Standard Operating Procedure for

Cryofracture for SEM



Procedure for freeze-fracturing samples

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

**Warning**: Liquid nitrogen may cause cryogenic burns or injury

1. Fix samples according to procedures worked out for your tissue type and dehydrate to 100% with an ethanol series.

2. Put on Safety glasses. Precool all tools that will come into contact with your sample in the liquid nitrogen, ie. single-edged razor blade clamped in a hemostat, and forceps.

3. Samples may be inserted into Parafilm sleeves for fracturing or placed directly into liquid nitrogen.

1. Prepare Parafilm sleeves by rolling strips of Parafilm to a desired width around an object with a diameter similar to your sample (be sure to leave room to crimp the ends).
2. Fill with absolute ethanol, add sample and crimp ends.

4. Move sample into LN2 and allow to freeze.

5. Fracture samples by placing the razor blade-hemostat over sample and striking with a hammer.

6. Pick up the fractured fragments with precooled forceps and place in fresh 100% ethanol. If the sample is still inside the paraffin sleeve, remove and discard the sleeve.

7. Dry sample with CPD (Critical Point Dry), lyophilizer or HMDS –whichever is appropriate.

8. Place fracture side up on a stub. The fractured face should appear smoother and shinier under the dissecting microscope than the other surfaces.

9. Samples are now ready to gold coat.