

## OTO Preparation of tissue culture cells for SEM

### Osmium – Thiocarbohydrazide (Kelley et al 1973)

1. Fix sample for routine EM prep (2.5% glutaraldehyde in 0.1 M phosphate buffer)
2. Post-fix in 1% OsO<sub>4</sub>
3. Rinse in buffer 5 – 10 times
4. Incubate in fresh 1% aqueous thiocarbohydrazide for a minimum of 10 minutes
5. Rinse 5 – 10 times over 15 minutes with distilled water
6. Incubate in 1% OsO<sub>4</sub>
7. Rinse 5 – 10 times over 15 minutes with distilled water
8. Dehydrate through an ethanol, cellosolve or iso-amyl acetate series
9. Critical point dry and mount

### Preparation of Thiocarbohydrazide

1. Heat .5 g TCH in 25 ml water to 60°
2. Cool to room temperature about 1 hour
3. Protect from light