

PROCESSING CELLS FOR SEM

1. Rinse cells 2-3 times with either serum-free medium or 0.1M PBS at 37°C to remove serum from the cell medium.
2. Fix the cells with 2.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.2 at 37°C. Wear gloves, and work in a fume hood when using glutaraldehyde. It will fix any tissue it comes in contact with, including your eyes and airways. After placing the cells in fixative, place the culture dish in a vapor tight container (Tupperware) in the refrigerator to fix overnight at 4°C.
3. Rinse the cells, 3x 10 minutes each, in the above buffer. Briefly rinse the cells in distilled water, 2 rinses, 2 minutes each, to remove buffer salts from samples.
4. Dehydrate the cells through a graded series of ethanol, 5-15 minutes per change, depending on if the cells were grown on Petri dishes, cover slips, or extracellular matrix. Coverslips in Petri dishes trap fluid between the coverslip and the dish they are in, requiring longer dehydration times. It may be possible to gently move the coverslips in their dishes to insure fluid flow under them, in which case 5 minutes time in the lower grades of ethanol will be sufficient. Extracellular matrix may be thick, and require extended dehydration times in each step.
 - 35% ethanol in distilled water 5-15 minutes
 - 70% ethanol in distilled water 5-15 minutes
 - 95% ethanol in distilled water 5-15 minutes
 - 100% ethanol 3 changes, 10-15 minutes each
5. Pipette off the last change of ethanol, and replace it with 100% Hexamethyldisilazane (HMDS). HMDS should be handled while wearing gloves, and used in the fume hood. It is caustic and an airway irritant. Allow the cells to soak in HMDS for 10-15 minutes. Repeat this step with fresh HMDS, again for 10-15 minutes.
6. Pipette off all of the HMDS from the dish. Air dry the sample with the dish tilting vertically to keep the sample dry. With matrices, it may be necessary to dry them in a vacuum, or in a 45°C oven for a few hours. With coverslips in Petri dishes, dry them overnight under vacuum to insure all of the fluid under the coverslips dries.