

Development of a Cryo-Electron Microscope for Simultaneous Observation of SEM and Transmission Images

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Researchers in areas of medicine and biology have long awaited a user-friendly, low-accelerating-voltage cryo-EM for a wide variety of applications. New low voltage cryo-scanning transmission electron microscope (STEM) has been developed based on conventional high-resolution SEM, which enables to observe a transmitted image and a secondary electron (SEM) image simultaneously in a fresh frozen state [1].

New cryo-transfer holder enables to observe specimens at -190°C beneficial for low beam damage (Figure 1). We also added a vacuum system to convert the liquid nitrogen into a slush. In this way we succeeded ultimately in vibration-free observation (Figure 2). Specimen chamber is kept clean with anti-contamination trap reached at -210°C using slush nitrogen. Frozen specimens are mounted easily on the holder in liquid nitrogen by using a cryo-station. Simultaneous observation is advantageous to distinguish structures on surface or inside (Figure 3). Image processing for drift correction is applied to improve contrast and resolution.

[1] E. Usukura, A. Narita, A. Yagi, S Ito & J. Usukura, An unroofing method to observe the cytoskeleton directly at molecular resolution using atomic force microscopy, *Sci. Rep.*, 6: 27472 2016

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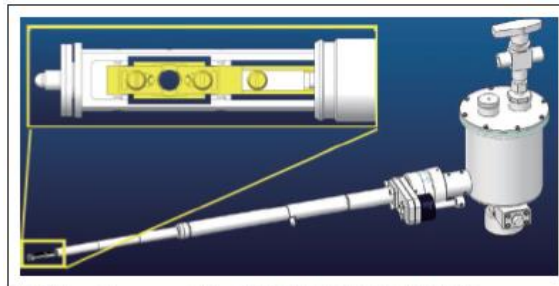


Figure 1. CAD diagram of the prototype cryo-transfer holder. The inset (upper left) shows an enlarged view of the specimen holder at the tip.

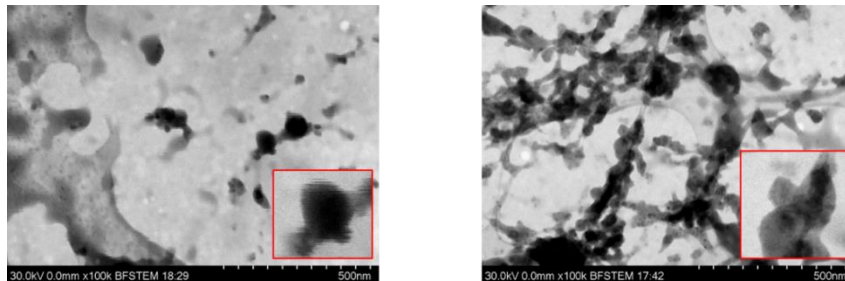


Figure 2. Images taken under liquid nitrogen cooling (left) and slush nitrogen cooling (right)

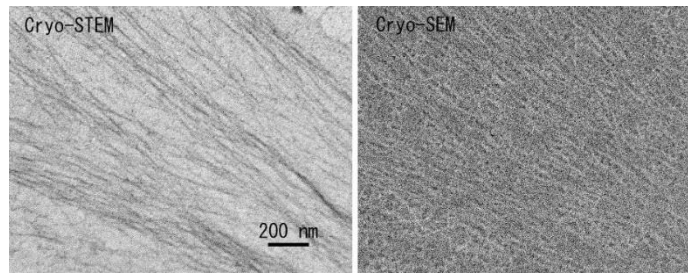


Figure 3. Simultaneously recorded cryo-STEM (left) and cryo-SEM (right) images