

SEM Processing Protocol

Lydia-Marie Joubert, lydiaj@stanford.edu

Fix samples 4hrs to overnight in 2%Glutaraldehyde with 4%Paraformaldehyde (PFA) in 0.1M Na Cacodylate Buffer (pH 7.3). Samples should be either cut to appropriate sizes, grown on 12-25mm coverslips, or centrifuged to concentrate cells. Cover samples completely in abundant fixative, never allow to dry out. Keep samples at 4C
BRING TO LAB ON ICE

Wash 5 min in same buffer

Post-fix 1-2 hrs in 1% OsO₄ (Osmium Tetroxide) – Highly toxic!! Use in hood only!!

Wash 2x 5 min in mQ H₂O

Dehydrate in increasing concentrations of ethanol: 30-50-70-90-100-100 % (10-30 min each, depending on sample size).

If you need to keep overnight, keep in 70% EtOH.

CPD (Critical Point Dry) in 100% EtOH with liquid CO₂, **OR** dry with HMDS

Put samples with double-sided carbon tape, or Colloidal Graphite, on **Al stubs**.

Sputter-coat with Pd/Au - 100Å layer ~10 min with the Denton apparatus in lab

Supplies needed (for Training Sessions you can get all supplies from CSIF)

Suppliers:

Electron Microscopy Sciences <http://www.emsdiasum.com/microscopy/default.aspx>

Ted Pella <http://www.tedpella.com/>

Fixatives:

16% PFA (EMS Cat #15710)

8% Glut (EMS Cat #16020) with

0.2M NaCacodylate (EMS CAT #11652)

Mix equal volumes (1 vial each) of PFA and Glut with 20 ml Buffer for the required fixative concentration

2% OsO₄ (EMS Cat #19192) – dilute with water to 1%conc.

HMDS (Hexamethyldisilazane) (EMS Cat #16700)

Colloidal Graphite: Ted Pella #16053

Specimen Mounts: Ted Pella #16111 (1/2 dm slotted head)

Carbon conductive Tape: Ted Pella #16073

Pelco Tabs12mm: Ted Pella #16084-1

Pelco Tabs 25mm Ted Pella #16084-2

Carbon conductive sheet (x10): Ted Pella #16085-1

Round Coverslips (12mm) Ted Pella #26023

