

**Protocol graciously provided by: Dr. Mariano Viapiano, Viapiano Lab protocols (© 2006-2014)**

**N. Fixation Procedure for SEM (cells on nanofiber scaffolds)**

1. Rinse any residual protein off of surface with PBS 1 X 5 min (optional)
2. Fix cells in 4% paraformaldehyde (or 10% formalin) in PBS 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 5 min
  - b. 70% EtOH balance with distilled water I X 5 min
  - c. 80% EtOH balance with distilled water 1 X 10 min
  - d. 95% EtOH balance with distilled water 1 X 10 min
  - e. 100% EtOH 2 X 10 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
6. Mount on SEM stub and sputter coat