

SEM Fixation Procedure - Cells on nanofiber scaffolds*

1. Rinse your samples with fresh buffer (e.g PBS) in the fume hood – repeat three times.
2. Fix cells in a 4% paraformaldehyde (or 10% formalin) in PBS solution for 30 minutes at room temperature.
3. Rinse with PBS 1 X 5 minutes.
4. Dehydrate in graded ethanol series:
 - a) 50% EtOH balance with distilled water 1 X 10 min.
 - b) 70% EtOH balance with distilled water 1 X 10 min.
 - c) 80% EtOH balance with distilled water 1 X 15 min.
 - d) 95% EtOH balance with distilled water 1 X 15 min.
 - e) 100% EtOH 2 X 15 min.
5. Hexamethyldisilazane (HMDS) chemical drying series:
 - a) 3:1 EtOH:HMDS 1 X 15 min.
 - b) 1:1 EtOH:HMDS 1 X 15 min.
 - c) 1:3 EtOH:HMDS 1 X 15 min.
 - d) 100% HMDS allow to air dry.

Note: All HMDS steps need to be carried out in the fume hood wearing the necessary personal protection gear as it is highly toxic.
6. Mount on SEM stub and sputter coat.

*Suggested procedure, please adjust according to your experimental needs.