**Animal Protocols**

**Fixation**

1. Fix tissue in 2.5% Glutaraldhyde in 0.1M cacodylate buffer pH 7.3-7.4 at 22 C
   * Perform under Vacuum
   * Power level 1 (about 100W)
   * 2 min on, 2 min off, 2 min on
   * Repeat without changing

**Buffer rinse**

1. Rinse in cacodylate buffer at 22 C
   * Do not perform under vacuum
   * Power level 2 (about 200W)
   * 40 sec
   * Repeat three times
   * If using tannic acid with buffer, rinse in butter once without tannic before continuing to osmium step.

**Osmium Tetroxide Fix**

1. 1% osmium tetroxide in 0.1M cacodylate buffer at 22 C
   * Perform under vacuum
   * Power level 1 (about 100W)
   * 2 min on, 2 min off, 2 min on
   * Repeat without changing

**Water rinse**

1. Rinse sample in distilled water and change to new water
   * Do not perform under vacuum
   * Power level 2 (about 200W)
   * 40 sec

**Dehydrate**

1. Ethanol at 22 C
   * 50% ETOH
   * 70% ETOH
   * 90% or 95% ETOH
   * 100% ETOH
   * 100% ETOH
   * 100% ETOH
   * Do not perform under vacuum
   * Power level 2 (about 200W)
   * 40 sec

**Critical point dry**

1. Either conventionally in a CPD or in the microwave with Hexamethyldisilizane (HMDS) at 22 C
   * Do not perform under vacuum
   * Power level 2 (about 200W)
   * 40 sec
   * Replace with new HMDS
   * Repeat two more times
   * Place in 60C oven for 5 mins
   * Remove excess HMDS
   * Place back in 60C oven until HMDS has evaporated

No two specimen types are the same. You might have to double the timings for some depending on how difficult it is to penetrate any cuticle and how big they are.