

Introducing Cryo Scanning Electron Microscopy

1. Basics

Cryo scanning electron microscopy is effective in imaging of samples containing moisture without causing drying artifacts. Figure 1-1 shows an external view of a general cryo SEM.

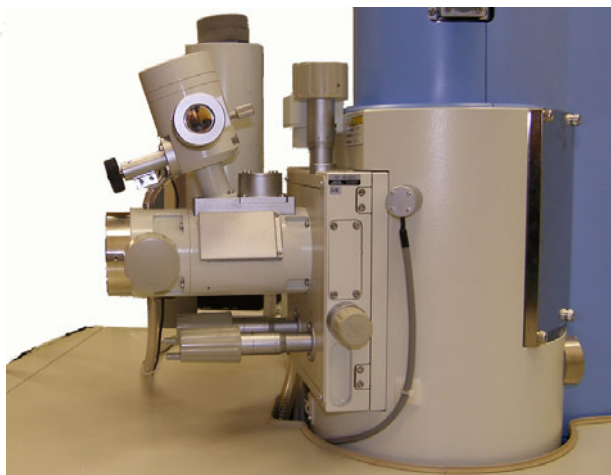
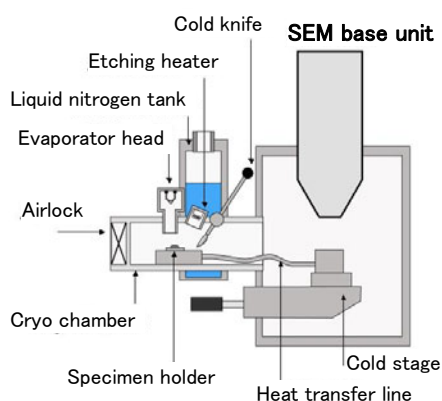


Figure 1-1. External view of general cryo SEM

Figure 1-2 shows the components of this general cryo SEM.



The cryo SEM comprises a cryo chamber for cleaving

Figure 1-2. Components of general cryo SEM

and coating of frozen samples and a cold stage for SEM imaging. The system in the figure integrates the cryo chamber and the cold stage, allowing a single liquid nitrogen tank to cool the chamber and the

stage. The cryo chamber incorporates a resistance heating vacuum evaporator to coat samples with gold. It also incorporates a heater for etching (subliming) the ice formed in samples. Figure 1-3 shows a typical operating procedure of this cryo SEM. A moisture containing sample needs to be rapidly frozen with liquid nitrogen (physical fixation) before it is loaded into the cryo SEM. The frozen sample is loaded onto the specimen process stage through the airlock system of the cryo chamber. The sample can be cleaved with a cold knife integrated in the chamber for imaging of its internal structure. The cleaved surface is etched in a controlled manner to remove the ice using the heater (ice sublimation) as needed, and is coated with Au for SEM imaging (or can be imaged at low kV without metal coating). There are two crucial points in the operating procedure. One is the etching process where the ice formed inside the sample is sublimated. And the other is the preliminary freezing (physical fixation) technique. These will be described in detail below.

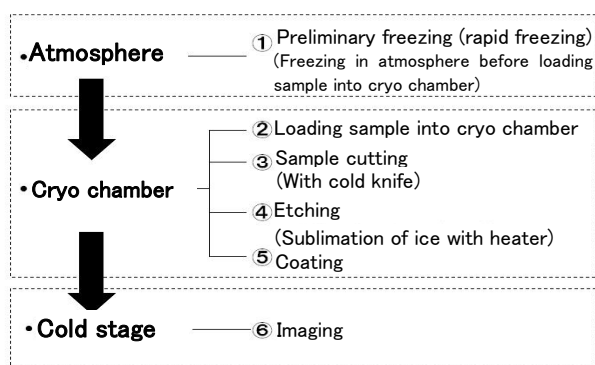


Figure 1-3. Operating procedure of cryo SEM

2. Etching

This process controls the temperature of the frozen sample in vacuum, allowing only the ice

formed in the sample to sublimate. It provides information on the water distribution in the moisture containing sample. The temperature at which the ice sublimates varies depending on the level of vacuum in the cryo SEM (pressure in the SEM chamber). The sublimation temperature can be identified from the ice vapor pressure curve shown in Figure 2-1. Figure 2-2 shows the emulsion of oil drops in water (O/W), an example of effectiveness of the etching process. The image taken after the sample was etched shows the distribution of ice (water).

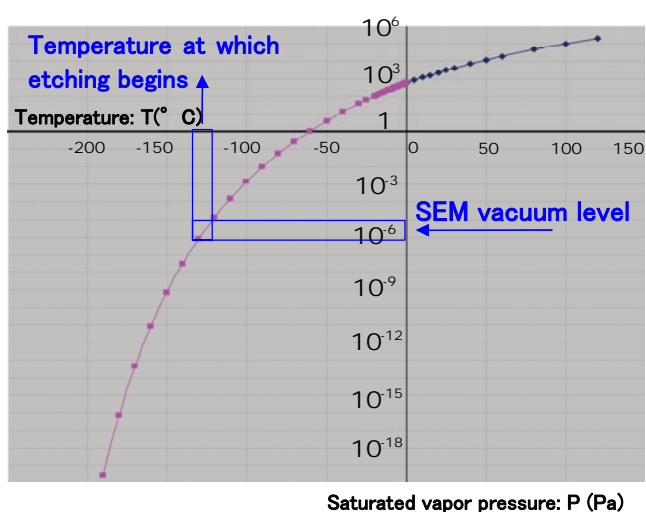


Figure 2-1. Ice vapor pressure curve

This is an ice vapor pressure curve. Etching (ice sublimation) begins when the vapor pressure is higher than the vacuum (pressure) level in the SEM chamber.

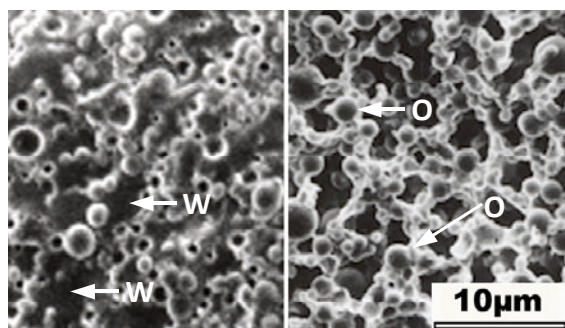


Figure 2-2. Changes in O/W emulsion resulting from etching

Left: Before etching; right: after etching The ice (W) was etched, leaving the oil (O), showing O/W emulsion in the sample.

3. Notes on preliminary freezing

Preliminary freezing is called physical fixation as opposed to chemical fixation. At the preliminary freezing process, a moisture containing sample is frozen with liquid nitrogen before the sample is loaded into the cryo chamber. However, since water has the smallest cubic volume at 4°C, it will expand its volume as it freezes, likely to destroy the sample structure. If liquid nitrogen is used for direct freezing of the sample, the freezing speed will slow down due to boiling of the liquid nitrogen. To avoid this, a technique to increase the freezing speed (rapid freezing) is needed. For example, in the metal contact technique (impact freezing), a sample is pressed onto a metal plate cooled with liquid nitrogen or slush nitrogen obtained by evacuating liquid nitrogen. Figure 3-1 shows an acrylic polymer emulsion sample frozen by liquid nitrogen, while Figure 3-2 shows the same sample prepared by the metal contact technique. Individual particles are clearly observed in the acrylic emulsion frozen by the metal contact method. Those in the other are indeterminate in form due to ice crystal growth damage.

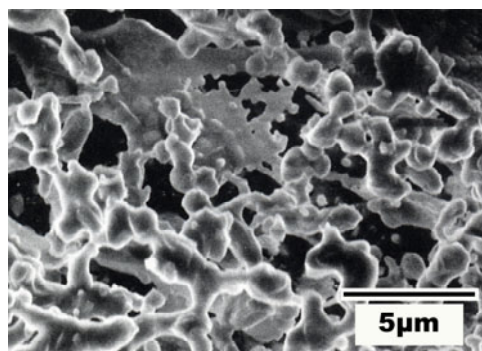


Figure 3-1. Direct freezing with liquid nitrogen

Polymer emulsion particles are indeterminate in form in the slow freezing process due to liquid nitrogen boiling.

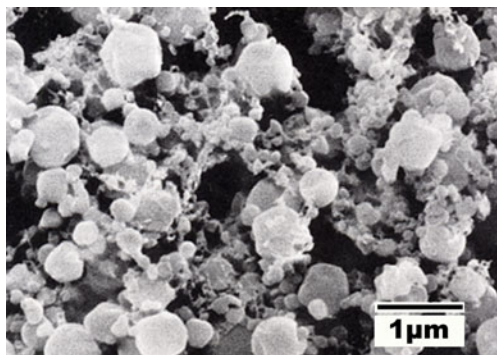


Figure 3-2. Freezing by metal contact

The sample was pressed onto a metal plate cooled down to the liquid nitrogen temperature for rapid freezing. Individual particles of polymer emulsion are visible.

Figures 3-1 and 3-2 confirm that the ideal sample should be as small as possible and be frozen as fast as possible.