

Preparation of Tissue for Scanning Electron Microscopy

1. Fix tissue in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer pH 7.3 with 3mM CaCl_2 .
2. Rinse 0.1M cacodylate buffer
3. Fix in 1% OsO_4 in 0.1M cacodylate.
4. Rinse in cacodylate buffer, then transfer to distilled/deionised water
5. Saturated aqueous solution of thiocarbohydrazide (0.5% in water, stirred, filtered just before use)
6. Rinse water
7. 1% aqueous OsO_4
8. Rinse water
9. Repeat steps 5-7.
10. Dehydrate in ethanol series to dry 100% ethanol
11. Critical point dry from liquid CO_2 .
12. Mount on SEM stubs using silver paint.
13. Sputter coat.

Supplies:

- Glutaraldehyde solution (25%): 18426 from Ted Pella; unless MBL has already
- Sodium cacodylate
- CaCl_2
- OsO_4 ; 4% aqueous solution – 18459 (10x2ml) from Ted Pella (or MBL)
- Thiocarbohydrazide (from Sigma; No. T-2134)
- Dry ethanol
- Specimen support stubs for SEM (For JEOL 840 – probably 12.5 mm: 16232, pack of 50, from Ted Pella or from MBL [but I am checking on stub size with Louie Kerr at MBL])
- Silver paint (16040-30 from Ted Pella)