Preparation of Cell on a Coverslip for SEM

1. Fix coverslips for one hour or as long as overnight with 2.5 % glutaraldehyde in 0.1 M Sorensen’s phosphate buffer, pH 7.4.

2. Rinse three times for 15 minutes each with 0.1 M Sorensen’s buffer.

3. Post fix for one hour at 4°C in 1 % osmium tetroxide in 0.1 M Sorensen’s buffer.

4. Rinse three times with 0.1 M Sorensen’s phosphate buffer, 15 minutes each.

5. Dehydrate for 5 minutes each in 30, 50, 70, 80, 90, 95 and two changes of 100 % ethanol or acetone.

6. 2 changes of Hexamethyldisilizane (HMDS) for 10 minutes each.

7. Remove all but a few drops of HMDS and let air dry in the hood overnight.

8. Mount on aluminum stub with conductive paint and let outgassing of adhesive overnight.

9. Sputter coat with gold.

10. Image them on an Amray 1910 FE-SEM.