Nanoscale Biomedical Imaging Facility
(CMEM sub-core)
Standard Operating Procedures

Procedure: Standard fixation and embedding

Purpose: To prepare sample blocks for sectioning and observation in the transmission electron microscope.

Method: Place the tissue in a drop of fixative and chop into 2mm cubes. The tissue is fixed, rinsed, post-fixed, rinsed, dehydrated, and infiltrated according to the following schedule. After infiltration with resin the sample pieces are placed in embedding molds and polymerized in an oven at 65 C overnight

Step	*Solution*	*Time*
1	2% glutaraldehyde in 0.1M sodium cacodylate buffer pH 7.3	> 2 hrs
2	0.1M sodium cacodylate buffer with 0.2M sucrose pH 7.3	10 min
3	1% osmium tetroxide in 0.1M sodium cacodylate buffer pH 7.3	1.5 hrs
4	0.1M sodium cacodylate buffer with 0.2M sucrose pH 7.3	10 min
5	70% ethanol	10 min
6	90 % ethanol	10 min
7	100% ethanol	10 min
8	100% ethanol	10 min
9	100% ethanol	10 min
10	propylene oxide	10 min
11	propylene oxide	10 min
12	50/50 propylene oxide/Spurr resin	1 hr
13	Spurr resin	1 hr
14	Spurr resin	overnight

The plastic blocks are sectioned (90nm thick) on an ultramicrotome using a diamond knife. The sectioned are collected on 200 mesh copper grids, stained with uranyl acetate and lead citrate.