# Directions for Processing Biological SEM Samples

**NOTE: CARRY OUT ALL WORK INVOLVING FORMALDEHYDE, TETRAMETHYLSILANE, and $OsO_4$ IN CHEMICAL FUME HOOD. These are hazardous chemicals, see MSDS. Exposure to $OsO_4$ can cause blindness and death**.

**NOTE**: All wash and stain volumes are at least twice the volume of the sample. 0.1M sodium cacodylate buffer can be substituted for the 1x PBS.

1. Fixative is removed and the sample is washed twice with 1x PBS, at least 10 minutes each wash.
2. Remove 1x PBS. 1% $OsO_4$ is added to the sample to completely cover. The sample is sealed and covered ($OsO_4$ is light sensitive). Stain for one hour.
3. Remove the $OsO_4$ and rinse with 1x PBS, 3 times at least 10 minutes each time.
4. After removing the last buffer rinse, wash twice with 30% ethanol, at least 10 minutes each time.
5. Remove the 30% ethanol and wash twice with 50% ethanol, at least 10 minutes each time.
6. Remove the 50 % ethanol and wash twice with 70% ethanol, at least 10 minutes each time.
7. Remove the 70% ethanol and wash twice with 90% ethanol, at least 10 minutes each time.
8. Remove the 90% ethanol and wash three times with 100% ethanol (200 proof), at least 10 minutes each time.
9. Remove the 100% ethanol and add Tetramethylsilane (TMS). Wash 3 times with TMS, at least 10 minutes each.
10. Remove the TMS and allow samples to dry in the hood for at least 10 mins. Place samples in desiccator for storage or coat samples for imaging. See SOP on operation of the Bal-Tec sputter coater.