Fixation and embedding of cells grown on plastic petri dishes (using propylene oxide).

- Remove the medium
- Aldehyde fixation in PBS, 0.1M cacodylate, 0.2M PIPES or HEPES pH7.2 30 min
- Wash in 0.1M cacodylate buffer 3 times during 15 min
- 1% w/v OsO_4 in cacodylate buffer (2 mls per 5.5cm dish) 30 min
- Wash in cacodylate buffer 3 times during 15 min
- Wash in distilled water 3 times during 15 min
- Dehydration in 70,90,100,100,100% ethanol each for 10 min
- Remove the cells from the dish with propylene oxide (pipette the solvent over the cells until they come off in sheets). At this point the sheets can either be left intact* or broken into small pieces by pipetting before centrifugation and pelleting.
- Wash cells once with propylene oxide to remove solubilised plastic
- Centrifuge at 14,000 xg for 3 min
- Resuspend pellet in propylene oxide/Epoxy resin ratio 1:1 (v/v) in a beam capsule pushed into the top of a plastic Eppendorf tube.
- Pellet at 14,000xg 2 mins and resuspend in Epoxy resin.
- Repeat centrifugation as short 10 sec bursts at 14,000xg to place evenly at the bottom of the beam capsule
- Polymerise the resin
- If cell sheets are left intact they can be handled with a glass rod through the dehydration and infiltration procedure and embedded flat for sectioning.