SEM – Microwave Fix and Dehydration



**Protective Equipment**; Safety glasses, Lab coat, gloves and Chemical Fume hood

Remember to **label all vials/tubes** with; sample name, fixative type, your name & date

**Solution preparation:**

 All solutions on ice prior to microwaving -  (fixatives and buffers)

 For plants; For animals;

 Fixative; Fixative;

 2% PFA 2% PFA

 2% Glut 2% Glut

 0.05 M PIPEs 0.1 M Phosphate or Cacodylate buffer

 Ethanol Dehydration series; 30%, 50 %, 60 %, 70%, 80%, 90%, 100% 

**Sample preparation:**

Dissections should occur prior to fixation

Prepare dummy tube for temperature probe place on ice until ready to use

Epindorf tubes 600 µl fixative - for small samples (1-5 mm). Do not put more than 3 samples in each tube

Scintillation vial approx. 1 ml fixative (samples over 5 mm). Do not crowd samples in vial

**Fix**;

Place containers and dummy tube in rack in ice bath (= ice + water), place in microwave

2min 30 sec, 250 - 750 Watts, Cut off temp 30° C

* Remove from microwave and rest at room temperature for 5 minutes

**Rinse**;

Buffer 2x, 5 min ea

ddH2O 1x, 5min

samples may be transferred from tubes to baskets in divided petri plate if desired.

**Dehydration**;  Ethanol

30%, 50 %, 60 % - 1x 40 sec each step

70%, 80%, 90%, 100% - 2x, 40 sec each step

after microwave replace ethanol with fresh 100%, can be stored overnight at room temp prior to critical point drying

Notes;

if you have a specific protocol requiring another fixative use what is recommended