IMF – FEI TEM Quick Start Guide – Basic Imaging

1. **Sign into the computer** (ex. Login: User)

2. **Run the Server**: If the microscope server is off, turn on "Microscope Software Launcher" and click on run to start the microscope applications.

3. **Turn on the Flu camera and TIA** (TEM Imaging & Analysis) software.

4. **Vacuum Checks** before removing sample holder:
    a. Check that the TEM vacuum is ready
    b. Column valves should be closed (*TEM touchscreen will also show closed valves image*) and AVAILABLE. Makes sure the column valves are closed or you will destroy the vacuum.

5. **Remove the sample holder** from the TEM (follow the on touchscreen directions), use appropriate gloves as necessary. Directions for single tilt are below:
    a. Press the touchscreen for remove sample to re-initialize stage
    b. ***Slowly*** pull the holder out directly until there is resistance to stop.
    c. Turn clockwise until it naturally stops.
    d. Finally, pull the holder directly out.

6. **Place your sample in the sample holder** (follow directions specific for the type of holder you chose: single tilt, double tilt, tomography, cryo-holder). For the Single tilt holder, use the pin unhinge the sample and then load your sample.

7. **Insert the sample holder** into the TEM (follow the touchscreen directions). General directions for single tilt holder:
    a. Presss load sample on the touchscreen
    b. Align the pin on the sample holder to the insert sample holder into the TEM until it cannot be pushed in any further and hold it in place (Make sure you do not fidget the holder. Place the sample in straight with the pin aligned).
    c. The sample chamber will start pumping. On the TEM touchscreen, select the holder you are using and let go of the TEM holder (the pump should hold it in place).
    d. Wait until the pumping is complete (~2 minutes and it's status is on the touchscreen).
    e. Turn the sample counter clockwise until the holder naturally stops.
    f. Slowly insert the holder into the TEM with BOTH Hands and guide the holder to be completely inside the TEM. There will be suction, but do not let holder slam into TEM.
    g. It should be completely in, if not, adjust the holder so the holder is completely in.
    h. If there is an error and you broke the vacuum while inserting the sample holder: go to vacuum tab in the software, expand the tab, and hit "recover" in the control tab in the expanded window. On the TEM touchscreen, press "Ready to Vacuum". Then try inserting the sample holder in again after the vacuum has recovered (~10-30 minutes).

8. **HT and camera Checks:**
    a. HT should be on (if not, contact lab manager)
    b. Accelerating voltage is set at 200kV
    c. For basic imaging, check that the beam size in in "microprobe" with spot size set between 3-5.

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d. Screen should be inserted to protect camera

9. **Open the column valves** from the software icon (sample will be exposed to TEM beam). This is found on the setup tab under the vacuum window.

10. **Search for the beam**
    a. Lower the magnification, spread the intensity, or move the stage to find the beam.
    b. If you do not find your sample, you may be on a grid bar.

Alignments (do all alignment on Clockwise side of the beam). Double check that the MF knobs are calibrating/aligning the correct component and be sure to turn them off so the alignments aren't accidentally adjusted after aligning a particular part.

11. **Center the Condenser Aperture:** Check that the beam spreads evenly. If the beam does NOT spread evenly from the center, then you need to do the following:
    a. Bring the beam to crossover (small point) with the intensity knob.
    b. Use the beam shifts to center the crossover point into the center of the screen.
    c. Spread the beam on the clockwise side with the intensity knob.
    d. In the "tune" tab, under Aperture, next to the Condenser aperture, click on "Adjust". Use the MF knobs (Aperture X & Y) to center the beam into the center of the screen. Hit Adjust when done. Then check if the beam spread out evenly, if it doesn't repeat the steps above.

12. **Condenser Stigmatism**: Check that the beam is circular. If not:
    a. Bring the beam to crossover and center the beam with beam shifts.
    b. Spread out the beam.
    c. In the "tune" tab, under "Stigmators", click on "condenser".
    d. Adjust the MF knobs (Cond. Stig X & Y) until the beam is circular. When completed, click on "None" in the Stigmator tab or click on the Stigmator button to turn off this alignment.

13. **Set Eucentric height** (When done correctly, the sample will not move when tilting)
    a. Find an area on your sample at low magnification and spread out the beam.
    b. Focus your sample.
    c. In the "Search" Tab, hit the expand button on the Stage window (arrow key pointing right), this will give you access to the Control tab.
    d. Hit the "Wobble" button in the expanded search window where it says alpha wobbler.
    e. Adjust the z-height (buttons on the right side of the control panel) to make it so that the sample does not translate, but just pivots on one axis.
    f. Hit "Wobble" again when done.

14. **Align Beam tilt X & Y:**
    a. Bring the beam into cross over and center the beam at the center.
    b. In the "Tune" tab, under Direct Alignments, click on "Beam tilt ppX".
    c. Adjust the MF knobs, to fuse the two spots together.
    d. Clock on "Beam Tilt ppY"
    e. Adjust the MF knobs to fuse the two spots together.
    f. Click Done under "Direct Alignments" when completed.

15. **Beam Shift Alignment**
    a. Bring the beam into crossover.
    b. In “Tune” tab, under “Direct Alignments”, click on “Beam Shift”.
    c. Using the MF knobs to bring the spot into the center of the screen. (Important: the MF knobs here are aligning the beam shift, so do not use the beam shift ball to center the beam for this alignment.)
    d. Click Done when completed.

16. **Rotation Centering** (If this alignment is done correctly, the sample won’t move when you focus)
    a. Spread out the beam clockwise and find a region of interest.
    b. Increase the magnification close to where you plan to image samples.
    c. Focus your sample (minimal contrast or spread out circle in FFT)
    d. In “Tune” tab, under “Direct Alignments”, click on “Rotation Center”
    e. Adjust the MF knobs so that the sample does not translate (it should appear as a heartbeat when aligned correctly)
    f. Click done when completed.

17. **Image Contrast**: If you need more contrast in your image then use the objective aperture. *However, if you want to look for lattice fringes, do not use the objective aperture*.
    a. Press Diffraction button on the keyboard.
    b. Adjust the intensity to sharpen the diffraction pattern. You may click on the “Manual tab” in the FluCamera, then scroll click and scroll to increase the lighting.
    c. In the “Tune” tab, under “Apertures” insert an Objective aperture.
    d. Click on the “Adjust” next to the Objective aperture.
    e. Use the MF knobs to move the aperture to center around the transmitted beam in diffraction.
    f. Click on “Adjust” when done to complete.
    g. Press the diffraction button on keyboard again to go back into imaging mode. Adjust the intensity as need to view the image.

18. **Objective Stigmatism**: Correct this if the FFT does not provide circular rings.
    a. Find a sample of interest at high magnification.
    b. Click on FFT on the FluCamera.
    c. Adjust the focus until rings appear on the FFT (at focus, there will be no rings, so you can bring the image to underfocus to get rings)
    d. Press the Stigmator button on the keyboard (or access the Stigmators in the “Tune” tab and hit Objective”)
    e. Adjust the MF knobs (Obj. Stig. X & Y) to make the rings circular. You can also check that its circular by using the focus knob to see if the rings expand uniformly out and in as a circle.
    f. When completed, press the Stigmator button on the keyboard (or press the “None” icon on the Stigmators window).

19. **Capturing an image (DO NOT Change Magnification or Intensity when using the BM-Ceta Camera or it will damage the camera**
    a. Before capturing an imaging, make use that the focus and objective stigmation is good (under focused image with circular FFT).
    b. Make sure that the beam is spread (screen intensity should be less than 1) and the magnification is set to what you want.
    c. In the FluCamera, click on "Insert Screen" on the top of the window to remove the screen (this allows access to the BM-Ceta Camera and pauses the FluCamera).
    d. In the "Camera" Tab, "Insert" the camera and click on "Search". You may need to adjust the integration time to get a good exposure time for the camera.
    e. Adjust the focus as needed.
    f. Hit "Acquire" to capture the image. Adjust the integration time while in "Acquire" if needed. Hit Acquire again if you want to take a new image with the new integration time.
    g. Hit "Insert Screen" on the FluCamera window when you want to search for another area (This unpauses the FluCamera, and protects the BM-Ceta Camera). Make sure the screen is down when you're searching. Only take out the screen when acquire images. No not take diffraction images as it will burn the camera.

20. **Saving an image**
    a. On the image you just acquired, click on File -> Save as to save the image as an *.emi file. This is your raw data.
    b. To save as a *.tiff file, right click on the image and export data. Save as full resolution tiff with scale marker. Be sure to have the scale marker to have a scale bar in your tiff image.

21. **Uploading files to the transfer computer**
    a. On the TEM computer, click on the "Transfer" network.
    b. Login is "user" and password is "imf".
    c. Move all files for uploading on the transfer folder.
    d. In the transfer computer, click on my computer and click on the C: drive. The transfer folder is located within. Use the transfer computer to upload your saved images or grab the images using USB on the transfer computer.

Emergency shutdown

1. In Setup (FEG Control Expert), turn off FEG (a few minutes to turn off the gun)
2. Turn off “Power” (HT will automatically stop)
3. In Microscope Software Launcher, hit Stop all
4. Turn off computer
5. In the cabinet, turn off all breakers (Right to Left) then turn off.
6. Turn off chiller
7. Turn off UPS and Electrical box.

Start-up Procedure (Supervisors only)

1. Turn on Chiller
2. Run on UPS and Electrical box
3. Turn back on breakers (Left to Right)
4. Turn on computer
5. In Microscope Software Launcher, press play
6. If Vacuum is off, hit recover (in Vacuum overview, turn on IGPa Manually, then hit recover, evacuate all)
7. If TEM has been off for a week, it needs a bake out. Turn on Power, then FEG. May need a warm start if off for less than 8 hours or a cold start if off for more then 8 hours. (call Thermofisher for bakeout)
8. Turn on HT and slowly ramp up the HT.

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