Chemical fixation

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Instructions for chemical fixation

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1. Excise a piece of leaf and immediately immerse it in fixative solution (2.5% glutaraldehyde +2% prarformaldehyde in cacodylate buffer (0.1M, pH = 7.4)).
2. Cut leaves into small pieces (1x1 mm2) in a big drop of fixative solution in a petri dish.
3. Place the samples into vials containing the fixative solution (the samples will float on the fixative).
4. (can be skipped) apply vacuum to the samples until they are at the bottom of the fixative. Start fixation time once the samples have sunk,
5. Fix the samples first for 2-3 hours at room temperature (to avoid chilling injuries on the tissue) and then transfer the samples to 4C for 24 hours. If the samples were not vacuum filtrated, then the samples should be fixed for ~48 hours.
6. Rinse the sample 3x20mins in 0.1M cacodylate buffer (pH=7.4).
7. Postfix sample in 1% osmium tetroxide in 0.1M cacodylate buffer (pH=7.4).
8. Rinse the sample 3x20mins in 0.1M cacodylate buffer (pH=7.4).
9. Rinse the sample in the following acetone series:
   - 10% acetone 15 mins
   - 20% acetone 15 mins
   - 30% acetone 15 mins
   - 40% acetone 15 mins
   - 50% acetone 15 mins
   - 60% acetone 15 mins
   - 70% acetone 15 mins
   - 80% acetone 15 mins
   - 90% acetone 15 mins
   - 96% acetone 15 mins
   - 100% acetone 3 x 15 mins
10. Make up Spurr′s resin and infiltrate samples in Spurr's resin:
    - 25% Spurr′s overnight
    - 50% Spurr′s 3-4 hours
    - 75% Spurr′s overnight
    - 100% Spurr′s 5 hours
    - 100% Spurr′s overnight
    - 100% Spurr′s overnight
11. Embed the sample in Spurr′s resin in silicone molds. Polymerize the resin in oven for 2 days at 60C.