# SEM Staining Protocol

1. **Fix tissue with 2% Glut/ 2% PFA for tissue, 2-2.5% glut for cells**
2. **Rinse with buffer until all Glut is gone**
3. **1% Osmium for 1hr at RT ( this is Hazordous waste). If there is a lot of collagen do Tannic acid**
4. **Rinse with PBS – this is hazardous waste**
5. **0.45um Syringe filter, 1% Glut / 1% tanic acid in PBS for 1hr at RT**
6. **Rinse well with PBS**
7. **1% Osmium at RT for 1hr**
8. **Rinse well with PBS**
9. **1% Glut / 1% tannic acid in PBS for 1hr at RT**
10. **Rinse well with PBS**
11. **1% Osmium at RT for 1hr**
12. **Rinse well with PBS**
13. **70 % EtOH 15 mins x2**
14. **95% EtOH 15 mins x2**
15. **100% EtOH 20 mins x2 for cells, 30 mins x2 for tissue.**
16. **Critical point dry**
17. **Mount**
18. **Sputter coat**
19. **Silver paint**

**Osmium in small benchtop fridge**

**Tannic acid on chemical shelves**

## Critically Point Dry

**Place sample in an appropriate holder, DO NOT USE permonox, thermonox or polystyrene.**
**Sample may shrink up to 60 %**
**Put some EtOH into chamber so saples don't dry out. Make sure no EtOH on rubber.**
**Finger tight valves, then tighten fully, tighten again**
**Turn machine on, turn on bone dry CO2,**
**Open cool to -5C, open fill.**
**Vent to remove EtOH, close vent, fill and re-cool, open fill.**
**Rest for 5mins**
**Flush x2. All valves closed.**
**Turn heat on – go above critical point indicated by red line. When pressure reaches 1500 it self vents. Let cycle x2.**
**When finished turn heat off, and vent real slow until pressure reaches 250, then open all the way.**
**Move each screw a little, to release evenly, until they are all off.**
**Power off, put screws on loose.**

## Mount

**Need Brush , standard carbon adhesive tabs, acetone, stubs, pen ,forceps.**

**Label stub and clean with acetone.**
**Stick on tab, use a razor to remove excess.**
**Place sample on adhesive tab, press around the edge.**
**Sputter coat**
**Vortex silver paint, and coat around the edges**