

Artifacts In Cryo Preparation for Electron Microscopy

Kim Rensing¹

¹Fibics Inc. Ottawa, ON, Canada

Wet and soft samples such as bulk biological materials do not naturally lend themselves to study by electron microscopy. Samples need to be chemically and structurally modified to withstand the high vacuum and the high energy electron beam for both scanning (SEM) and transmission (TEM) electron microscopy. In the standard protocol, the samples are chemically preserved (“fixed”), then dehydrated and infiltrated with liquid resin which is subsequently solidified. These solid samples can be thinly sliced (sectioned) using an ultramicrotome and the sections mounted onto a metal grid for observation in the TEM. This is necessary because untreated biological material is destroyed by the harsh conditions inside the EM, and a TEM beam can only pass through very thin material. However, all this manipulation induces large scale artifacts via the slow process time, the extraction of biological material and the gross structural changes. Cryo-preparation methods can avoid many or all of these problems depending on the techniques used.

Using a high pressure freezer, samples up to 200 μm thick can be frozen into a vitreous state within milliseconds, often without exposure to chemicals. They can then be dehydrated while in the stable, frozen state through the freeze substitution process, and embedded in resins for sectioning and observation by room-temperature TEM. The ultrastructure is similar to that of chemical fixation, but greatly improved because it avoids many of the above-mentioned drawbacks. Alternatively, it is possible to observed frozen samples directly by TEM. Advances in cryo-ultramicrotome technology have made cryo-transmission electron microscopy of vitreous sections (CEMOVIS) more easily accessible, although still technically challenging.

For the study of membranes, deeply frozen samples can be fractured to expose membrane surfaces and their constituents. A electron beam cannot pass through such a sample, so for TEM, a casting of the fractured face is made by “spraying” a very thin layer of metal onto the surface from a single direction angled to the surface plane. Following stabilization of the thin metal layer with a second layer of carbon, the samples are brought to room temperature where all the biological material is dissolved away. The remaining replica can then be floated onto a grid and observed by the TEM. The surface topography induces shadows in the metal coating which ultimately result in the scattering contrast in the TEM.

With the advent of field emission electron sources, scanning electron microscopes are able to achieve resolution approaching that of the TEM. Since they are designed to observe surface features, the SEM is well suited to examination of exposed fracture planes. With a cryo-stage on these instruments, it is now possible to avoid the technically challenging replica technique, and directly observe frozen, fractured samples in an SEM. For stability, the surface can be coated evenly by metal sputtering and it is not necessary to remove the biological material prior to observation.

With any processing technique, there is a possibility of inducing artifacts. It is important to know what successful results look like; these can be reviewed in current literature. However, with these advanced cryo preparation methods, it is important to also have an understanding of what the samples look like when the process fails. The ability to interpret images of processing artifacts makes it possible to diagnose the problems and correct them. The figures shown here show just a few examples.

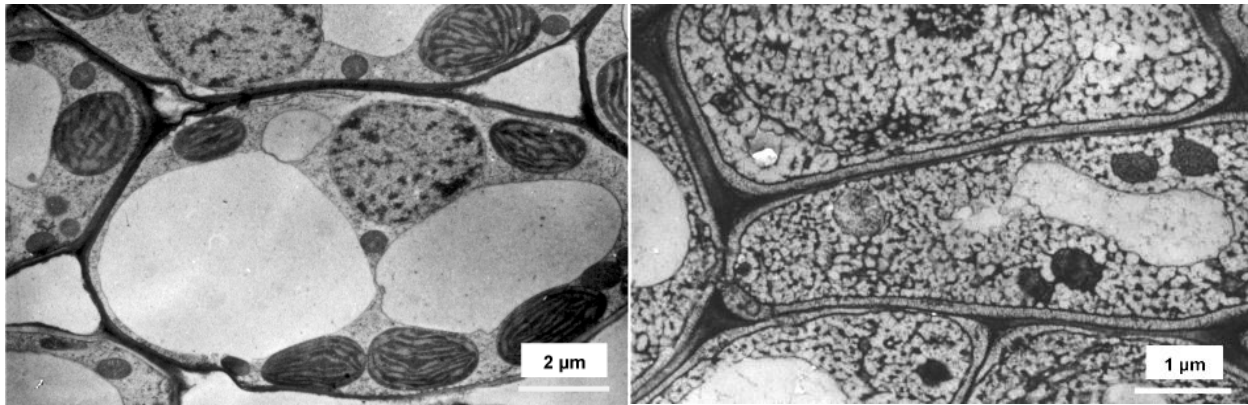


Fig. 1. a) Well high pressure frozen and preserved leaf tissue. b) Poorly frozen leaf tissue indicated by net-like cellular constituents.

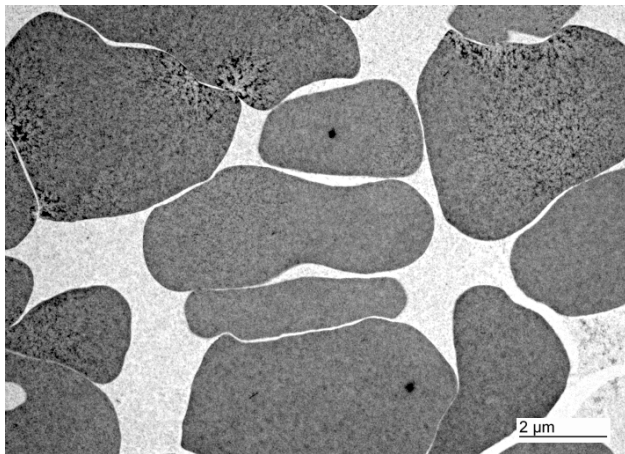


Fig. 2. Predominantly well-frozen red blood cells with areas of ice-crystallization in-growth.

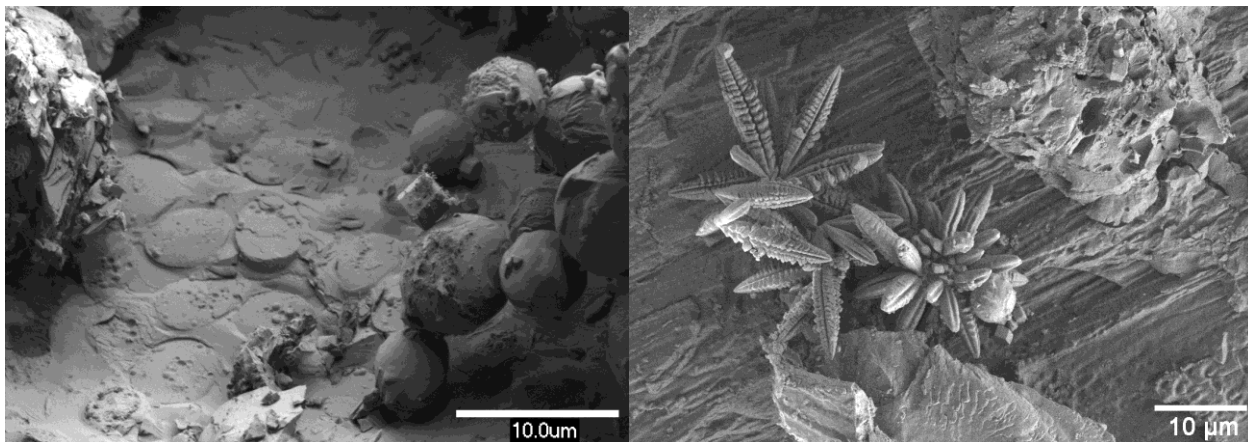


Fig. 3. a) Fractured yeast preparation with hexagonal and vitreous ice contamination. b) Ice crystal growth on fractured materials following freezing.