Biological Sample Fixation for SEM

Reagents:

2% BUFFERED GLUTARALDEHYDE
5.96 grams HEPES in 10 ml 50% glutaraldehyde. Bring to 250 ml with DI water.

1-2% OSMIUM TETROXIDE
4 ml 4% Osmium tetroxide, 4 ml 0.4M HEPES (23.83 g in 250 ml DI water), and 8 ml DI water, PH to 7.0.

0.1M HEPES BUFFER
5.96 grams HEPES in 250 ml of DI water, PH to 7.0

50%, 70%, 95% and 100% ETHANOL

Hexamethyldisilazane (HMDS)

Protocol:

1. Fix sample with 2% buffered glutaraldehyde, let sit for overnight. Fixative should be 10 to 20 times the volume of the sample.

2. Rinse with 0.1 M HEPES buffer 3 times for 5 minutes each with gentle agitation.

3. If the sample is tissue: Post fix in 1-2% Osmium tetroxide for 1 hour. You can skip this step for non-tissue sample.

4. Alcohol series dehydration:
    1. 50 % ethanol, 2 times for 10 minutes each with agitation.
    2. 70 % ethanol, 2 times for 10 minutes each with agitation.
    3. 95 % ethanol, 2 times for 10 minutes each with agitation.
    4. 100 % ethanol, 3 times for 15 minutes each with agitation.

5. Drying: Choose either Critical Point Drying (CPD) or Chemical Drying

    Option 1: Critical Point Drying with Tousimis Autosamdri-931

    Option 2: Chemical Drying
    1. (2 parts 100% EtOH : 1 part HMDS) for 15 minutes.
    2. (1 part 100% EtOH : 1 part HMDS) for 15 minutes.
    3. (1 part 100% EtOH : 2 parts HMDS) for 15 minutes.
    4. HMDS alone for 15 minutes, 3 times.
    5. Let the last HMDS evaporate in a fume hood overnight.

6. Mount samples on specimen stubs, sputter coat with 10 nm Au/Pd and imaging.