ESEM Theory

1) The specimen chamber in the ESEM is separated from the microscope column by a pair of pressure limiting apertures (PLAs). The differential pumping system keeps a high vacuum in the column, yet allows a lower vacuum and water vapor in the specimen chamber.

2) The water vapor in the specimen chamber is used to:
   a) amplify the signal from the specimen
   b) suppress charge build-up on the specimen
   c) keep the sample from drying out.

3) Special gaseous detectors that can work in a wet environment are used in wet mode.

4) To keep water in your sample from evaporating, the chamber pressure must be 4.6 Torr or higher, and the sample must be chilled with the Peltier stage. See the ESEM humidity chart to determine appropriate operational temperatures and pressures.

Detector Choice

1) The final pressure limiting aperture is located in the gaseous detectors.

2) Large apertures allow a larger field of view (for low magnification work), but also let more water vapor leak into the column. This means that the larger the aperture chosen, the lower the allowable pressure in the specimen chamber.

3) The detector should be chosen for the best trade-off between field of view and prevention of evaporation. See the description of the various detectors in the Controls & Detectors section of the blue notebook.

Pumpdown Considerations

1) With the appropriate detector, the sample can be viewed under saturated water vapor (it neither gains or loses water). During pumpdown, the goal is to replace the dry air in the chamber with the appropriate pressure of water vapor, without drying out the sample in the process.

2) This is accomplished by using the Purge function in the Vacuum controls. You can either use Auto Purge (pre-set based on your choice of detector), or customize your own Purge routine.

3) Helpful hints:
   a) Chill the sample and stub before inserting in the ESEM.
   b) Put water drops on the corners of the stage.
   c) Flood the chamber, then vent it to insert the sample. This will leave some residual humidity in the chamber.
Optimizing Image

Working distance (from sample to detector), beam voltage (kV), spot size, and pressure/humidity all interact to determine the quality of the image.

1) **Pressure.** Higher pressure = more water vapor. If you need to keep your sample hydrated, you will need to work at pressures above 4.6 Torr. In all cases, you need enough water vapor to amplify the electron signal from the sample, but not so much that the electron beam is swamped.

2) **Working distance (Z).** Another way to change the amount of water that the electron beam has to traverse and the amount available for signal amplification is to change the distance between the detector and sample (more distance = more water). Often, changing $Z$ is more feasible than changing the pressure, particularly if you are trying to maintain hydration in a delicate sample.

3) **Beam voltage (kV).** If the electron beam has to pass through too much water, it won’t penetrate well. Possible adjustments:
   a) Increase kV (will depend on delicacy of your sample)
   b) Reduce humidity (decrease pressure, but beware of drying your sample)
   c) Decrease working distance (reduce Z)

4) **Spot Size.** Spot sizes are given in relative numbers of 1 to 8, with 1 being the smallest. Higher magnifications require smaller spot sizes; 2 – 6 is the most likely range that you would use.

5) **Charging.** Sample charging can be reduced by decreasing kV, or increasing the water vapor (by increasing pressure) in the chamber.

6) **Sample Flooding.** If you have water lying on top of your sample, you won’t be able to see any detail, since the electron beam does not penetrate water. You can dry the water off of the sample by lowering the pressure (in small steps). Once it is dry enough, increase the pressure to the appropriate point (depends on temperature) to prevent further dehydration.