TEM Morgagni Procedure

1. Check the vacuum. Vacuum overview button in the pulldown menu on the bottom right or under the Vacuum tab on top left. The column should be a light blue color and the status in the vacuum window should show “Vacuum ready- All Vac”.
   If the vacuum is down, restart the vacuum from the panel by pushing the vacuum off and then on buttons. It may take ~15 minutes for the ODP temperature to reach the necessary temperature and for the column to be pumped down.
2. Turn on the High Tension voltage from the panel. You should hear a whistling noise. The voltage should always be set to 80kV.
3. Filament should heat up when the HT is turned on. This is indicated by the heating button in the filament panel under the High Tension tab being yellow. If it is gray, the heating should be turned on by pressing the heating button.
4. Check the emission. The value should be >3.5. If it is too low, increase the bias. Don’t push the emission higher than necessary, because this will shorten the lifetime of the filament.
5. Set the spot size to 1. This controls the first condenser lens. The knob to do this is the leftmost knob on the left panel. The outer ring on this knob controls the intensity of the beam.
6. Verify that the condenser aperture is inserted by wiggling the large knob on the top aperture in CW direction. If it turns easily CW, this means it was out. Make sure it’s on the first aperture (no more than one click CW). The Condernser aperture should NEVER be out.
7. Turn off the room light and look for the beam. If you don’t see the beam, lower the magnification while adjusting the intensity at the same time. Ideally mag should be around 4.4kx.
8. Shift the beam to the center of the screen using the Shift x and Shift y buttons on the panel.
9. Changing the intensity, check that the beam stays centered while going through cross over (i.e the beam does not “swing”). If the center of the beam moves when going through crossover, the condenser aperture needs to be centered. Do this by using the small knob on the aperture and the small knob on the right side of the column. Continue checking the intensity while doing this.
10. Check the shape of the beam. If the beam is oval, this indicates it condenser lens astigmatisms. In the Stigmators panel under the *Tune* tab click the Condenser button. This activates the Multifunction X,Y knobs on the panel. Change these until the beam is round.
11. At a magnification of 4.4Kx, make sure the beam is centered and the intensity spread out enough to almost cover the fluorescent screen.
12. Open the camera software on the left screen (to move the mouse cursor between the screens, press the black button next to the mouse). The camera software is called AMT Capture Engine.
    a. Pull up correct exposure time settings by pressing the Recall button in the software and choosing RegularSettings (if you decide to set up your own exposure time and gain settings you can do that by using the Set/Save button).
    b. Raise the screen to allow the beam to reach the CCD
    c. Click the *Live Image* button
    d. Center the red histogram by adjusting the intensity.

    The image should be uniformly gray. If it isn’t, it’s a good idea to collect a new background image: New background images should be collected if you change the exposure settings.
    a. Under the Correction tab click on “Acquire Backgrounds”
    b. Assuming no specimen and adjusted intensity and beam position, click on Proceed
    c. Click once more on Live Image, to make sure that the background collected was correct. You should see a uniformly gray image.
13. Place a sample in the holder. Do not touch the area above the o-ring without gloves, this can cause contamination in the column.
    a. Use the small needle to lift the clasp on the holder.

b. Place your grid in the circular indentation. Once it is on you can tap the side of the rod with the needle to nudge it into place.
c. Lower the clasp carefully.

14. Insert the sample into the chamber. Have the vertical rod in the 1 o'clock position as you insert, once it is past the initial divot turn to 12 o'clock and stop. A red light should have turned on while the airlock is being pumped down. Turn the sample to the 6 o'clock position and insert the rest of the way. Push the button on the end to release the sample, hold it down while you extract the rod. You will have to turn it from 6 to 12 o'clock when it is pulled back half way.**If there is too much resistance while turning it from 6 to 12, that could be because the head isn't fully inserted. DON'T FORCE the holder back to the 12 o'clock position. You may accidently shear off the tip of the head. Instead re-insert the holder and tap the head farther into the column, than take the holder out.**
15. **When moving the stage, pay attention that there is no clicking sound when turning the rods. If there is, this means that the stage is at the end of it's range and continuing to turn the rod in the same direction will cause the stage to jam.**
16. Find a grid square you'd like to image. Lower the magnification to zoom out on the sample. Once past Shadowmag you will have to remove the objective aperture to get a larger field of view. The Objective aperture can be removed by turning the large knob on the column CCW.
17. After the square is selected, reinsert the aperture by turn one click CW. Increase magnification, while making sure the beam remains centered and the intensity remains bright enough..
18. Make sure the Objective aperture is centered (note: there are several positions for this aperture. The first position, aka smallest aperture, is the most commonly used one. This step, as well as the astigmatism step, will have to be done for whichever position you are using).
    a. Go to the 20kx magnification (higher is fine as well), while keeping beam centered and intensity bright enough.
    b. Make sure the fluorescent screen is down
    c. Press the *Diffraction* button on the panel, a window will pop up on the computer screen asking you to verify that the fluorescent screen is down (this is to ensure that the bright diffraction beam does not damage the camera).
    d. Use the knobs for the Objective Aperture to center the halo (objective aperture) around the bright spot (condenser aperture). If you see other things in your image, they are coming from the grid, you can move slightly over if they obstruct the halo.
    e. Press the *Diffraction* button again to stop diffraction imaging.
19. Make sure the image is in focus and there is no objective lens astigmatism
    a. Go to a magnification of about 20kx and adjust the focus. One can also look at the FFT to learn about the focus.
        i. In the camera software, click on the FFT button. When adjusting the focus you will see a central brightspot and a larger ring in the. Adjust the focus until the circle/ring fully expands and then contract again. If you cannot see a ring it's possible you are too far from focus and need to first adjust focus in the image by eye. True focus is when the ring is fully expanded in between the two contracted forms of the FFT.
        ii. With the FFT fully expanded, in the microscope software in the Parameters panel under tune click the Reset defocus button. This will reset the Defocus number in the bottom panel to 0. Negative stain images are usually collected at a defocus of -2um.
        iii. If the ring of the FFT is oval, this indicates Objective lens astigmatism. In the Stigmators panel under the *Tune* tab click the Objective button. This activates the Multifunction X,Y knobs on the panel. Change these until the FFT is round.

20. Acquire your images. You can open a *Case* to make saving images faster. For more information on operating the camera, you can read the manual on the desktop.
21. To take out the grid, insert the holder in the same manner as in step 14. This time push the button while inserting and release it when the holder is fully in. This will allow the holder to grab the head. Take the holder out (without pressing the button again, or you will release the head), similarly to the way you did in step 14.
22. When finishing your session, turn of the High Tension and close the camera software.
23. You can retrieve your images using the PC computer located in the computer bay. Open up Finder and go to the Morgagni linked drive. There is a folder called ImagesAMT that should have your folder with images inside it. Either bring a flash/external drive to place images on or upload them to a cloud storage.