

PROTOCOL:

SEM Biological HMDS Processing

Processing biological samples for secondary electron (SE) sampling in the Scanning Electron Microscope (SEM) requires the removal of fluids from their matrix. This is typically done using Critical Point Drying (CPD) or Freeze Drying (FD). Hexamethyldisilazane (HMDS), a chemical with extremely low surface tension, is a great alternative approach to CPD or FD for SEM samples. HMDS's low surface tension allows previously fixed and dehydrated samples infiltrated with HMDS to air dry without the artifacts typically associated with air drying.

Setup requirements

- Get ORGANIZED!! Have equipment and solutions ready to go because this process moves rapidly!
- Setup requirements for microwave if appropriate.
- Program microwave for desired processing times.
- Setup water load and reference temp probe.
- Setup vacuum chamber and/or agitation if needed.

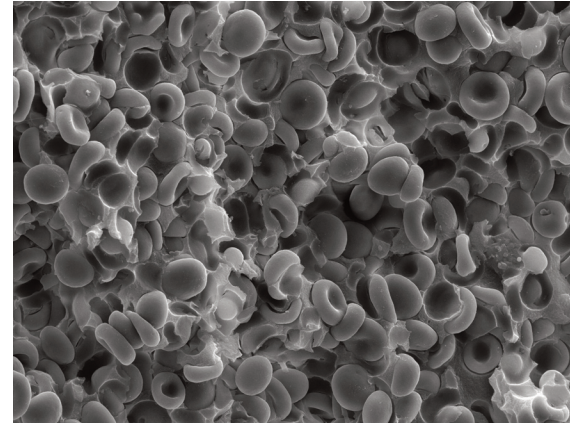
PROCEDURE:

NOTE: All of the following steps can be carried out in the 1.7 ml microfuge tubes, scintillation vials, or Petri dishes.

Step	Temp.	Time for Method Used		
		Microwave	Benchtop (ambient)	
1. Initial fixation (Karnovsky's)	37°C	2:30 min.	2 hr.	
2. Buffer rinse — 3 changes	37°C	60 sec. ea.	10 min. ea.	
3. 2-4% OsO ₄ ** in DI water	37°C	2:30 min.	2 hr.	
** Sometimes 2% Potassium Permanganate in DI is used for plants and bacteria.				
4. Water rinse — 3 changes	37°C	60 sec. ea.	10 min ea.	
5. Ethanol dehydration	a. 50%	45°C	60 sec.	10 min.
	b. 70%	45°C	60 sec.	10 min.
	c. 80%	45°C	60 sec.	10 min.
	d. 90%	45°C	60 sec.	10 min.
	e. 100%	45°C	60 sec.	10 min.
	f. 100%	45°C	60 sec.	10 min.

Transfer to Critical Point Drying holders immersed in ETOH if appropriate

6. Infiltration — ETOH : HMDS				
Plant	3:1	Room Temp.	No Microwave HMDS	15 min.
	2:1	Room Temp.		30 min.
	1:1	Room Temp.		30 min.
	100% HMDS	Room Temp.		30 min.
	100% HMDS	Allow to evaporate in fume hood with no fan on overnight		



Mouse Lung Tissue: Trachea area. Red blood cells inside blood vessel in the lung.

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EMS Catalog supplies used

Cat. No.

Fixatives

Karnovsky's Fixative EM Grade	15732-10
2% OsO ₄ Aqueous Solution	19172

Buffers

Phosphate	19340-72
Cacodylate	11652

Dehydrants

Ethanol	15055
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HMDS

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