Cryo EM tissue preparation.

1. Fix tissue mildly. Trim into small (<1mmsq) blocks.
2. Infuse tissue with a cryoprotectant for at least one hour (PVP/ sucrose).
3. Mount onto cryo pins, and freeze rapidly in liquid nitrogen.
4. Store pins in cryo vials in liquid nitrogen dewar.

Immunolabeling Experimental protocol.

1. Cut ultra thin sections (50-80nm)
2. Pick up section with sucrose drop.
3. Place on formvar / carbon coated Nickel grid.
4. Wash off excess sucrose.
5. Block for nonspecific binding with 10% FCS 1 hour.
6. Place grid section side down on 10ul drop of diluted antibody. for several hours / overnight.
7. Wash with blocking buffer.
8. Incubate grids section side down on secondary antibody, (5-10nm IgG colloidal gold).
9. Wash blocking buffer/ wash water.
10. Stain 20 mins with neutral UA.
11. Wash with water.
12. Stain and embed section in a monolayer on PVA / Methyl cellulose.
Let dry and scope.

Protocol taken from the W.M. Keck Microscopy Facility at The Whitehead Institute website