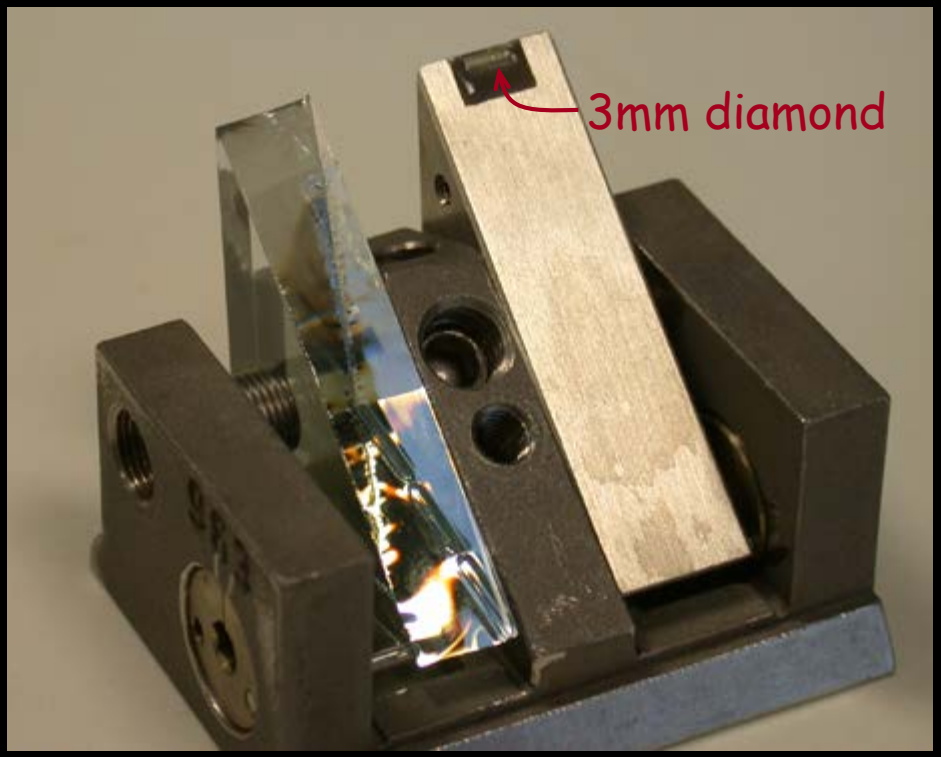
Ultramicrotomy

Sections:
50-70 nm thick
0.1-0.5 mm square

Knives
Glass
Diamond

Resin
good morphology

Cryo
good immuno-labelling



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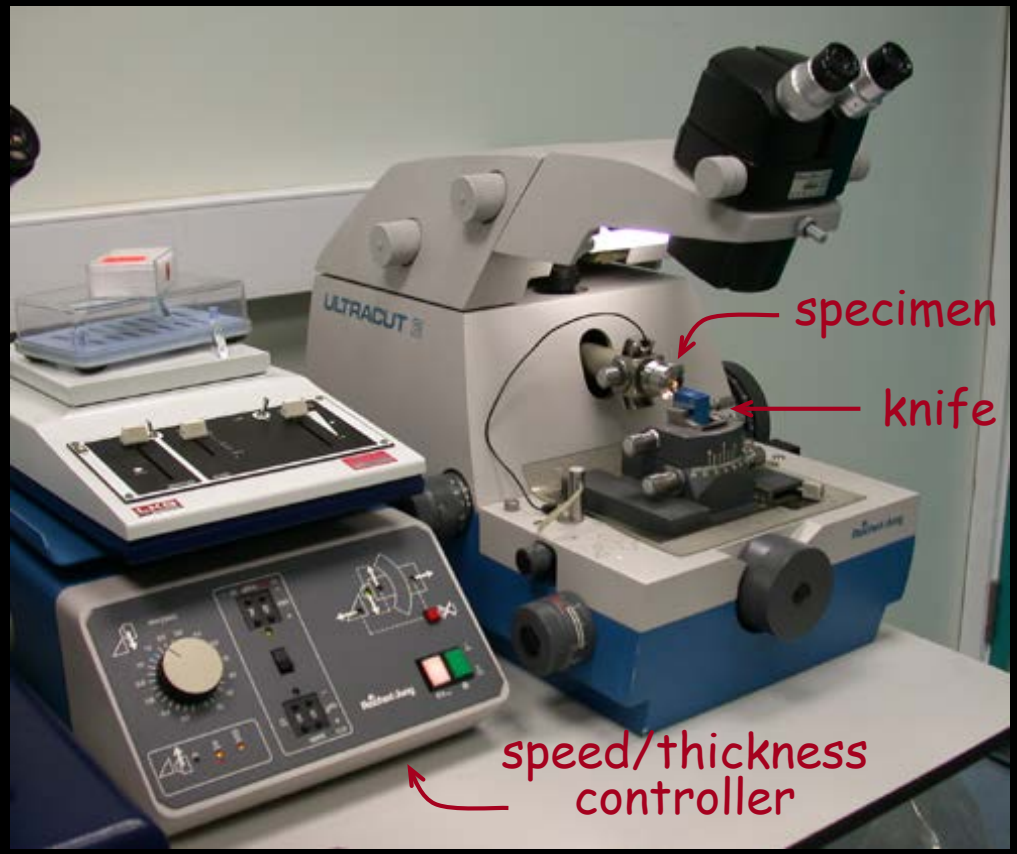


Processing for resin sectioning

Sample:

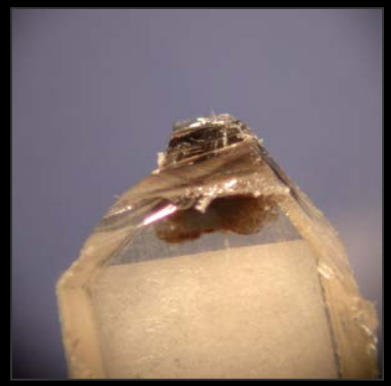
eg. cells in 1 x 6cm dish. Final pellet ≤1mm^3

Protocol:

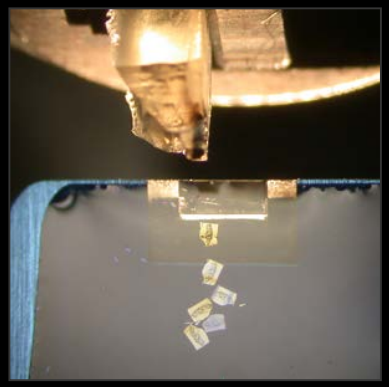
1.	Fix (4% paraformaldehyde, 2.5% glutaradehyde)	4hrs-o/n
2.	Wash and quench (50mM glycine)	5 min
3.	1% OsO_4	1.5hrs
4.	5% alcoholic uranyl acetate	1.5hrs
5.	Dehydrate (50%, 70%, 90%, 100% ethanol, 100% acetone)	1.5hrs
6.	Infiltrate (araldite resin; 30:70, 70:30, 100%)	4 hrs
7.	Polymerise 60°C	o/n

Processing for resin sectioning

1. Trim block (≤0.3mm^2 face)



2. Sectioning 50-70nm (silver/gold colour)



3. Collect sections on EM grid