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Development of Cryo-STEM for Scanning Electron Microscopy

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We have developed a cryo-STEM holder which is compatible with commercial cryo-transfer stages for scanning electron microscopes (SEM). The new cryo-STEM setup allows the image and analysis of frozen specimens in both transmission and conventional secondary electron modes down to liquid nitrogen temperatures.

In these days most electron microscope manufacturers offer scanning transmission electron microscope (STEM) detectors for their SEMs. The solid state STEM detectors are based on doped semiconductors with low work-functions, where the incident electrons generate multiple electron-hole (E-H) pairs and the free charge carriers can be collected and further processed. Currently, all commercially available STEM detectors are built for EM observations at room temperature. However, FEI Company recently introduced a so called “Wet-STEM” detector for imaging of wet and hydrated specimens. The STEM design includes a Peltier stage, where the specimen temperature can be lowered to around 0°C.

The new cryo-STEM design involves two parts. First, the main cryo-holder in the SEM sample chamber has been modified to house a state-of-the-art second generation STEM detector. The STEM detector consists of 14 diode segments as illustrated in Figure 1. Similar to a dedicated STEM instrument the arrangement of the diodes allows obtaining true bright and dark field images. Second, the sledge has been redesigned to meet following four requirements: (1) The TEM grid can be loaded into the sledge in a protected and cooled environment, such as in a FEI Vitrobot™ or cryo chamber of an ultramicrotome. Further, (2) the sample needs to be maintained at liquid nitrogen temperature during transport and transfer into the cryo transfer chamber. Also, (3) the frozen sample has to be protected from humidity in air or liquid nitrogen, to avoid ice formation on the sample surface and finally, (4) the sledge needs to be compatible with the stage in the cryo-transfer chamber.

Photographs of the cryo-holder and sledge are shown in Figure 2. The TEM grid sits on a rod which slides into a protective cavity during transport and transfer (Figure 2a). After the transfer into the SEM cryo-transfer chamber, the fracture knife is used to move the sample back into the open STEM position (Figure 2b). Another feature of the sledge is that the clamp, which holds the grid in position, is manufactured from a low background material to minimize the signal from the holder during the EDS analysis.

In this presentation we will introduce the cryo-STEM/SEM design and show first results of frozen tissue, cell monolayers and polymers.

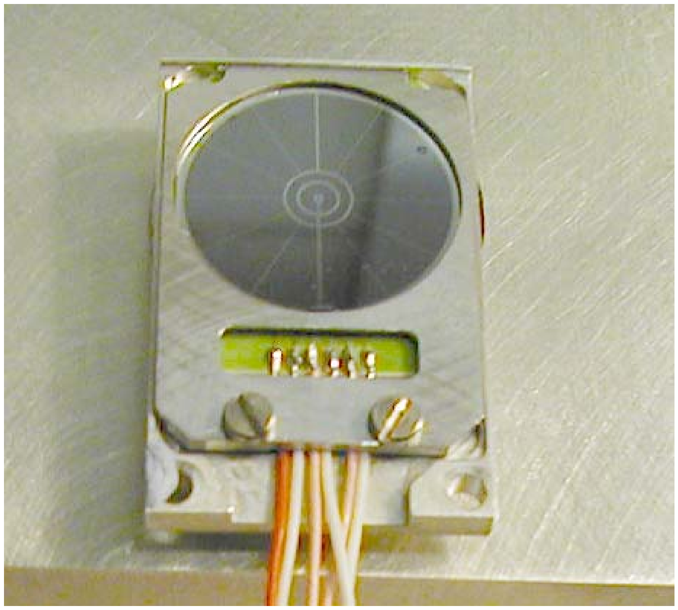
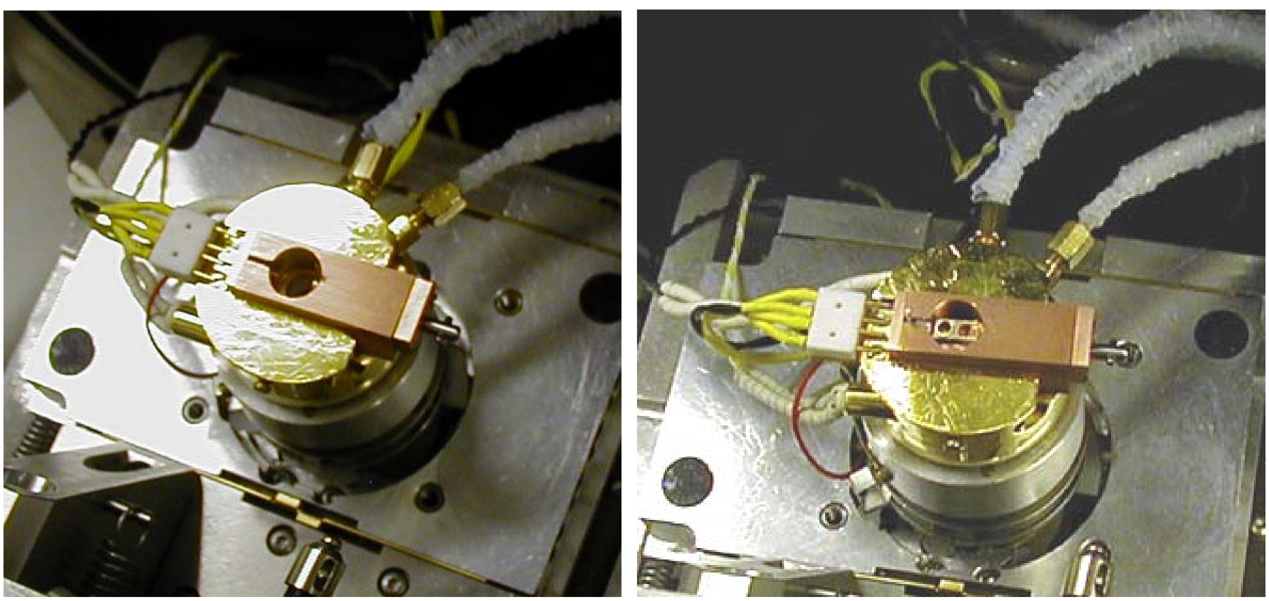


FIG. 1. Photograph of second generation FEI STEM detector.



(a) (b)

FIG. 2. Photographs of the sledge and cryo-holder. Figure 2a shows the sledge in the closed position, where the TEM grid sits inside a cavity to protect the sample from icing, while Figure 2b shows the sledge in the open STEM position.

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