Standard SEM Sample Preparation



Procedure for fixation and dehydration of samples for Scanning Electron Microscopy

**Protective Equipment**; Safety glasses, Lab coat, gloves and Chemical Fume hood

Remember to **label all vials** with; sample name, fixative type, your name & date

Primary Fixation: 

2- 4% Paraformaldehyde/2% - 3% Glutaraldehyde Overnight, 4° C

 in buffer\*

Rinse: With buffer 3x 5 – 10 min ea

Secondary fixation (optional):

Osmium tetroxide (1-2%) 2% -1h room temp (r.t.),

 Do not use PIPES buffer with Osmium Or 1% - overnight 4° C

Rinse: With ddH2O after OsO4 3x 5 -10 mins each

(If freeze dring after ddH20 rinses - See freeze dry protocol and contact staff for help)

Dehydration: 

 30% Ethanol 10 mins, r.t.

 50% Ethanol 10 mins, r.t.

 70% Ethanol 10 mins, r.t.

 90% Ethanol 10 mins, r.t.

 100% Ethanol 3x, 10 mins ea, r.t.

Final Drying options;

- check with staff for which procedure applies and for help/training.

* Freeze dry – immediately after water rinses, do not dehydrate with ethanol
* HMDS   - animal & microbial samples
* CPD (critical point dry – plant, animal, microbial

\*Buffers - The buffer used in the fixative is determined by the tissue type.

 The most commonly used buffers in our lab are the following;

 0.05 M PIPES buffer (usually used with plant tissues)

 0.1 M Phosphate buffer

 0.2 M Cacodylate buffer 