Grid Preparation

Making Formvar-carbon coated grids

1. Prepare 1.0% Formvar in 100% chloroform. Can adjust concentration to get thinner films (0.5%).
2. Dip precleaned microscope slides into a beaker containing the Formvar solution (should cover slide ~3/4 up).
3. Slowly, pull out the slide at a constant rate.
4. Air-dry the slide in a slide holder.
5. Meanwhile, fill a plastic container (1 ml tip box cover works well) all the way to the top to where it is about to overflow.
6. Once the slide is dry, scratch the edges of the slide to allow the plastic to come off (use a razor blade).
7. Slowly and gently dip the slide into the water such that the plastic film comes off the slide and floats on the surface of the water.
8. Observe the floating plastic film from the side so that so can see the thickness (by the color of the plastic).
   Purple/gold …….too thick
   gray……too thin
   Silver……..Perfect!
9. Drop grids on top of the film with the dark side down.
10. Pick up the film containing the grids using a piece of parafilm such that you end up with grids sandwiched between the parafilm and the plastic film.
11. Allow the grids to air-dry overnight before you carbon coat them

Making Carbon Coated Grids

- Making the carbon film
    1. Take a piece of mica and cut off a square or rectangular piece that is approximately 2 x 3 cm.
    2. Cleave the mica with a razor blade or scalpel that has been cleaned with acetone. Once the mica has begun to separate along a cleavage plane, forceps may be used to pull the mica completely apart. It is better to do this than to scratch the surface of the mica with the razor blade. Mica, as purchased from most EM supply companies, is rather thick and a new piece may be split around 4 times.
        - This mica should be cleaved immediately before coating as the freshly cleaved surface is clean and hydrophilic, but it becomes contaminated (and thus hydrophobic) over time time.
    3. Attach the mica to a filter paper, with sticky tape, with the freshly cleaved plane facing upward.
    4. Place the filter paper in a vacuum evaporator for carbon coating and deposit a film of carbon onto the mica surface.
    5. After coating, the filter paper should be light gray in color (compare the filter paper behind the mica with that which was exposed, and therefore carbon coated). This is a good indicator of the film thickness.

Carbon Coating Grids

1. Turn the MAIN and MECHANICAL PUMP switches on, as well as the water valve on the back wall.
2. Move the Black-knobbed handle to the BACKING position.
3. Make sure the meter switch is set to EVAPORATOR.
4. Open the air inlet (little black valve) and remove the bell.
5. Plug in the carbon coating leads to ground (E) and (1).
6. Remove the cover on the carbon coating apparatus and release the tension spring.
7. File the edges of the carbon electrodes (replace if rod is < 3mm long). One rod must be flat (left) and the other (right) must be a sharp point.
8. Adjust the rod to ~1mm distance and replace the spring and cover.
9. Place your sample in position (along with folded paper to see amount of carbon being deposited).
10. Replace the bell and close the air inlet valve.
11. Move the black-knobbed handle to ROUGHING. Let the vacuum reach 0.04 torr on the bell jar gauge.
12. Move the black-knobbed handle back to BACKING. Turn on the DIFFUSION PUMP.
13. Leave the diffusion pump on (to warm up) for ~ 20 minutes.
    13B. Could also put in the grids at this point, while in backing.
14. Now move the handle to ROUGHING and let the vacuum reach 0.04 torr on the bell jar gauge.
15. Fill the baffle with liquid N_2.
16. Move the handle to BACKING.
17. Move the RED-knobbed handle to OPEN.
18. Let vacuum reach 2-5X10^{-6} on the discharge gauge. (30-45 minutes).
19. Turn the electrode selector to #1.
20. Turn the electrode switch ON.
21. Turn up the voltage and warm up 30 seconds.
22. Evaporate 3 seconds at a time. Monitor the amount of carbon by observing shadow in paper.
23. When done, turn electrode switch OFF, Turn RED-knobbed handle to close.

24. Open air inlet, remove grids, replace bell and close air-inlet
25. Move handle to roughing and let vacuum go to 0.04 torr.
26. Move handle to BACKING, turn OFF the diffusion pump and let it cool for 20 minutes.
27. Move handle to Close position.
28. Switch off pump, power and water.

Staining Samples on Grids

1. Prepare a 1% uranyl acetate solution in water
2. Place 5 µL of your diluted virus preparation (dilute to concentration of 100 µg/mL) on the glow discharged grid. The time it is left on the grid before staining is not critical (~2 minutes is good).
3. Blot off most but not all of the virus sample from the edge of the grid with a piece of whatman filter paper (do not allow remaining sample to dry out).
4. Immediately add 5 µL of water or buffer to rinse off unwanted compounds (ie. Sucrose or glycerol). Leave on for ~2 minutes before blotting off water as before.
5. Repeat step 4 as needed .
6. Immediately add 5 µL of the uranyl acetate stain.
7. Immediately blot off and add another 5 µL of the uranyl acetate stain. Leave the stain on the grid for a minimum of 2 minutes.
8. Blot off the stain using a piece of Whatman filter paper. Blot from the edge of the grid and try to remove as much liquid as possible without touching the surface to be looked at on the microscope.
9. Place the grid on the surface of a circular Whatman filter paper with the sample side down.
10. Air-dry the grid before looking at it in the electron microscope.

Using the EM 420 Electron Microscope

1. Press the on button labeled HT (this will be on the left-hand panel).
2. Turn on the panel lights by pulling on the panel light knob (pull out).
3. Fill the liquid nitrogen container.
4. Switch on the emission, which is on the far left hand side of the left panel. About 10 or 11 clicks is enough (until you see the image area illuminated).
5. Adjust the intensity to where you get a small spot, then adjust the emission until the filament shadow disappears.
6. You now have to align the beam.

A. On the microscope column, you will find three aperture control mechanisms

Only move the outer knob

This opens aperture

B. What you want to do is center the intense beam in the middle of the circle.

Image area
lighted area (with intense beam centered in middle)

C. Another way is to press the deflection button on the right panel (normally on magnification).

Then center the beam using the deflection knobs on the left and right panels.

7. You then need to put the grid on the specimen holder.
8. Place the specimen in the microscope by gently inserting it in with the notch on the right hand side. The holder will be sucked in and you have to wait until the red light goes out. You then rotate the specimen holder clockwise until it stops, slide it in until it stops, rotate back, and insert the rest of the way in.
9. Start off with low magnification, until you find an area on the grid on which to focus the microscope (the edge of a grid mark).
10. The focus knob is on the right panel and consists of an inner and outer knob. The inner knob is to set the step and the outer knob is to adjust. Useful step settings are from 5-7 for coarse adjustment and lower for fine adjustment. Focus by adjusting the image to where you get a sharp edge on the side of the grid.
11. Scan around using the two handles on the side of the viewing area.
12. To remove the specimen, pull out the holder until it stops then turn clockwise. Remove the rest of the way using your thumb for leverage (remember that it is held in by a vacuum and this is the resistance you will feel).
13. To take photographs, adjust the intensity until the exposure time reads 0.5 seconds. Lift up the stage and the photograph button illuminates. Press the button and wait until the photograph is taken. You can then replace the stage and continue.
14. To shut down the microscope, remove your sample as stated above (remember to plug up the specimen hole with the rubber stopper). Turn down the emission filament, turn off the scope with the HT button and push in the panel light knob.