

SEM Biological HMDS Processing

Endless Possibilities ...

## 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.

## Set Up

- **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.

**EMS Catalog Supplies** 

3ciences

**HMDS 16700** 

## The Procedure

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

STEP		Temperature	Time for Method Used		
			Microwave	Bench Top (ambient)	1
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 <sub>4</sub> ** in DI water		37°C	2:30 min.	2 hr.	1
**Sometimes	s 2% Potass	ium Permanganate	e in DI is used for	plants and bacteria.	
4. Water rinse - 3 c	changes –	37°C	60 sec. ea.	10 min ea.	nC
5. Ethanol dehydration	on a. 50%	45°C	60 sec.	10 min. 50\	8/,
VIICLOS	b. 70%	45°C	60 sec.	10 min.	
*LOU W	c. 80%	45°C	60 sec.	10 min.	
	d. 90%	45°C	60 sec.	10 min.	
077	e. 100%	45°C	60 sec.	10 min.	
Election	f. 100%	45°C	60 sec. 8	10 min	
Transfer to Critical P	oint Drying h	olders immersed i	n ETOH if approp	riate	
6. Infiltration – ETOH : HMDS				201	
Plant 3:1	07	Room Temperature	No Microwave	15 min.	
2:1		Room Temperature	HMDS	30 min.	
114/10		Room Temperature	18 Electron,	30 min.	1
100% HMDS		Room Temperature		30 min.	
100% HMDS		Allow to evaporate in fume hood <i>with no fan on</i> overnight			~1
	1. Initial fixation (Kar 2. Buffer rinse - 3 3. 2-4% OsO <sub>4</sub> ** in D  **Sometimes 4. Water rinse - 3 o 5. Ethanol dehydratio  Transfer to Critical P 6. Infiltration - ETOH Plant 3:1 2:1 1:1 100% HMDS	1. Initial fixation (Karnovsky's)  2. Buffer rinse - 3 changes -  3. 2-4% OsO <sub>4</sub> ** in DI water  **Sometimes 2% Potass  4. Water rinse - 3 changes -  5. Ethanol dehydration a. 50%  b. 70%  c. 80%  d. 90%  e. 100%  f. 100%  Transfer to Critical Point Drying R  6. Infiltration - ETOH: HMDS  Plant 3:1  2:1  1:1  100% HMDS	1. Initial fixation (Karnovsky's) 37°C  2. Buffer rinse - 3 changes - 37°C  3. 2-4% OsO <sub>4</sub> ** in DI water 37°C  **Sometimes 2% Potassium Permanganate  4. Water rinse - 3 changes - 37°C  5. Ethanol dehydration a. 50% 45°C  b. 70% 45°C  c. 80% 45°C  d. 90% 45°C  e. 100% 45°C  f. 100% 45°C  Transfer to Critical Point Drying holders immersed in  6. Infiltration - ETOH : HMDS  Plant 3:1 Room Temperature  2:1 Room Temperature  1:1 Room Temperature  1:00% HMDS  Room Temperature	1. Initial fixation (Karnovsky's) 37°C 2:30 min.  2. Buffer rinse — 3 changes — 37°C 60 sec. ea.  3. 2-4% OsO <sub>4</sub> ** in DI water 37°C 2:30 min.  **Sometimes 2% Potassium Permanganate in DI is used for  4. Water rinse — 3 changes — 37°C 60 sec. ea.  5. Ethanol dehydration a. 50% 45°C 60 sec.  b. 70% 45°C 60 sec.  c. 80% 45°C 60 sec.  d. 90% 45°C 60 sec.  e. 100% 45°C 60 sec.  f. 100% 45°C 60 sec.  Transfer to Critical Point Drying holders immersed in ETOH if approping forms and the second proping forms are presented in ETOH if approping forms and the second proping forms are presented in ETOH if approping forms and the second proping forms are presented in ETOH if approping forms are pre	STEP

