

- 1) Update the Gain Reference on the K2 Camera
 - a) K2 computer-Check that the camera is at -20°C . The camera temperature can be found in Digital Micrograph-Top Left hand panel.
 - b) Polara-move C2 and Objective apertures to position #4. C2=100uM and Obj=Empty
 - c) Follow Directions for collecting a gain reference/dark reference (see protocol)
- 2) Once the gain reference has been updated prepare the scope for the first step of alignment
 - a) Place the C2 aperture to either 50uM or 30uM depending on experimental needs
- 3) Align the C2 aperture
 - a) Use the intensity knob (left control panel) to condense the beam
 - b) Use the intensity knob to move back and forth through crossover
 - c) Manually adjust the C2 using knobs on the front and side of the aperture
 - d) *The goal of this alignment is to have the spot be in the same position + & - crossover
 - e) Once the C2 is aligned end the procedure by adjusting the intensity knob to the right. Always work on the right side of crossover.
- 4) Gun Tilt Alignment
 - a) Go to "Tune" in the FEI user interface
 - b) Click "Gun Tilt"
 - c) Condense Beam using the intensity knob
 - d) Center the beam using the trackball on the left control panel (make sure trackball is set to center the beam--FEI user interface bottom left)
 - e) Bring up mini screen - button on the left side of the microscope
 - f) Use the multi-function X/Y knobs (left and right control panels) to adjust exposure time
 - g) *The goal of this alignment is to minimize the exposure time and/or to maximize the dose. These values are found in the bottom-center of the user interface
 - h) End this alignment by clicking "done" in the "Tune" window
- 5) Gun Shift Alignment
 - a) Go to "Tune" in the FEI user interface
 - b) Click "Gun Shift"
 - c) This alignment needs to be done at 2 spot sizes (spot 6 and spot 2)
 - d) Start with spot size 6
 - e) Use the trackball or multifunction X/Y to center the beam (beam shift)
 - f) Click next arrow in the "Tune" panel. This should shift the multifunction X/Y knobs to gunshift and the beam to spot 2.

- g) Use the multifunction X/Y knobs to shift the beam to the middle (should be using gunshift)
 - h) Move back and forth between spot size 6 and spot size 2 and continue adjusting the beam until it doesn't shift when changing spot sizes (from spot 6 to spot 2)
 - i) End this alignment by clicking "done" in the "Tune" window
- 6) Optional Alignment - Spot Size Dependant Alignment
- a) Go to "Align" - "Gun" - "Spot size dependant"
 - b) Follow protocol in the window
 - c) Start with spot size 10
 - d) Set L2 (In FEI User Interface) to "normalize all"
 - e) Click L2 (normalize all) before and after each step of this alignment
 - f) Condense the beam using the intensity knob
 - g) Center the beam using "beam shift" (Trackball or multifunction X/Y)
 - h) Click L2 to "normalize" the beam
 - i) Make sure beam stays centered then shift to the next spot size
 - j) *The goal of this alignment is to center the beam at all spot sizes
- 7) *Optional - Can set up the Camera/Sample dose at this point
- 8) Insert Objective
- a) Insert the objective choosing either 100uM or 70uM depending on the experiment
 - b) Check to make sure the beam can still be seen on the screen
 - i) If the beam is not there:
 - (1) go to lower magnification (until a beam can be found)
 - (2) Center the beam using beam shift
 - (3) Increase the magnification and keep centering the beam (using beam shift) until you reach your desired working magnification
- 9) Insert Platinum Iridium Grid
- a) This should only be done if you are cleared to insert a sample
 - b) Follow cartridge insertion procedure
 - c) The alignment (Platinum Iridium) grid should always be at position 6 in the MSC (multiple sample chamber??)
 - d) Check to make sure the beam can still be seen on the screen
 - i) If the beam in not there:
 - (1) move the stage to make sure that the grid is not on a grid bar
 - (2) go to lower magnification (until a beam can be found)

- (3) Center the beam using beam shift
- (4) Increase the magnification and keep centering the beam (using beam shift) until you reach your desired working magnification

10) Centering the Objective Aperture

- a) Select the “diffraction” button on the right control panel
- b) Typically the beam will shift in “diffraction mode”
- c) DO NOT USE BEAM SHIFT TO CENTER THE BEAM
- d) *The objective of this alignment is to center the objective “halo”
 - i) Use the knobs on the front and right of the objective aperture to center the halo
- e) When done, return to imaging mode (SA mode) by selecting the “diffraction” button on the right control panel

11) Eucentric Focus

- a) Click on the “Eucentric Focus” button on the right control panel
- b) The beam will flicker/flash or it will move and come back

12) Find Eucentric Height

- a) Find a spot/particle on the grid
- b) Use “wobble” to adjust the eucentric height
 - i) “Wobble” is found in the FEI User Interface under:
 - (1) As of 12/31/15 do not use the wobble button on the right control panel
 - (2) Search-Stage-Control-Wobble
 - (3) Adjust z-axis using ***** on the right control panel
 - (4) This process can be expedited by start at z height of -20

13) Beam Tilt X and Y

- a) Condense beam using the intensity knob. You want a small pin sized beam
- b) Select Beam Tilt Y
 - i) location
 - ii) use the multifunction x and y knobs to minimize the beam movement
 - iii) When finished select done
- c) Redo the same procedure for Beam Tilt X
- d) Center the beam using
 - i) Use beam shift under Tune>>>>>
- e) Check Beam Tilt X and Y after adjusting the beam

14) ALIGNMENT FINISHED!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!

