Chemical Fix Protocol
(Danforth Plant Science Center, Integrated Microscopy Facility)

1. Fix in buffered primary fixative. Small tissue pieces/cut under fixative/2% glutaraldehyde (as freshly distilled as possible) in PIPES buffer, pH 6.8, 1.5-2 hours at room temperature.

2. Rinse three times in buffer.

3. Post fix in buffered 2% osmium tetroxide, 1.5 hours.

4. Rinse three times in water.

5. Dehydrate in an ethanol/acetone series. % ETOH: 5,10,20,30,50,75,95; 15-20 minutes each. 30 minutes in 100% ETOH. 15 min in 100% acetone, 45 minutes in a second change of 100% acetone.

6. Infiltrate with resin dissolved in acetone. For plant tissue, Spurr's resin is recommended. Slowly infiltrate in vials laid on their side on a shaker with rotation. Our protocol: 5% 12 h, 10% 12 h, 25% 24 h, 50% 24 h, 75% 24 h, 100% 24 h.

7. Embed: put into a fresh change of resin and polymerize 1-2 days at 60 degrees C.