

SEM Protocol

Gail's version of Josh's protocol

NATURAL SEM PROCEDURE

Turn on the "DISPLAY" switch on the control panel under the counter under the chamber

1. Turn on camera so that you can see inside the chamber (use white button on front of right television screen)
2. Move the Robinson Detector into the chamber by unscrewing the knob on the top of the detector and lifting the bottom bar that is holding the detector to the SEM and slowly guiding it into the chamber. The vacuum will pull it slowly. Do this slowly because the detector is very sensitive. You can tell that it is inserted correctly when the housing of the detector is flush with the chamber opening. (Gail's version: I never check it, I just push it in all the way, and I find that I need to push it kind of hard at first)
3. Press the white button on the front of the right television screen to see the black left screen again. Turn the vacuum off to empty the chamber, i.e. depress the Air/Evac button under the left screen.
4. Press the VACUUM SET (F2) button on the computer keyboard. Set this to N-SEM by moving the cursor with the left arrow key (so that you highlight NSEM) and press enter. Then use the arrow keys to highlight the # of pascals (pa). Scroll down to 30pa using the left arrow key. Press enter, then press the vacuum set button again to take this off the screen.
5. Press the BEAM CURRENT (F10) button on the keyboard and move the beam to the R of LARGE (use the right arrow key; press the shift key at the same time as the right arrow key to make it go faster). Press F10 to make this display disappear.
6. Load your sample as soon as the chamber is loose (you need to push down a little before pulling out). The sample should be placed with nail polish and tape on a metal peg.
7. Put the sample on the SEM stage. Push the door shut and switch to EVAC setting. It will take about 2 minutes to evacuate the SEM.
8. When the vacuum is ready, the ACC/VOLTAGE light will blink. Wait for it to stop blinking- about another minute (Easiest to just set a timer for 3 minutes starting from when you press Air/Evac button). Turn the ACC/VOLTAGE button on.

THIS IS WHERE GAIL'S PROTOCOL DIVERGES FROM JOSH'S. GAIL HAS NICER IMAGES THAN JOSH SO FOLLOW THIS ONE!!!

The rest of the settings will be made on the SEM keyboard/panel.

10. Switch "Image Tone" to manual.
11. Switch ACC/Voltage up to 10kv (use down/up buttons).
12. Switch focus to manual. Press "fine" briefly, and a decimal number (probably ~0.15) should appear in the magnification display box. Keep pressing "fine" until the number

scrolls down to 0.11 (it will probably beep at you a couple of times, but don't worry about this). Now switch focus back to auto.

Gail's note: at this point, its time to saturate the filament. First of all, I don't bother to look at waveforms at all. Secondly, I saturate part way, then focus, center, etc., and only at the last minute do I saturate all the way. This way, the strong current doesn't hit the sample until the last possible moment, so you lessen the risk of burning your sample.

Before saturating the filament, make sure the computer is ready to go (see computer page).

13. Press the key that's marked with a single arrowhead over a double arrowhead, twice. Make sure the double arrowhead is now displayed in the upper right hand corner of the screen.

14. Turn the filament clockwise. Anywhere between high noon and two o'clock, you should be able to see an image. If you don't, turn up the contrast knob until you do. This corresponds to the first peak of filament saturation.

15. Use the Z knob on the chamber casing to coarsely focus the sample. Use the X, Y, and rotating knobs to properly position the sample. Press the magnification buttons to get up to 180X (i.e. the standard for a Drosophila eye). Press the "autostigmat" button to finish the autofocusing procedure -- it will freeze the image for about 5 seconds, then beep.

16. NOW continue to turn the filament button clockwise. The image should darken/disappear, then as you continue clockwise, it should brighten up/reappear. Go just a hair past the brightest point.

17. Adjust the contrast/brightness buttons so that the image is pretty bright but not blinding.

18. Press the photo button. A slow scan of your sample will appear simultaneously on the left SEM screen as well as on the computer screen. Once this scan is complete (the machine will beep), press the upper left "live/freeze" button on the SEM computer keyboard to return to the live image. If you are unsatisfied with your image, you can play around with the contrast and brightness, or you can autostigmat at higher magnifications at different places on the sample.

19. When you are done with your picture, turn down the filament very slowly by turning the filament knob counterclockwise all the down.

20. Turn off the ACC/VOLTAGE button.

21. Set your timer for three minutes. Wait 3 minutes for the filament to cool. If you do not do this, you will break the whole machine and many people will be mad at you!!!!!!!!!!

During these **3 minutes**, you can keep yourself entertained by saving your files on the computer (again, see computer page).

22. Now that you've waited for **3 minutes**, release the vacuum in the chamber by pressing the VACUUM/Evac button.

23. When the door has loosened (about 2 minutes) take out your sample. If you are going to take more pictures go to #7, but you can skip steps 10-13.

IF YOU ARE DONE:

24. Reset the BEAM current so that the bar is only half full. Also, turn the N-SEM setting to the SEM setting like when you started. Pull out the Robinson detector and tighten the screw as soon as the lever on the bottom falls down.

25. Shut the chamber door and turn the VACUUM on again.

Turn off the display switch on the front of the SEM.