

SCANNING ELECTRON MICROSCOPES

Poor Man's Cryo-SEM

SMART - POWERFUL - FLEXIBLE

Cryo-SEM imaging is a powerful tool in studying the structures of electron beam and vacuum sensitive materials. These materials include: fragile biological structures such as fungi, plants, cells, etc. as well as soft or volatile samples and even liquids. Cryo-SEM offers some clear advantages by rapidly freezing a sample prior to imaging, thus maintaining the sample as close as possible to its natural state. Long dehydration and chemical fixation steps can be avoided. Inhibiting dehydration helps maintain delicate structures without shrinkage. Moreover, volatile or even liquid samples are stabilized under the electron beam. Cryo fracturing techniques allow for study of the internal microstructure of these types of vulnerable materials as well. A few of the disadvantages are that for efficient freezing, the sample size must be small and the price may not be in everyone's budget for a state-of-the-art cryo system with freezing station, cold stage, vacuum transfer system etc.



Figure 2: Polymer screws isolate cryo-stub from holder

A low cost alternative to a complete cryo-system that has been demonstrated to provide good results for many applications is a simple Cryo-Stub. The technique can be thought of as 'Poor Man's Cryo' and is a simple and cost effective means of looking at fully hydrated materials or other electron beam and vacuum sensitive samples. It involves pre-freezing a Cryo-Stub in liquid nitrogen and then contact freezing the sample prior to placing it in the SEM. The advantage of this technique is that it is simple, cost effective and the sample will in situ freeze dry in the SEM. The disadvantage is that there is no temperature control and the sample will in situ freeze dry inside the SEM.

The supplies you'll need are a thermos container or something suitable to put a small amount of liquid nitrogen in, a Cryo-Stub and a cryo-glue or clamps to fix your sample to the stub.

The sample preparation procedure is as follows:

- 1. Immerse the Cryo-Stub in liquid nitrogen and let it equilibrate
- 2. Once fully cooled, remove the Cryo-Stub and quickly contact freeze your sample and place inside the SEM
- 3. Image as normal. You need to remember to insulate your cooled brass block from the basic holder simple teflon screws or spacers would provide such insulation.

Below are some examples of materials images with this 'Poor Man's Cryo' method.

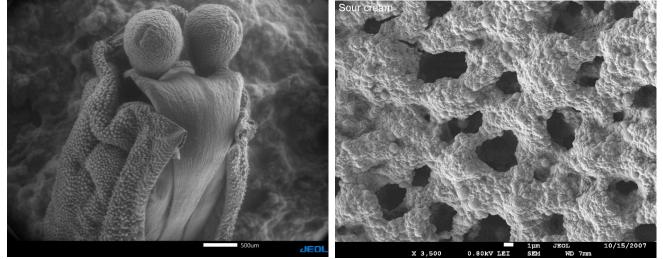


Figure 2: Orchid- Stigma anther

Figure 3: Sour cream