

(Submission Date)

(Name of primary investigator)

(Tissue type, Case number, special characteristics)

1. Tissue Processing

Primary fixation	Fix tissue segments in 2% glutaraldehyde, 2.5% formaldehyde in 0.1M Na cacodylate buffer, pH 7.4, for 2 h.
Wash	Wash at least 3X in 0.1M Na cacodylate, pH 7, 20 min each.
Post-fixation	1% OsO ₄ with 0.5% K ₄ Ferro(CN) ₆ in 0.1M Na cacodylate buffer for 1 h at RT followed by 1 rinse in buffer then water.
Dehydration	50, 75, 80, 90, 95% ethanol, 10 min each, 100% ethanol, 5 min X 3 Propylene oxide X 2
Infiltration	1:2 Epoxy:PO 2 h (Epoxy = Epon Araldite) 1:1 Epoxy:PO 2 h 3:1 Epoxy:PO 2 h 100% Epoxy 2 changes ON on rocker.
Polymerization	Transfer 5 segments to fresh epoxy beds in BEEM caps, heat cure at 65C for 2 days.

2. Ultramicrotomy, post stain thin sections
3. TEM examination, photography
4. Film development, Scan and post images for PI