5. Load grids into cassette and place cassette into the Krios. See Grid Loading Operating Procedures (DONE BY CORE MEMBER).
6. Under the autoloader tab press the arrow in the top right corner of the first panel. In the options panel, click on inventory button. The inventory will take a few minutes.
7. Label the grids on the inventory panel.
8. To load a grid into the scope, click on the grid that you want to load and then click the Load button. For direct alignments, a sample carbon grid or the cross-grating grid can be used. Gold sample grids do not perform well with the AutoCTF function.
9. Open the EPU software. Press ignore on the alert about being connected with the dongle.
10. Under the Tune tab, check to make sure the Objective aperture is removed from the column.
11. Under the Setup tab, select the Col Valves Closed button to open the column valves.
    a. When this button is yellow, the column valves are closed.
    b. When this button is gray, the column valves are open.
12. Press R1 button if you do not see an image of the grid in the Krios software. The R1 button inserts and retracts the Flu screen.

Rough Eucentric Height (Microscope conditions: Atlas and Grid Square)

1. In EPU, select Atlas under the Preparation tab and then click Set
2. Insert Flu screen by pressing R1 on right TEM control panel if not already inserted
3. Open column valves in Vacuum tab of TEM software if not already open
4. Use the joystick in the control panel to center a prominent feature (e.g. dirt spot). Make sure that the grid square that contains this feature is intact.

5. Go to the Stage+ tab in the TEM software
    a. Ensure alpha angle is -20, then select alpha (this will tilt the sample by -20 degrees)
    b. Use the Z-axis +/- buttons on the control panel to shift the prominent feature back to the center
    c. Select alpha again to return to 0 degree tilt and verify that prominent feature remains in about the same place. Repeat steps 4 and 5 if needed.

Fine Eucentric Height and AutoFocus in EPU (Microscope conditions: Hole Eucentric Height)

1. In EPU, select Hole Eucentric Height under the Preparation tab and then click Set
2. Retract Flu screen by pressing R1 on right TEM control panel
3. Select the Autofunctions tab
4. Select AutoEucentric Height by Beam Tilt. Be careful not use the Auto Calibrate menu.
    a. Click the ▶ play button at the top of the screen to start the sequence
    b. Wait until the sequence has successfully completed
5. Select Autofocus. Be careful not use the Auto Calibrate menu.
    a. Click the ▶ play button at the top of the screen to start the sequence
    b. Wait until the sequence has successfully completed
6. Insert Flu screen by pressing R1 on right TEM control panel
7. Press the L2 button to normalize all

C2 Aperture Alignment (Microscope conditions: Hole Eucentric Height)

1. In EPU, select Hole Eucentric Height under the Preparation tab and then click Set
2. In the TEM software go to the Column tab, and the Beam Settings panel.
    a. Select Free Ctrl (button will turn yellow)
    b. Select C3 off in the Free Ctrl tab.
3. Reduce the size of the beam to the diameter of the small circle on the screen using the intensity knob on the control panel
4. In the drop down menu located on the right bottom side of the TEM display, select Direct Alignments
    a. Select Beam Shift under Direct Alignments
    b. Use the Multifunction X and Y knobs on the TEM panel to center the beam
    c. Once centered, click Done at the bottom of the Direct Alignments menu
5. Increase the size of the beam to the diameter of the large circle on the screen using the intensity knob on the control panel
6. In the Column tab of the TEM software, select Condenser 2 Adjust in the Apertures panel
7. Center the beam over the large circle in the display using the Multifunction X and Y knobs on the TEM panel
8. Repeat steps 4-8 until the beam is centered on the small circle when beam size is decreased and centered on the large circle when beam size is increased
9. De-select Condenser 2 adjust in the Apertures panel
10. In the beam settings panel, select TEM and then select Nanoprobe (this reactivates the C3 lens)
11. Reduce the size of the beam to a small spot on the screen using the intensity knob on the control panel

12. In the drop down menu located on the right bottom side of the TEM display, select Direct Alignments
    a. Select Beam Shift under Direct Alignments
    b. Use the Multifunction X and Y knobs on the TEM panel to center the beam
    c. Once centered, click Done at the bottom of the Direct Alignments menu

C3 Condenser Aligment (Microscope conditions: Mag at 18,000x)

Note: This alignment is difficult to view for an extended period. Moving farther from the display screen can help with perspective on how the beams are centered. It is helpful to turn the red circle screen display off, then manually draw circles around the smallest spot of the beam and the largest spot of the beam to aid in visualizing how the beams are shifting. The beam does not have to be in the middle of the screen when pulsing.

1. Using the magnification knob on the TEM panel, slowly increase the magnification to 18,000x
2. Adjust the size of the beam to the diameter of the small circle on the screen using the intensity knob on the TEM control panel
3. In the drop down menu located on the right bottom side of the TEM display, select Direct Alignments
4. In the Direct Alignments menu, select Beam Shift
    a. Use the Multifunction X and Y knobs on the TEM panel to center the beam
    b. Once centered, click Done at the bottom of the Direct Alignments menu
5. In the Direct Alignments menu, select Condenser Center TEM. The beam will start pulsing between small and large diameters
    a. Use the Multifunction X and Y knobs on the TEM panel to center the largest beam with the smallest beam
    b. Once aligned, click Done at the bottom of the Direct Alignments menu
6. Verify that the condenser is centered by adjusting the intensity knob on the TEM control panel from small to large beam diameters. If any shift is observed while adjusting the beam size, repeat step 4.

Condenser Astigmatism (Microscope conditions: Data Acquisition)

1. In EPU, select Data Acquisition under the Preparation tab and then click Set
    a. Spot size should be 4 and exposure set to 5 seconds
2. Select the FFT button at the bottom of the TEM display screen to view the FFT (Thon rings)
3. Reduce the size of the beam to a small spot using the intensity knob on the control panel
4. In the drop down menu located on the right bottom side of the TEM display, select Direct Alignments
    a. Select Beam Shift under Direct Alignments
    b. Use the Multifunction X and Y knobs on the TEM panel to center the beam
    c. Once centered, click Done at the bottom of the Direct Alignments menu
5. In the Column tab of the TEM software, select Condenser in the Stigmator panel
    a. Use the Multifunction X and Y knobs on the TEM panel to make the beam as round as possible. The Thon rings in the FFT view should be perfectly circular.
    b. Once complete, select the None button in the Stigmator panel

Eucentric Height and AutoFocus in EPU (Microscope conditions: Data Acquisition)

1. In EPU, select Data Acquisition under the Preparation tab and then click Set
2. Reduce the size of the beam to a small spot using the intensity knob on the control panel
3. In the drop down menu located on the right bottom side of the TEM display, select Direct Alignments
    a. Select Beam Shift under Direct Alignments
    b. Use the Multifunction X and Y knobs on the TEM panel to center the beam
    c. Once centered, click Done at the bottom of the Direct Alignments menu
4. Use the intensity knob on the control panel to enlarge the beam to fill the TEM display
5. Retract Flu screen by pressing R1 on right TEM control panel
6. Select the Autofunctions tab
7. Select AutoEucentric Height by Beam Tilt. Be careful not use the Auto Calibrate menu
    a. Click the ▶ play button at the top of the screen to start the sequence
    b. Wait until the sequence has successfully completed
8. Select Autofocus. Be careful not use the Auto Calibrate menu
    a. Click the ▶ play button at the top of the screen to start the sequence
    b. Wait until the sequence has successfully completed
9. Press on eucentric focus on the keyboard (upper right side)
10. Insert Flu screen by pressing R1 on right TEM control panel

Beam Tilt Pivot Points (Microscope conditions: Data Acquisition)

1. Reduce the size of the beam to a small spot using the intensity knob on the control panel
2. In the drop down menu located on the right bottom side of the TEM display, select Direct Alignments
    a. Select Beam Shift under Direct Alignments
    b. Use the Multifunction X and Y knobs on the TEM panel to center the beam
    c. Once centered, click Done at the bottom of the Direct Alignments menu
3. In the Direct Alignments menu, select “nP Beam tilt ppX”
    a. Use the Multifunction X knob on the TEM panel to align the two pulsing spots as closely together as possible
    b. When complete, click Done at the bottom of the Direct Alignments menu
4. In the Direct Alignments menu, select “nP Beam tilt ppY”
    a. Use the Multifunction X knob on the TEM panel to align the two pulsing spots as closely together as possible
    b. When complete, click Done at the bottom of the Direct Alignments menu
5. In the Direct Alignments menu, select Beam Shift
    a. Use the Multifunction X and Y knobs on the TEM panel to center the beam
    b. Once centered, click Done at the bottom of the Direct Alignments menu

Condenser Astigmatism (re-check) (Microscope conditions: Data Acquisition)

1. Follow procedure for Condenser Astigmatism on page 3 to recheck the astigmatism

Eucentric Height and AutoFocus in EPU (Microscope conditions: Data Acquisition)

11. In EPU, select Data Acquisition under the Preparation tab and then click Set
12. Reduce the size of the beam to a small spot using the intensity knob on the control panel
13. In the drop down menu located on the right bottom side of the TEM display, select Direct Alignments
    a. Select Beam Shift under Direct Alignments
    b. Use the Multifunction X and Y knobs on the TEM panel to center the beam
    c. Once centered, click Done at the bottom of the Direct Alignments menu
14. Use the intensity knob on the control panel to enlarge the beam to fill the TEM display
15. Retract Flu screen by pressing R1 on right TEM control panel
16. Select the Autofunctions tab
17. Select AutoEucentric Height by Beam Tilt. Be careful not use the Auto Calibrate menu
    a. Click the ▶ play button at the top of the screen to start the sequence
    b. Wait until the sequence has successfully completed
18. Select Autofocus. Be careful not use the Auto Calibrate menu
    a. Click the ▶ play button at the top of the screen to start the sequence
    b. Wait until the sequence has successfully completed
19. Insert Flu screen by pressing R1 on right TEM control panel

Reset Defocus and Close EPU software

1. Press L1 on the TEM control panel to reset defocus to 0
2. Use the Focus knob on the TEM control panel to set the defocus to -1um
3. Close the EPU software

Objective Stigmation and Coma Free Alignments using Sherpa software (Microscope conditions: Data Acquisition)

1. Use the joystick in the control panel to center over a carbon region of the sample. If using the cross-grating, center in the middle of a square
2. Open the Sherpa software
    a. Select the software organizer icon at the bottom of the display. The icon is a red cube in a black/white box
    b. Select the tools tab in the software organizer window
    c. Select Sherpa
3. Sherpa Software Setting:
    a. Select the AutoCTF tab
    b. Under the Camera panel the settings should read:
        i. Type: BM-Falcon
        ii. Exposure Time (s): 2.5
        iii. Binning: 1
        iv. Readout Area: Full
        v. Autofocus to (um): Not checked

4. Objective Stigmation
    a. Select Measure in Objective Stigmation panel
        i. Want stigmation to be close to 0nm
        ii. Defocus to be -1 to -2
    b. Adjust defocus if needed, then select Correct in Stigmation and ensure stigmation is close to 0 (less than 2 is acceptable)
5. Coma Free
    a. Select Measure in Coma Free panel to measure initial coma value
    b. Select Correct in Coma Free panel
        i. Should pass after a few iterations (will do a max of 10)
        ii. If doesn't pass, try adjusting defocus more negative
6. Remeasure (and re-correct if needed) Objective Stigmation