

High Resolution Electron Microscopy Facility

Protocol for Transmission Electron Microscopy (TEM)

*For grant or paper submission

Samples were fixed with a solution containing 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.3). Samples were then washed in 0.1M sodium cacodylate buffer and treated with 0.1% Millipore-filtered cacodylate buffered tannic acid, post-fixed with 1% buffered osmium and stained en bloc with 0.1% Millipore-filtered uranyl acetate. The samples were dehydrated in increasing concentrations of ethanol and then infiltrated and embedded in LX-112 medium. The samples were then polymerized in a 60°C oven for approximately three days. Ultrathin sections were cut using a Leica Ultracut microtome (Leica, Deerfield, IL) and then stained with uranyl acetate and lead citrate in a Leica EM Stainer. The stained samples were examined in a JEM 1010 transmission electron microscope (JEOL USA, Inc., Peabody, MA) using an accelerating voltage of 80 kV. Digital images were obtained using an AMT imaging system (Advanced Microscopy Techniques Corp., Danvers, MA).