

## Gatan CCD camera on the JEOL 1010 TEM

The software used currently (spring, 2016) is Gatan Microscopy Suite version 1.7 and DigitalMicrograph version 1.72.53. This software is the same as that on the JEOL 3200FS.

1. Start the computer.
2. After the computer has started, simultaneously press **ctrl-alt-del**.
3. Enter the account name (JEOL) and the password (EM185).
4. Check the water flow rate. The flowmeter is located on the back wall (behind the 1010). It should be between 10 and 15 liters per hour (these rates are indicated by the two red markers on the gauge). This water chills the cooling system for the camera, and the camera will be damaged if there is no cooling water. Intense brightness will also damage the CCD camera. This could happen if a diffraction pattern, or anything else that is intensely bright, is imaged without reducing the brightness and without a beam stop. For this reason **do not take images of diffraction patterns** with the CCD camera. The water for the camera cooler comes from the chiller next to the sink in the main lab, the same source as the cooling water for the 1010's diffusion pump and lenses.
5. The control box for the CCD camera should be on, the Peltier cooler switch should be set to Cool, the Shutter switch should be set to Auto, and the Camera switch should be set to Computer. If the Peltier cooler is not already cool, it will take at least two hours for the camera temperature to stabilize.
6. Double click on the icon for Gatan DigitalMicrograph (DM). The icon is somewhere along the left side of the computer monitor.
7. There are small windows (or modules) inside DM that do various things. The modules on the right are for image acquisition. The modules on the left show information about the images and the imaging process, and can be used to alter the display of any of the images open in DM. There is also a module on the left at the bottom (Image Info) that allows the user to annotate an image. Such annotation is stored in the header of dm3 files (but not TIFF or JPEG files). The Results window is sometimes available at the bottom of the DM window. It shows information about previous actions that were taken within DM, and can be used to display things like min/max/avg/std dev of any image or sub-region of an image.
8. Documentation about DM and the CCD camera is in a Help folder on the desktop. Additional info can also be obtained from Help and Search in the DM itself.

Note: The documentation in the desktop folder that deals specifically with cameras does not apply to the current camera, but rather to the 4k x 4k camera that was replaced in the spring of 2016.

9. Click on Camera in the menu bar at the top. Scroll down and click on Insert Camera. While the CCD camera is inserted and the large view screen is raised, the beam is automatically blanked and it is not possible to use the TEM film camera: any negatives taken will be blank.
10. Get a focused, properly stigmated image on the TEM. **Set the current density on the TEM monitor to 55 pA/cm<sup>2</sup> when reading the beam current from the large screen.**

At high magnifications, it may be necessary to spread the beam larger (fewer pA/cm<sup>2</sup>, a longer reported exposure time) to ensure that the illumination of the image is even. Even with the beam spread, it should still be possible to record good images using an exposure time of 1 s (or less).

**11. Put the plastic cover over the TEM viewing window.**

12. Lift the large viewing screen by pressing **PF1** and then **return**. While the TEM view screen is raised, there will be no read out on exposure time or current density on the TEM monitor.
13. The computer monitor for the TEM blanks out when the large screen is raised. Turn it back on by pressing **PF10** (this is also marked Page).
14. Optional use of Camera View module for searching and focusing: Instead of the work described in step 10 that involves looking at the 1010's viewing screens, samples can be located using the Camera View module in search mode. Focusing and stigmation can be done using Camera View in focus mode. Both imaging modes will produce an image that is normally poorer in resolution than the 1024 pixel by 1024 pixel full-sized image. In both modes, the Focus Loupe can be used to reduce the area viewed and allow for faster image acquisition. How easy it will be to search and focus will be determined by the exact setting of the search and focus modes.
15. Go to the Camera Acquire module. With the illumination set as describe in step 10, **exposure times of 1 s or less should produce good images.**

Note: For most purposes, binning should be set to 1. If the binning is not set to 1, the exposure time should be reduced in order to acquire good images.

16. Press the Start Acquire button located in the Camera Acquire module.
17. Enter the magnification and kV in the window that pops up (and Mode should be set to Imaging).
18. **Read-out of the image will take ~10 s.** The unbinned (bin set to 1) images are 1k by 1k. The magnifications for the CCD camera images are approximately 40% greater than the magnification shown on the monitor for film. The increase in magnification is caused by the CCD camera being further along the optical path than the film camera.

Note: The pixel size of the current camera (24  $\mu\text{m}$ ) is larger than that of the older, 4k x 4k camera (15  $\mu\text{m}$  pixels), so that even though the new camera images are only 1k x 1k, the field of view is larger than would be expected based on the number of pixels. It is in fact about 40% that of the original 4k x 4k camera. **In order to see roughly the same field of view using the current camera, images should be recorded at half the mag used with the old camera.**

19. If you consistently set the same electron dose on the TEM using the main screen current density reading, you can also use a consistent exposure time for the CCD camera in Camera Acquire module.
20. Press **PF2** then **return** to lower the TEM view screen in order to scan your sample for a new location to image.

21. When you are finished with your TEM session, retract the CCD camera by going to Camera in the menu bar at the top of the computer screen. Scroll down and click on Retract Camera.
22. Images can be saved by going to File in the menu bar at the top of the computer screen and scrolling down to Save. The same thing can be accomplished by typing **ctrl-s**. This saves the active (dark blue bar across the top) window. Save the image to your folder on the Users hard-drive (E:). **Always save images as dm3 files** (*i.e.*, do not save any image directly as a TIFF or JPEG file).
23. It is possible to convert the .dm3 files to TIFF or JPEG using the Batch Convert (found under File in the menu bar across the top of the program). Press OK after the last file has been converted. The .dm3 format is readable only in DM, ImageJ/FIJI and a few other, uncommon image display programs. **Always keep the original dm3 files since they contain information (metadata) that is lost upon conversion to any other file format.**

Note: There is an offline version of DM available from the Gatan website that anyone can install on a laptop or lab PC, but it does not have the full capability of a licensed copy.

#### Additional DM tricks:

- 1) It is possible to build file names automatically using DM, and to save files using a single keystroke. Click on File in the menu bar across the top of the screen, and then on Global Info in the drop-down menu that appears. One of the options in the new window that appears is "Save numbered". Click on that option and fill in the requested information. There are text boxes for the location to save the images (*i.e.*, a folder on the E: drive), the basic name of the image series (*e.g.*, myImagesFromToday), and the image number to start the series. After setting these values, close the window. Any image can be saved by making it active (click on the window, causing the blue bar across the top of the image to turn intensely blue) and typing **ctrl-y**. The file will be saved with the current image name and number (*e.g.*, myImagesFromToday\_0001.dm3) and the image number will be automatically incremented for the next image.
- 2) You can close all the image windows in a DM session without being prompted to save any changes by typing **alt-ctrl-w** and clicking the "x" in the upper right corner of any image. You can also close windows by typing **alt-ctrl** and then **w** multiple times: each time you type **w**, the active window (the one at the front) will close. Finally, you can close all the windows in a DM session (including the results window if it is open) by typing **alt-ctrl-shift-w**.