

Electron microscopic analysis of negatively stained specimens using the FEI Tecnai G²

1. Fill the liquid nitrogen dewar (can be done up to an hour prior to using the microscope)
2. Log on to the microscope computer
 - Username: emusers
 - Password: 12345
3. Check to make sure that the CCD camera is on and is cooled (should read -24.9°C) on the device behind the microscope.
4. Click Start on the Application Launcher to open up the software needed to run the microscope.
5. When completed the microscope will be ready for use (can activate application launcher to observe)
 1. Left monitor will display the GUI for the operating the microscope (TEM User Interface)
 2. Right will display the GUI for operating the CCD camera to record images (DigitalMicrograph).
6. Under the setup tab in TEM User Interface:
 1. Set the High Tension to 120kV (should be set already) and turn it click the “High Tension” grey button to turn it on(grey=off;yellow=on). Wait until the current stabilizes below 1 µA.
 2. Click the “Filament” button to turn on the filament (takes about 75 seconds to heat up). When finished, current should be greater than 10 µA.
 3. Click “Col. Valves Closed” to open the column valves.
7. Beam should be visible on the phosphor screen.
 1. Set the magnification to 1,050x using the magnification knob on the right control panel.
 2. Use the “intensity” knob on the left control panel to spread or condense the beam so that it is just slightly smaller than the phosphor screen
 3. If the beam is not properly centered on the phosphor screen, use the trackball on the left control panel to reposition it.
8. Once beam is located and centered at 1,050x magnification, close the column valves by clicking “Col. Valves Closed”.
9. Center the stage by opening and expanding the “Stage²” panel and then clicking the reset “Holder” button.
10. Remove the sample holder from the microscope.
 1. Pull the holder straight out until it resists
 2. Turn clockwise until it resists
 3. Pull out the rest of the way.

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11. Place the grid in the sample holder and use the “O-ring” to lock it in place. Avoid touching the copper part of the holder to minimize contamination of the column vacuum.
12. Insert the sample holder straight into the column.
 1. Align the small metal protrusion on the holder with the closed mark on the CompuStage (at 5 o’clock) and manually insert the holder until the sensor to acknowledges it.
 2. On monitor indicate which holder (e.g. single tilt), hit CR icon
 3. Wait until the red light goes off and the pump is no longer running, then turn the sample holder counterclockwise until the vacuum from the column starts to draw the holder in.
 4. The holder should only need to be guided during the final insertion step, the column vacuum should provide the necessary force.
13. In the setup tab in TEM User Interface:
 1. Make sure that both the High Tension and Filament are still on, if the vacuum crashed during holder removal or insertion, these may need to be turned back on.
 2. Look at the vacuum for “Gun/Column”, when this drops below 8, open the column valves by pressing “Col. Valves Closed”.
14. An initial, low magnification view of the specimen should be visible on the phosphor screen.
15. Move the stage to find a dark particle with the trackball on the right control panel.
16. Place the dark particle in the center of the stage and raise the magnification to ~10,000x with the magnification knob on the right control panel. While increasing the magnification, continually adjust the intensity so that the beam is visible and fills the phosphor screen.
17. In the expanded “Stage²” panel, set the wobbler slider to 5 degrees and click wobbler.
 1. The stage will oscillate from +5° to -5°.
 2. Using the Z-axis buttons on the right control panel, minimize the movement of the stage on the screen.
 3. When movement is minimized, click the wobbler button again to stop the oscillation.
 4. Click tracks in search stage then click add (to add xyz and tilt angle) then enter ‘eucentric height’ in window, CR icon, check icon.
18. Using the trackball on the right control panel, move the stage to an area that looks slightly darkened or cloudy (an indication of good stain deposition).
19. Place the darkened region in the center of the stage and raise the magnification to ~60,000x with the magnification knob on the right control panel. While increasing the magnification, continually adjust the intensity so that the beam is visible and fills the phosphor screen.

20. Spread the beam using the intensity knob on the left control panel so that the beam appears uniform across the screen.
21. In DigitalMicroscope, insert the CCD camera (Camera—>Insert Camera).
22. Under Camera View on far right, click the toolbox to setup the camera.
 1. Options should be: 0.1 second exposure
 2. 8 Binning
 3. Gain Normalized Correction
23. Raise the screen by clicking R2 on the right control panel (Screen Lift) to allow the beam to reach the camera.
24. Start acquisition by clicking “Start View”.
25. Using the intensity knob on the left control panel, put the white box in the center of the green area.
26. Open an FFT to focus the beam on the specimen (Process—>Live—>FFT).
 1. Using the focus knob on the right control panel, adjust the focus until Thon rings are visible.
 2. Adjust the focus until the zero defocus cross-over zero defocus is found (the point where no rings are visible in the FFT).
 3. Click the R1 button on the right control panel to “Reset Defocus”
 4. Set the focus to -1500nm with the focus knob on the right control panel. (If well aligned this will place the first ctf zero at about 18 A.)
27. Click “Stop View” to stop the imaging.
28. Under Camera Acquire on far right, click the toolbox to setup the camera.
 1. Options should be: 1 second exposure
 2. 1 Binning
 3. Gain Normalized Correction
29. Click “Start Acquire” to record an image.
30. If the image looks good, move the stage to a nearby area and collect another image. Repeat until enough images have been recorded.
31. If image is highly astigmatic:
 1. Press R2 to lower the screen
 2. Raise the magnification to ~150,000x with the magnification knob on the right control panel. While increasing the magnification, continually adjust the intensity so that the beam is visible and fills the phosphor screen.
 3. Press R2 to raise the screen
 4. Use the defocus knob to try to find the cross-over zero defocus point
 5. Click the R1 button on the right control panel to “Reset Defocus”
 6. Set the focus to -1500nm with the focus knob on the right control panel.
 7. Turn on the “Stigmator” from the dialog box in the lower right part of the screen
 8. Selective “Objective” from the new window to adjust the objective aperture

9. Click “Start View” to view the image and its FFT.
10. Turn the multifunction knobs on the two control panels to correct the shape of the visible Thon rings in the FFT window.
11. Adjusting the focus with the focus knob on the right control panel may be helpful if the Thon rings are too distorted by the astigmatism or their signal is too weak compared to background.
12. Click none on the Stigmator panel to save the corrections.
13. Click “Stop View” to stop the imaging.
14. Press R2 to lower the screen
15. When rings appear round, reduce the magnification to ~60,000x with the magnification knob on the right control panel. While decreasing the magnification, continually adjust the intensity so that the beam is visible and fills the phosphor screen.
16. Press R2 to raise the screen
17. Click “Start Acquire” to record an image to check that the image is no longer astigmatic.
18. Move to a new area with the trackball on the right control panel and repeat the image acquisition process from step 24.
32. If the stain is poor, reduce the magnification to ~6,000x with the magnification knob on the right control panel. While decreasing the magnification, continually adjust the intensity so that the beam is visible and fills the phosphor screen.
33. Select another area for imaging and repeat steps 19, 20, 23, 24, 25 and 27.
 1. Camera does not need to be retracted between images
 2. The live FFT window will automatically update when “start view” is pressed
34. When finished imaging a grid:
 1. Set the magnification to 1,050x using the magnification knob on the right control panel.
 2. Use the “intensity” knob on the left control panel to spread or condense the beam so that it is just slightly smaller than the phosphor screen
 3. Center the stage by opening and expanding the “Stage²” panel and then clicking the reset “Holder” button.
 4. Camera retract.
 5. Close the column valves by clicking “Col. Valves Closed”
 6. If another grid will be inserted, follow steps 9 - 11.
 7. If finished for the day:
 1. Remove grid sample and replace grid holder.
 2. Turn off the filament by pressing “Filament” (takes ~40 seconds to turn off)
 3. Turn off the high tension.
 4. If last user, start the cryo cycle (Setup → Vacuum → Cryo Cycle)

5. **Log off computer.**

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