

SEM PROTOCOL

Gloves must be worn throughout this procedure. All work must be done in a fume hood.

- Put perfusion fixed tissue in 2.5% glutaraldehyde in 1x PBS (pH 7.4) for 60 minutes.
- Cut up or pin tissue for proper orientation.
- Wash thoroughly in 3 changes 1x PBS for 15 minutes each.
- Fix tissue in 1% OsO₄ for 60 minutes.
- Wash thoroughly in 3 changes 1x PBS for 15 minutes each.
- Dehydrate in graded series of alcohol for 15 minutes each:
 - 30% ethanol
 - 50% ethanol
 - 70% ethanol
 - 90% ethanol
 - 100% ethanol x 3

Critical point dry (See Critical Point Drying Procedure) specimen or dehydrate in Hexamethyldisilazane (HMDS) for 15 minutes and allow to air dry.

- Mount on studs.
- Sputter coat specimen (See Operation of the Cressington Sputter Coater).
- Store in desiccator.