

Gavin Murphy's Protocol for Imaging Biological Samples on the JEOL 3200

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Important Points or Concepts

- It is important to align the microscope from the top to the bottom, though some steps can be done out of order, like just checking if the condenser aperture is right and the specimen is at eucentric focus.
- For collecting data, usually will be above 25,000x mag. At 30,000x mag or less, use alpha 3. Above 30,000x, use alpha 2. If you're ever above 200,000x, use alpha 1.
- TEM mode is for standard imaging
- Try to keep track of how many revolutions of a knob you make if you are unsure of which direction to turn. It will help in recovering from a mistake.
- Aperture #1 is the largest, #4 the smallest.
- Start out in Mag1. You'll take images in Mag1.
- U & P is JEOL
- Ctrl F gives you FFT of an image on digital micrograph.
- MDS adds shifts, tilts, stigmation, etc. to whatever was done in MDS off. This could be good, but could be very bad if someone added an undesirable shift to the last used MDS state. This can be fixed by saving the Def/Stig settings while in Off mode in the Lens/Def Memory menu item, then loading them in the MDS modes.
- Alignments can be saved.
- In diffraction mode, PL def/stig is used to center the pattern, and IL STIG is to make the pattern circular.
- If you're doing cryo, consider first cooling down the station, then doing alignments while it is cooling. Or, do alignments on the waffle grid in the morning, then cool down stage.
- If you can't find a tool, it might be in the oven warming up.
- The waffle grid square has squares that are 0.46 μm wide (the ones seen at e.g. 100 kx)
- Vacuum preliminaries: Green light should be on for ion pump on far console. The needle should be to the left close to the green tape. PEG1 should be reading 9 or less. The pumps should read EVAC ready.

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0. Startup

A. Initial Setup

When you enter the room to use the JEOL JEM 3200FS for the first time each day, check the following items:

1. The nitrogen tank (to left when entering room) should be open. Do not simply read the pressure gauges but instead, make certain the main valve is open several turns.
2. The anti-contaminator (ACD) should be cold. If it is not cold, fill with liquid N₂ and remember to top it off after 10-15 minutes. If it is already cold, top it off. [If the warming device is in, you remove the plug and cylinder assembly. Set aside. Pour in nitrogen through funnel.]
3. Fill a second time after the Leiden frost effect/boil. Then refill every few hours.

4. Check the accelerating voltage. If it is already at 300 kV, the instrument is ready to use. If it is at 260 kV (Stand By mode), click the "Normal" button in the High Voltage Control window (and also answer "yes" to the pop-up window that confirms the desire to return to "Normal" conditions.). It will take a bit less than 15 minutes for the accelerating voltage to go from 260 kV to 300 kV.
5. The magnification should be between 30k and 50k; if this is not the case, adjust the magnification and let the [director](#) know that the 3200FS was not in the correct state when you found it.
6. Once the microscope is at 300kV, push the "Degauss" button located in the lower right corner of the "Filter Tuning" window.
7. Check that the vacuum readings are normal. All readings in the TEMcon GUI should indicate "Evac Ready" and the two needle gauges on the large electronics cabinet in the corner of the room should be in the correct range. The bottom gauge needle should be to the left of the green tape. The green bar called PEG1 should be less than 9.
8. If the vacuum conditions are met, then you can open the beam valve. If the beam valve icon is greyed out, then it is not possible to open valve.

B. Holder removal

1. Make certain that the goniometer is in the neutral position: x/y/z, x-tilt & y-tilt are all zero; This can be accomplished by double-clicking on the "Stage Neutral" area of TEMcon (lower right corner of the main window)
2. Click OK
3. While the holder is in the column, the switch below the airlock should be in the "PUMP" position; toggle the switch to "AIR" before doing anything else
4. Slowly pull the holder straight out of the column; it will pull out about 6 cm and then stop; if you release the holder at this point, the vacuum will pull it back into the column
5. Rotate the holder $\sim 80^\circ$ counter-clockwise
6. Pull out a further 1 cm and rotate the holder an additional $\sim 10^\circ$ counter-clockwise
7. Release the holder and wait for the vacuum reading in the GUI on the PC to read $>230 \mu\text{A}$ (this should take $< 1 \text{ min}$)
8. At this point, pull the holder completely out of the column

Note: Do not leave the airlock empty for significant amounts of time. If you are going to delay more than 15 minutes before re-inserting the holder (or a different holder), please put one of the other holders into the microscope.

C. Room temperature holder insertion

1. Load grid into your stage of choice, usually the high tilt holder because we leave the waffle grid in the low tilt one.
2. The stage should still be in neutral
3. Change the entry in the text box above the stage position values in TEMcon to reflect the holder that you are about to put into the microscope.

4. The switch beneath the specimen airlock should be toggled to "AIR" before putting a holder into the microscope
5. Align the pin on the rod of the holder with the slit at "9 o'clock" in the opening of the specimen air lock
6. Gently push the holder as far into the airlock as it will go and toggle the switch to "PUMP"
7. The yellow light above the switch should come on and you should hear valves open and close, and a pump will start after a short while
8. After several minutes, the second (green) light will also come on
9. For a sample that may significantly outgas, wait 5-10 minutes after the green light turns on before proceeding to the next steps
10. Rotate the holder $\sim 10^\circ$ clockwise and let the vacuum pull the holder a short way (~ 1 cm) into the column; the yellow light will turn off after this 10° rotation.
11. Rotate the holder a further $\sim 80^\circ$ clockwise and again let the vacuum pull the holder the rest of the way into the column; a very light tap on the end of the holder can be used to make certain that it is seated properly
12. While following steps 9 & 10, watch the vacuum gauge on the large electronics cabinet behind the JEOL PC monitors; it will probably deflect fully to the right but should IMMEDIATELY begin to recover; if recovery does NOT begin immediately, ask for help
13. Once the holder is fully inserted into the column, the yellow light will remain off and the green light will remain lit
14. Once the column vacuum gauge on the large electronics cabinet has reached the proper reading (to the left of the drawn mark) and the "Beam Valve" monitor in TEMcon (left side of main panel) is colored green and the reading is better than $40 \mu\text{A}$, it is safe to open the beam valve

NOTE: Until the holder is fully inserted into the column, the "Beam Valve" region of TEMcon will remain grey and the beam valve cannot be opened; this is a lock-out feature of the microscope to prevent X-rays from leaking through the specimen airlock.

NOTE2: Remember not to force the holder in any way – there should be very minimal resistance during all these steps, and there is absolutely no need to push the holder into the column.

D. Cryo Holder Insertion.

1. Leave holder in the pump station.
2. Connect temp control.
3. Pour nitrogen into it.
4. Monitor temperature. If it never gets cold, then there's a vacuum problem.
5. *This is a good time to do alignments while waiting for it to cool down.*
6. When temperature drops to -120°C , cool down transfer station. Insert the silly little piece of Styrofoam under the right side to incline it.
7. Cool down a Styrofoam dewar.
8. Around temperature -160°C , transfer your grids to the Styrofoam dewar.

9. When temperature drops to $-170\text{ }^{\circ}\text{C}$, move holder to transfer station.
 - a. Turn off pump
 - b. Wait 5' for turbo pump blades to slowly stop. The green lights on the pump will go off.
 - c. Tighten all the zeolite valves on the two holders.
 - d. Close the valves that lead to the zeolite inlet valves.
 - e. Remove holder
 - f. Put it in the transfer station.
10. Move your grid box into the station's box receptacle.
11. Slide open the shield and remove the clip by screwing it CW and pulling up.
12. Remove old grid and dispose.
13. Some people wait for the nitrogen level to decline below the grid-loading "stage", so that it is easier to put a grid in the holder.
14. Put in grid.
15. Stick the clip in and screw it down CCW with the pencil.
16. Clean up.
17. Move temp control to microscope and plug it in. Put the wires on their hooks.
18. Move station to microscope and put it on the Styrofoam square.
19. Click on stage neutral.
20. Remove unwanted stage.
21. Tilt stage to $+15^{\circ}$ with "+x". (Standard -> Stage.)
22. Make sure glass window is covered, because nitrogen might crack it.
23. Put cryo holder in. Some nitrogen will pour out.
24. Hold it a bit and make it lean counterclockwise. (Or else the weight of the stage's dewar will make it lean clockwise, and pump won't engage.)
25. Turn on pump by pulling out and switching it from "air".
26. Wait till green light, then turn it like Section C says.
27. Refill dewar a little.
28. One must wait 45 minutes for thermal equilibration for the first cryo grid of the day. Leave shield on.
29. Turn stage back to zero with "-x"

E. Cryo conditions.

The scope has probably been aligned before cryoholder insertion. The eucentric height must be found.

Redo Section III A.

When taking a first look at grid, in order not to over-dose the specimen, use low mag 200x, spot 5 and spread the beam.

If the sample is good, proceed to redo alignments quickly.

F. Cryo Holder Removal

1. Put stage at neutral
2. Slide shield down
3. Close valves
4. Remove holder like in Section B.

If you are done for the day, then

5. Put it in the vacuum station.
6. Attach Thermocouple
7. Start warm up cycle by pushing knob and turning to it.
8. Back at scope, put in the room temperature low-tilt holder.

Otherwise,

5. Put the holder in the cooled transfer station and load a grid.

I. Gun-to-Condensor

Explanation: To align the gun lenses to the condenser lenses.

- **Be in MDS off and image the waffle grid.**

A. Centering the condenser aperture – *Where we pick a condenser aperture.*

Alpha: 3

Mag: I use 40,000.

Apertures in?: Condenser 1,2 or 3. Probably 1.

Spotsize: 1

Slit: out

1. Find the beam
2. If no beam, then in this order,
 - i. move specimen around,
 - ii. lower mag,
 - iii. Strengthen brightness clockwise all the way, then converge counterclockwise slowly
 - iv. Go to condenser aperture 1
 - v. Select Low Mag to search for the beam
 - vi. Turn on SPCTR to perform Degauss
 - vii. Remove grid from specimen.
3. Insert Condenser aperture 1.
4. Center by converging brightness, centering with beam shift, expanding brightness to the outer hatches of the viewing screen, and centering with aperture controls.
5. Iterate step 4 once or twice.

B. Eucentric height

Explanation: It's important to be at eucentric height so that the optics are aligned properly. It's possible to accidentally align everything for a stage height that is not at the proper eucentric position, but there will be unintended problems.

Alpha: 3

Mag: I use 40,000.

Apertures in?: The condenser aperture you previously selected.

Spotsize: 1

Slit: out

1. Press the standard focus button
2. Turn on image wobble x

3. Reduce the movement by increasing and decreasing the z-axis control
4. Turn off image wobble x.

Faster way:

1. Condense beam.
2. Move the stage (probably down) until the diffraction pattern shrinks down to the beam diameter.

C. Gun-Condenser Alignment (Manual's way)

Explanation: So that the gun's optical axis is shifted and tilted to go through the condenser lens's optical axis.

Alpha: 3

Mag: 40,000

Apertures in?: Condenser 1,2 or 3. Prob 1. Mike says middle ap.

Spotsize: 1

Slit: out

1. Center with beam shift
2. Switch to spotsize 1
3. Condense brightness to crossover
4. Toggle gun alignment by pressing F2. Alternatively, select Operation -> Alignment-Gun. Now shift is gun shift and def/stig is gun tilt.
5. Center beam with gun shift.
6. Turn on anode wobbler.
7. Make the illumination wobble concentrically using gun shift and/or tilt.
8. Turn off anode wobbler.
9. Switch to spot size 5.
10. Toggle bright tilt. Now beam shift and beam tilt are being controlled by shift and def/stig. (The deflection coils under the condenser lens.)
11. Center with shift.
12. Repeat steps 2 through 11 until the electron beam does not shift from the center of the fluorescent screen, but you might be able to skip steps 6-8 after the first few iterations.

II. Condenser

A. Recentering the condenser aperture - Where we pick the spotsize.

Explanation: So the aperture is centered on the optical axis.

Alpha: 3

Mag: 40,000

Apertures in?: Condenser 1,2 or 3. Prob 1. Mike says middle ap. Use this aperture for the remainder of your session.

Spotsize: 3 - 5. 3 if doing room temperature EM, 4 or 5 if doing cryo.

Slit: out

1. Center by converging brightness
2. Center with beam shift
3. Expand brightness to the outer hatches of the viewing screen

4. Center with aperture controls.
5. Iterate steps 1–4 until there is no change.

B. Condenser lens astigmatism

Explanation: The condenser lens astigmatism should be corrected if the beam does not expand in a circular fashion when the beam is converged and diverged around crossover using the “Intensity” (LC) knob. Small amounts of astigmatism will not noticeably affect the imaging conditions.

Alpha: 3

Mag: 40,000

Apertures in?: The condenser aperture you previously selected.

Spotsize: 3 – 5. Use this spotsize for the remainder of your session.

Slit: out

1. Converge the brightness.
2. Press Condenser stigmatism “COND STIG” button.
3. Turn the brightness knob slowly clockwise and counterclockwise (strengthening and weaking C2, resp) through crossover.
4. If the beam is not circular, use the Def/Stig knobs to make it circular.
5. If you see the beam sweeping from side to side, it means your condenser aperture is not centered. If so, recenter it.

III. Objective lens

The objective lens is the most important and there are many alignments to perform. In addition, the alignments to be done depend on what imaging is to be carried out. Single Particle Analysis will aim for resolutions better than 30 Ångstroms, so more alignments are done. EM crystallography would aim for 3 Å. Some tomography might aim for 20 Å, but the resolution of the tomogram won't be better than 50 Å. The menu of alignments is written below.

For tomography do rotation centering, for single particle analysis do voltage centering.

A. Optional: Recheck Eucentric Height

Explanation: It's important to be at eucentric height so that the optics are aligned properly. It's possible to accidentally align everything for a stage height that is not at the proper eucentric position, but there will be unintended problems.

Alpha: 3

Mag: 40,000

Apertures in?: The condenser aperture you previously selected.

Spotsize: The spotsize you previously selected.

Slit: out

1. Press the standard focus button.
2. Pres IMAGE WOBB X or Y
3. Focus by changing z-height.
4. Turn off wobbler

B1. Horizontal Adjustment of Condenser Lens Deflector Coil (Manual's way)

Explanation: Adjusts the current ratio of the two deflector coils under the condenser lens so that the image does not shift off the screen when you tilt the incident beam – which is useful when you use MDS Focus.

Alpha: 3

Mag: 40,000x

Apertures in?: The condenser aperture you previously selected.

Spotsize: The spotsize you previously selected.

Slit: out

1. Converge brightness and shift beam to center
2. Toggle Wobbler-TiltX
3. Toggle Compensator-Tilt on Alignment Panel
4. Overlap the two split beams with only the def/stig knob X.
5. Turn off Wobbler TiltX
6. Center beam shift
7. Toggle Wobbler TiltY
8. Overlap the two split beams with def/stig knob Y
9. Turn off Wobbler Tilt Y.
10. Deselect Compensator-Tilt

C. Centering of the Objective Lens – where we pick the alpha and magnification

Explanation: There are 4 centers of the objective lens: the current (aka rotation), voltage, coma-free axis and reversal. Current centering is good for lower mags (less than 40,000) and is convenient for when you don't want the image to shift when you change defocus. Voltage centering is good for higher mags and is considered necessary for higher resolution work. Coma-free axis centering is for advanced, high-resolution usage. Do just rotation centering for low mag work or for low resolution tomography, or do voltage centering for single particle analysis and higher mag tomography or tomography aiming for higher resolution. You can do both if you want, but it's not necessary to do both. I'm still feeling out coma-free axis centering. It would definitely be good for STEM.

C1. Current Centering aka Rotation Centering

Explanation: So the image doesn't move when you change defocus (weaken or strengthen the Obj lens). This is good but not critical for tomography because as the stage is tilted, the defocus will vary along the specimen perpendicular to the tilt-axis.

Alpha: 3 if mag less than or equal to 30,000. 2 if between 40,000 and 200,000. Else 1. Use this alpha the rest of the time.

Mag: 100,000x

Apertures in?: The condenser aperture you previously selected.

Spotsize: The spotsize you previously selected.

Slit: out

1. Focus on some carbon or other information-rich area.

2. Spread the beam
3. Turn on objective wobbler.
4. Press Bright Tilt
5. Write down the CLA2 hexadecimal values in case you want to return to the starting conditions.
6. Set the center of the image expansion and contraction to the center of the screen with Def/Stig knobs.
7. Turn off objective wobbler.
8. If the beam moved a lot, you have to recheck the beam shift, pivot points (section III B1 & B2) and then redo the Rotation centering.

C2. Voltage Centering

Explanation: Good for single particle work and if the mag is better than 40,000x. Also, use this center if any EFTEM energy shifting like "Most probable loss" imaging is to be done, because that involves changing the accelerating voltage. Or any Energy Filter spectroscopy.

Alpha: 3 if mag less than 40,000. 2 if between 40,000 and 200,000. Else 1. Use this alpha the rest of the time.

Mag: 100,000x

Apertures in?: The condenser aperture you previously selected.

Spotsize: The spotsize you previously selected.

Slit: out

1. Move the stage to a specimen that has information on it and center the beam.
2. Spread the beam fully, at least to the edge of the screen, ideally all the way, but you probably wouldn't see any feature, so spread as far as you can while still seeing features.
3. Press HT Wobble. The image expands and contracts.
4. Press Bright Tilt.
5. Write down the CLA2 hexadecimal value in case you want to return to the original setting.
6. Set the center of the image expansion and contraction to the center of the screen with Def/Stig.
7. Turn off HT Wobble
8. Turn off Bright Tilt if you want.
9. If it needed a large shift, check the condenser aperture, stig and the beam tilt pivot points (III B).

D. Objective Aperture Centering – where we pick an objective aperture

Explanation: To center the In-Gap Objective Lens aperture is in the center of the back focal plane. The objective aperture will remove highly-scattered, diffracted rays, which have the most information, but are usually rich in noise. The objective aperture is like a low-pass filter.

[Maybe you should put in a specimen that has defined diffraction distances. Then, you could see what frequencies the different objectives exclude at given magnifications/camera lengths. Then use that info to guide people's selection of an aperture.]

Alpha: The alpha you previously selected.

Mag: 100,000x

Apertures in?: The condenser aperture you previously selected. Think of what objective aperture you will use.

Spotsize: The spotsize you previously selected.

Slit: out

0. Press SA MAG
1. Be at the mag and alpha that you used in Mag1
2. Spread the beam to past the screen edge
3. Insert SA Aperture 3.
4. Center it with the aperture controls roughly.
5. Select SA Diff
6. In diffraction mode, use the Diffraction Defocus to make a nice ring-like diffraction pattern. Center it with PL Def/Stig. You can make it more circular with IL STIG if it's oval.
7. Change the magnification to have a camera length of 25 cm.
8. Select OLA aperture, the in-gap objective aperture. Select the aperture number you want. Use 1 – 3. Some just 1. I like to use 2.
9. Center the aperture shadow in the diffraction pattern.
10. Return camera length to 100 cm.
11. Switch back to Mag1.
12. Remove SA aperture, if it was in.

E. Obtain an image with Digital Micrograph

Every state the same.

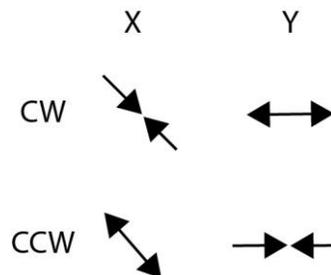
1. The CCD is usually cool (-25 °C), but make sure it is. Else it could get damaged.
2. Double click on Digital Micrograph, if it's not already open.
3. Go to camera -> Insert, as long as the vacuum is good.
4. Make sure the gain normalization was done for that week.
 - a. If not, make sure you are imaging a hole, a blank space.
 - b. Go to camera, gain normalization.
 - c. Use the default settings, a count of 8000.
 - d. Start it and follow the directions.
5. Setup search mode to have the right settings of time. 0.3 maybe.
6. Start view.
7. Go to process, live, fft to see the power spectrum. [Or reduced FFT. See which works.]
8. If it helps, add circle to FFT by clicking on circle tool. Then, move to center of image. It helps to click on the ruler icon to the left to switch from units of distance to units of pixels. Move to the center of the image. Shift-click on the center and expand the circle.
9. Stop and go on.
10. Ctrl Y will automatically name and number your images if it has been setup. Set it up by going to preferences, the wrench and hammer icon on top right of Digital Micrograph.

F1. Objective lens astigmatism correction (Digital micrograph way)

Explanation: Any objective lens astigmatism will impair image quality. Note that if you change mag or the objective aperture, this will have to be redone.

Every state the same.

1. Image some information-rich area on the screen.
2. Adjust brightness to be wide and center with beam shift.
3. Focus.
4. Now image on Digital Micrograph.
5. See if the rings in the FFT are circular. If they are elliptical, then there is astigmatism.
6. Press the objective stigmator OBJ STIG.
7. Adjust the Def/Stig buttons until the rings are circular.
- 8.



9. Exit OBJ STIG.

F2. Objective astigmatism on the phosphor screen.

Every state the same.

If at a mag between 20,000 and 100,000

1. Image a hole in the carbon. Adjust brightness, center with beam shift. Focus.
2. Press OBJ STIG.
3. Move focus to overfocus so you see a fringe.
4. Adjust image using Def/Stig knobs so that the fringe appears in equal contrast all along circumference.

If at a mag over 100,000

1. Image a hole edge in the carbon. Adjust brightness, center with beam shift. Focus.
2. Press OBJ STIG.
3. Go overfocus
4. Adjust def/stig until image of carbon appears as small independent particles.

IV. Energy Slit

Explanation: There are many alignment procedures for the energy slit, but we'll assume that it was well aligned. The energy slit removes inelastically scattered electrons which only contribute noise, not signal, to the image.

A. Inserting energy slit

Alpha: The alpha you previously selected.

Mag: The mag you want to use for imaging.

Apertures in?: The condenser and objective apertures you selected.

Spotsize: The spotsize you previously selected.

Slit: out, but soon to be put in

1. Drop the screen because the spectrum is bright and can damage the CCD.
2. Find some information rich area and insert the SA aperture.
3. Select SPCTR mode
4. Move the spectrum to the center of the screen using Diff Focus.
5. Press the slit button.
6. Select the slit width to be 30 eV.
7. If you see no beam, then it is blocked..
 - a. Widen the slit to 80 eV.
 - b. Move the slit in 10 unit increments to the left until you see the spot.
 - c. If you move all the way and still haven't found it, then move to the right.
 - d. Once you find it, center on it.
 - e. Narrow the slit and keep centering until you get to a width of 30 or 10 eV.
8. Move the slit with the Position arrows to pass through the spectrum tip.
9. Deselect SPCTR mode.
10. Remove SA aperture.

Or

1. Alternatively, one can position slit while imaging in Mag1.
2. Change the position of the slit, and pick the state in between when there is a darkened center or all darkness, but closer to the state where there is a darkened center.

V. Minimum Dose System and Taking Images

Explanation: Necessary for minimizing the dose to sensitive samples. It cycles between a low mag search, an image-shifted focus and a final exposure.

- **Make sure you move from search, to focus, to exposure back to search.**

The MDS adds to whatever alignment was done while MDS was off. Sometimes that creates problems.

Use the function keys. F3 Search. F4 Focus. F5 Exposure. F6 Blank. Between each step – e.g. Search then Focus – blank the beam.

You should setup the auto saving feature of Digital micrograph.

1. Click on the wrench icon at top right and navigate to the right place.
2. Set the file name template.
3. Try it out with Ctrl + Y.

A. When you need to focus

Everything the same.

If your mag is low, then

5. Pres IMAGE WOBB X or Y
6. Focus with Obj Focus
7. Turn off wobbler

If the image is limited by the objective aperture, then

Turn off Image Wobb X or Y and just focus it with the next section

If your mag is intermediate, then

1. Under- and overfocus on a hole in the carbon until you see neither a white or a black “halo” or “fringe” around the edge. Somewhere in between.
2. Note this value, and then slightly underfocus it. (Negative focus.)

If your mag is high, then

1. Focus on the fine structure of the carbon, or use the fringe method.

At the end, re-press Mag1 to force focus to be called “0” at that focus.

B. Setting up MDS

Everything the same.

Consider saving your alignments in Lens/Def Memory for the day, and load them for the three modes.

1. Make the initial settings for the 3 modes.
 - a. Set the after photo mode, beam blanking time, beam blanking method, and delay time. We use zero for both times. Just do what everyone else does.
2. **Confirm that the alpha, spot size and magnification are what you want.**
3. Cycle through the states 3x to work out the hysteresis.
4. Use the screen.
5. **Switch to Photo mode.**
 - a. Shift the beam to the center.
 - b. Center the stage on a noticeable feature.
 - c. Set conditions such as mag, brightness, and irradiation area.
 - i. The manual recommends a smaller beam size to limit collateral damage.
 - ii. Set exposure time to manual.
 - iii. Set Digital micrograph exposure for 0.3 – 1 second. [Joe uses a 0.5 sec exposure on Digital Micrograph at 50k or 40k mag. At 80k, Joe uses a 0.3 sec exposure.]
6. **Switch to search mode**
(Steps a – c are usually done already.)
 - a. Lower the magnification
 - b. Widen the beam almost fully.
 - c. Set DIFF mode
 - d. Shift Diff Focus largely to obtain a low mag image.
David uses a 100 cm camera length.

Joe uses 25 cm.

Andrej uses something in between.

- e. Adjust IL STIG in diffraction mode so beam is more spherical.
 - f. Adjust Proj Lens def/stig to move noticeable feature to the screen center. It's like an image shift.
 - g. If the image is blocked, then move the objective aperture out of the way. **[Though this is not ideal.]**
 - h. It is possible to take an MDS search image on the CCD (if the camera length is 100 cm.)
- 7. Switch to Focus mode** and set imaging conditions like mag, brightness and irradiation area.
- a. Increase magnification to at least 100,000x.
 - b. Condense the beam to CCD size.
 - c. Press Image Shift button.
 - d. Shift the electron beam's irradiation area using the Shift knobs to some microns away from your region of interest, (4 μm is good, others use only 1 or 2 μm .) while also bringing the image back to the center using Def/Stig knobs.
 - e. Unclick Image Shift button.
8. Cycle through the settings to confirm that they are stable and correct, and to work out any hysteresis.

C. Using MDS

Some people don't bother to blank and unblank at each step.

1. Switch to search
2. Take slit out
3. Lower screen.
4. Unblank beam.
5. Find a region of interest.
6. Blank beam.
7. Switch to focus.
8. Return focus to 0 nm.
9. Unblank the beam.
10. Focus on something.
11. Blank beam.
12. Set focus to zero by pressing Mag1
13. Switch to Photo.
14. Raise the screen.
15. Insert slit.
16. Apply the desired defocus.
17. Unblank the beam.
18. Take image with Acquire on Digital micrograph.
19. Blank the beam.
20. Save it with Ctrl + Y.
21. Repeat all these steps like a robot.

VI. Little used alignments

B2. Tilt Adjustment of Condenser Lens Deflector Coil (Manual's way)

To be done after III.B1

Explanation: Adjusts the current ratio of the two deflector coils under the condenser lens so that the image does not shift off the screen when you shift the incident beam – which is useful when you use MDS Focus.

Alpha: 3

Mag: 10,000x

Apertures in?: The condenser aperture you previously selected.

Spotsize: The spotsize you previously selected.

Slit: out

1. Select SA DIFF mode.
2. Center with Projection Lens def/stig.
3. Turn the brightness knob fully counterclockwise until a beep is heard.
4. Obtain the caustic spot (bright center spot) using the diff focus knob.
5. Select wobbler Shift X, and notice the beam splits in two.
6. Select Compensator-Shift on the Alignment panel.
7. Put the split caustic spot together using Def/Stig X only.
8. Turn off Wobbler Shift-X.
9. Select Wobbler Shift-Y.
10. Put the split caustic spot together using Def/Stig Y only.
11. Turn off wobbler-Shift Y and the Compensator Shift.
12. Switch back to Mag1.

G. Coma-free axis alignment [Can't distort the image like the protocol suggests. Needs work.]

To be done after III.F

Explanation: We will wobble the beam tilt then so that the image does not move. The wobble of the beam tilt will act as a lever, moving the image from point to wide circle, back to point, then to opposite wide circle and back. Useful for high resolution. You'd think if our illumination is intended to be parallel, this won't matter. . . . On the other hand, if we intend to do STEM, we will be scanning the beam with large tilts and shifts over the specimen, so coma-free axis alignment will be invaluable. If you change mag or objective aperture, this will have to be redone. Also, if you move to a different area of the specimen, this will have to be redone. Crazy.

Every state the same.

1. Take a search image of amorphous carbon, and do a Process -> Live -> Reduced FFT.
2. Go to maintenance page and click Freq/Amp
3. Change frequency to 3 or lower for the tilt/shift and amplitude to 4 for the tilt/shift.
4. Wobble Tilt X.
5. Press Bright Tilt to control Beam tilt with Def/Stig

6. Turn Def/Stig -X knob to minimize movement of the Live FFT. Note that the beams can't be made absolutely circular the first time. They will always sway elliptically, but the movement should be minimized.
7. Turn off wobble Tilt X
8. Turn on wobble Tilt Y
9. Turn Def/Stig-Y knob to minimize movement of FFT
10. Turn off wobble Tilt Y
11. Do an objective stigmatation calibration: Press Obj Stig and make rings circular.
12. Repeat steps 4 - 11 several times. At the end, the movement of the rings should be minimal and the rings should always be circular.

H. Parallel Illumination [Can't make this work without being in rotation center, and I don't want rotation center usually.]

Explanation: Parallel illumination is important to prevent aberrations of the objective lens from being worse. It means that the rays striking the specimen are parallel and not converging. You'd think we should have done this when we were tuning the condenser, and though we should have, we couldn't till we had aligned everything downstream of it. The only thing we will change is brightness, the knob that we thought we could do what we wanted with, but it is actually important that it is at the "right" setting, which is usually a wide spread.

Everything the same.

1. The image is focused and stigmatated and centered and well spread.
2. Take a CCD image and save it.
3. Change defocus to 5 micron and save an image.
4. Change defocus to 10 microns and save an image.
5. Go to process -> simple math and click a+b and select the 5 and 10 micron defocus images.
6. If there is movement of the gold beads, then the rotation center is off. [No kidding. The coma-free center is different than the rotation center.]
7. If you don't want to switch to the rotation center from the coma-free center, then see if changing the brightness will help.
8. Change to a different brightness and repeat steps 3 - 6. If the movement is worse, then dial brightness in the opposite direction, or vice versa.
9. Try to remember the brightness setting from the knob turn or better, the value of C3. [Confirm this for what mags people will use.]

B. Shifting the Energy Shift

To be done after inserting the energy slit.

Explanation: To capture a different part of the spectrum, like the Most Probable Loss. It is done by increasing by tens of eV the accelerating voltage, so the Voltage Centering should be the center you aligned.

Alpha: The alpha you previously selected.

Mag: The magnification you previously selected.

Apertures in?: The condenser aperture and objective aperture you previously selected.

Spotsize: The spotsize you previously selected.

Slit: in

1. Press energy shift.
2. Turn the Energy shift knob to capture the loss you want. Or use the arrows on the Filter Tuning window.

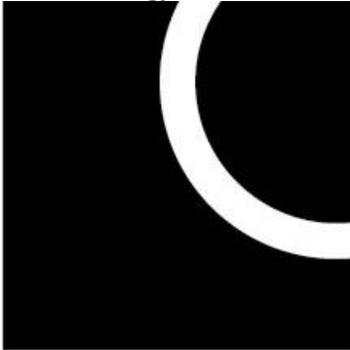
Some alignments done now and then.

C. Adjusting isochromaticity

Explanation: When you observe an image cutting, and you notice a pattern (Fig. 5.17) when you shift the energy slit.

Everything the same.

1. Manual says to shift to moderate brightness
2. Press SPCTR
3. Move zero loss spot to center using Diff Focus
4. Press Slit switch
5. Set the eV width
6. Move slit with position
7. Turn off SPCTR
8. Move energy selection slit to position so that it looks like



9. Adjust the isochromaticity on the Filter Tuning window so the arc radius is as small as possible.
10. Select DEF-Select-FLA on the Filter Tuning window
11. Move center of arc of image cutting to center of screen using def/stig knobs.
12. Set ev slit width
13. Move the slit so that the bright part of image becomes largest.

VI. Other imaging modes

A. Selected Area Diffraction

The microscope is adjusted to stage IV. A. 4 with a chosen objective lens centering.

The objective aperture is removed, all else the same I guess.

1. Press SA MAG
2. Be at your chosen mag
3. Move to an interesting area.
4. Insert SA Aperture you want. Probably 3.
5. Spread the beam.
6. Press SA Diff

7. Select camera length with Mag dial.
8. Use Diff Focus to make a clear pattern.

B. Bright Field Image

The microscope is adjusted to stage IV. A. 4 with a chosen obj lens centering.

The objective aperture is removed, all else the same I guess.

1. Do VI. A first.
2. Insert the objective aperture
3. Adjust the position and size so only the direct spot passes through.
4. Set SA Mag and set Mag.

C. Dark Field Image

The microscope is adjusted to stage IV. A. 4 with a chosen obj lens centering.

The objective aperture is removed, all else the same I guess.

1. Do VI. A first.
2. Insert objective lens aperture.
3. Adjust the position and size so only the direct spot passes through.
4. Press the Dark Tilt switch.
5. Adjust image so that only the diffraction spot you want can pass through the objective lens using Def/Stig.
6. Set SA Mag and set Magnification.
7. Adjust image brightness and beam shift.
8. Do you have to undo the Dark Tilt when finished?

D. Ultra Low Mag

Just press low mag. It should be fine. If not read the manual. The alignment is off. When finding grid squares in low mag mode, in the Mag1 mode it will be shifted to the right by a half-grid square. You should probably check this because it does move.

E. Zero-Loss Diffraction Image

F. Energy-Loss Diffraction Image

G. Energy Loss Spectrum

Slit Alignments

H. Adjusting achromaticity

Explanation: When you observe images or diffraction patterns, the projection lens is focused on the achromatic plane of the filter lens. Normally this focus has been done at instrument installation. However, when the adjustment is spoiled, the image can appear to be flowing in one direction. So then readjust it.

1. Presumably the axis alignment and focusing has been done.
2. Select mag1 or sa diff
3. If image mode, make a clear image. If in diffraction, focus with Diff Focus.
4. Turn on energy shift
5. Set energy shift value to 50 to 100 eV.

6. Adjust the Achromaticity so that the image movement by turning on or off the energy shift is minimum, however, don't expect to stop all movement.
7. Turn off energy shift.

I. Filter Lens Focus

J. Filter Lens Deflection Coil 1

K. Filter Lens Deflection Coil 2

L. Filter Lens Astigmatism Coil 1 or 2

M. Filter Calibration

VII. Shutdown.

When you are finished for the day, do the following things:

1. Make certain that you know whether someone is scheduled to use the 3200 after you finish. You will need to check the [calendar](#) and/or to check with the [director](#).
2. Leave the microscope at a magnification of 30k-50k, spotsize 1, alpha 3 and unblank the beam,
3. Retract the CCD camera and lower the large viewing screen.
4. Turn off the TV camera if you have used it.
5. Remove the specimen holder and replace it with a holder containing a grid intended to be left in the microscope during an ACD bake-out cycle, like the waffle grid.
6. Make certain that the column vacuum starts to recover before leaving the microscope room.
7. Close the valve on the nitrogen tank (to the right of the double doors as you leave the microscope room).
8. If you are the last user of the day, start the ACD bake-out cycle: insert the heating coil into the anti-contaminator (ACD) and push the "On" button below "ACD Heat" on the "Bake Out/ACD Heat" window. If there is a holder in the column, a dialog box will appear asking whether you really want to start the bake out. If the grid in the holder can withstand the heating cycle, answer "yes" and proceed. You will next see a dialog box asking you to remove all manual apertures. There are no manual apertures on the 3200FS, so simply continue.

Things left out

What is brightness zoom?

Do we have a dose meter?

Add section on what to do for vacuum problems.

Notes

Expose auto gives you a suggested dose time. That is, it measures the dose on the screen in seconds. Perhaps that is the dose one should use only when one is doing negative stain imaging, says Joe.

Joe had trouble with alignments. He redid it 3x and still the MDS focus was shifted way too much. The obj aperture was getting in the way. So he did a maintenance alignment instead of a normal alignment. It is accessed from the JEOLS menu instead of the Maintenance menu. I guess when in normal maintenance, when in MDS off, alignments will affect the 3 MDS modes. When in maintenance, it won't affect the 3 MDS modes. Don't do this unless you're a pro.

The way we inherit the previous user's MDS settings is frustrating.