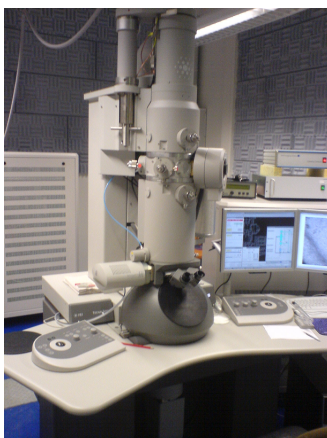

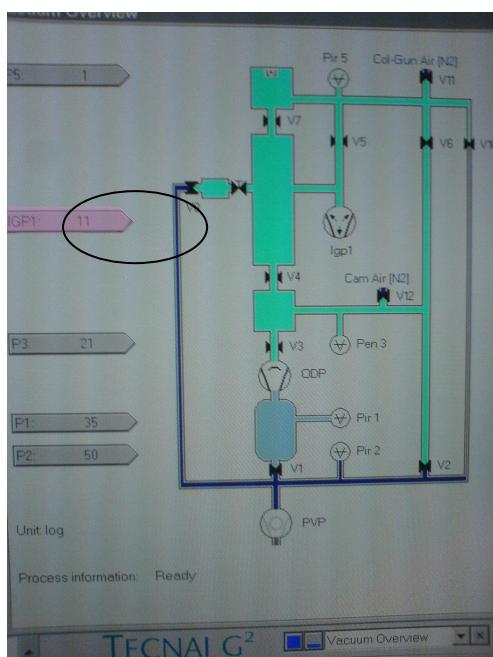



Basic EM Instructions




1. In “Set up”: Turn on High Tension and wait until the emission is steady (watch blue bars).
2. Turn on filament; emission should stay between $3\mu\text{A}$ and $10\mu\text{A}$.
3. Fill N_2 into the cold trap and insert the copper wires.
4. Keep the column valves closed.
5. Place a grid inside the sample holder; carefully lift the small grid-lid with the needle. Only touch the silver part of the holder, never touch it beyond the o-ring.
6. To insert the sample holder (stand up to do this), turn it so that the little pin points to 5 o'clock and push it this way into the stage until a resistance is felt and the red light turns on. Try to keep the holder as horizontal as possible.
7. Choose single tilt holder  on the computer and wait until the pump and the red light turns off and valve 8 is closed.
8. Then, turn the holder anticlockwise until it is sucked into the stage. Don't release it until it is completely inside.
9. Now, check that the IGP 1 (see circle on the pic. below) value is below 30. If so, it is ok to open the column valves. Otherwise, wait for better vacuum!



1. Lower the screen (R1) and set the magnification to about 10 000x.
2. Spread the beam across the entire screen. (Make sure, the beam spreads wider, when the knob is turned clockwise. Theoretically, it is possible to spread the beam anti-clockwise if it is focused over the crossover point, but avoid this)
3. It is very important to correctly set the eucentric height using the alpha wobbler. Choose a recognisable feature on your grid at 10K magnification and click on L1  on the left user pad or, alternatively go to “Search” →, “setup”, then activate the wobbler. Minimize lateral movement using z-axis buttons (on the right user pad not in the pict).



1. Then, press Eucentric Focus button on the right hand panel.
2. Spread the beam across the entire screen, and then lift the screen (press
3. R1 on right user pad) and cover the window with the rubber mat. Check that
4. the CCD and not the WAC CCD camera is selected in the camera tab.
5. To take pictures, click search and choose a hole. The magnification should be set to about 1100x to get a good overview. Zoom into the desired hole at a magnification of 67000x and click on preview and live FFT and see the Thon rings. Turn the focus button so that the rings become larger and larger until they disappear completely. Now the absolute focus or Scherzer focus is achieved.

6. Focus steps can be changed by turning the lower, wider dial of the same knob (should be 3 for fine and 4 for course focusing). Note, if the rings are not round, but oval or in extreme, like this ✖, check problems protocol!!!!
7. Reset the defocus (button R3) and defocus to -739nm to get some contrast (if you used a magnification of 110x, defocus -369nm)
8. Press Acquire to take a picture
9. Save the picture by clicking  in the camera window
10. When you are finished, close the column valves,
11. turn off filament,
12. turn off the camera (search/preview button, NOT camera software!!!)
13. and take out the sample holder: Click Reset holder and wait until the red light is off. Carefully pull out the holder while pressing the thumb of the other hand against the blue part of the stage. Turn holder clockwise until you feel the resistance and then pull it out completely. Always keep it parallel!!!
14. Then, insert the sample holder back into the Perspex cylinder for storage and remove your grid.
15. Take off the cold trap and turn on cryo cycle of the microscope if you are the last one using it. The cryo cycle can be found in Setup → vacuum (open flap) ➤ cryo cycle. ⚠ Do NOT click on cryo cycle before you haven't turned off the filament and waited until it is at zero!!!!