

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

Procedure: Standard fixation for scanning electron microscopy

Purpose: To prepare samples for observation in the scanning electron microscope.

Method: Place the tissue in fixative. The tissue is fixed, rinsed, post-fixed, rinsed, and dehydrated according to the following schedule. After dehydration the samples are critical-point dried, attached to aluminium stubs and gold sputter-coated.

<i>Step</i>	<i>Solution</i>	<i>Time</i>
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	20 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	20 min
5	70% ethanol	20 min
6	90 % ethanol	20 min
7	100% ethanol	20 min
8	100% ethanol	20 min
9	100% ethanol	20 min
10	Critical point dry	
11	Mount on stubs	
12	Sputter coat	