Fixation and embedding of cell pellets prepared from monolayer cultures

- Fix 0.5% glutaraldehyde in 0.2M PIPES, PBS or 0.1M cacodylate buffer pH 7.4 30 min
- Wash x3 in 0.1M PIPES buffer pH 7.4 over 10 min
- Scrape the cells in 1ml PIPES and break up sheets by pipetting through a plastic 1ml pipette tip
- Centrifuge at 15,000-20,000 xg 15 min
- Cut pellet into 0.5mm (or smaller) blocks
- Post-fix pellets in 1% OsO4 in 0.1M cacodylate buffer pH 7.2 30 min

If membrane contrast is needed then use reduced osmium tetroxide: 1% OsO4/1.5% potassium ferrocyanide (made by mixing 2 parts of 2% OsO_4 H_2O, 1 part O.4M cacodylate buffer pH 7.2 and 1 part 6% KFeCN)*

- Wash in cacodylate buffer three times for a total of 10 min
- Wash in distilled water three times for a total of 10 min

Can be done in plastic tubes up to this point but should be transferred into glass vials

- Ethanol 70,90,100,100 and 100% each for 10 min
- Propylene oxide washes x2 each for 10 min
- Epoxy resin/Propylene oxide 50/50 v/v at least 1hour
- Epoxy resin 100% 2 hr or longer
- Epoxy resin embedding in beam or gelatin capsules
- Polymerisation 60°C 1 day/overnight

increases in contrast may be achieved by following osmium tetroxide with
(1) 0.5% w/v magnesium uranyl acetate in water (1hour) (so called en-bloc staining) or

(2) 1% w/v tannic acid (30min) followed by a 10 minute rinse in 1% w/v sodium sulphate both in 0.1M cacodylate buffer pH7.2, followed by washes in distilled water and incubation in 0.5% w/v magnesium uranyl acetate in water (1hour). These modifications enhance the contrast of strucutres such as cytoplasmic filaments, ribosomes and vesicle coats.