High Resolution Electron Microscopy Facility

Protocol for Scanning Electron Microscopy

***For grant or paper submission**

Fixed samples containing 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.3) were washed with 0.1M cacodylate buffer (pH 7.3) and then post-fixed with 1% cacodylate buffered osmium tetroxide. Samples were washed with 0.1M cacodylate buffer and then with distilled water. Samples were then sequentially treated with Millipore-filtered 1% aqueous tannic acid, washed in distilled water, treated with Millipore-filtered 1% aqueous uranyl acetate, and then rinsed thoroughly with distilled water. The samples were dehydrated with a graded series of increasing concentrations of ethanol and then transferred to graded series of increasing concentrations of hexamethyldisilazane (HMDS) and then allowed to air dry overnight. Samples were mounted on double-stick carbon tabs (Ted Pella Inc., Redding, CA), that had been previously mounted to aluminum specimen mounts (Electron Microscopy Sciences, Ft. Washington, PA). The samples were then coated with platinum under vacuum using a Balzer MED 010 evaporator (Technotrade International, Manchester, NH) until they reached a thickness of 25 nm. Samples were then flash carbon coated under vacuum and transferred to a desiccator for examination at a later date. Samples were examined with a JSM-5910 scanning electron microscope (JEOL, USA, Inc., Peabody, MA) at an accelerating voltage of 5 kV.