Preparing tissue for the SEM (Jill's version)

Fixative solution

25mM Phosphate buffer @pH 7
3% Gluteraldehyde
@25mls = 6ml 0.1M PB, 3ml of 25% glut.

A. Fixation

1. Fill tube with fixative solution.
2. Cut tissue and place in tube with solution.
3. Place tube on its side to keep the air away from the tissue.
4. Incubate at 4oC for 12-24 hrs (overnight is good).

B. Osmium tetroxide step

1. Buy 0.5g capsules.
2. Place 25mls of water in a bottle.
3. Put capsule in bottle and break it open with a glass pipette. This makes about a 2% solution.
4. Let solution sit at room temperature to dissolve.
5. The solution can be stored in the cold room or freezer for about 1 month. If frozen, thaw at room temperature. This solution should be straw coloured. If it is purple it is no longer good.
6. Dilute to a 1% solution in 25mM phosphate buffer (25mM is the FINAL concentration of PB!).
7. Pour off the fixative and add the 1% osmium tetroxide solution.
8. Incubate in the cold room overnight to several days. The osmium turns black.

C. Dehydration of tissue

1. Pour off osmium solution and rinse 3 times with 25mM PB. Be sure to put the first wash into the osmium waste.
2. Put tissue through an alcohol series. 15 to 30 mins each step.
   a. 30%
   b. 50%
   c. 65%
   d. 75%
   e. 89%
   f. 95%
   g. 100%
   h. 100%
   i. 100%
   j. 100% overnight
   k. 2 more 100% soak next day

   The tissue can be stored in 100% alcohol permanently.

D. Critical point drying

1. Put tissue into baskets.
2. Fix with alcohol.
3. Dry in the SEM facility.