Cryogenic imaging of biological specimens using Helium Ion Microscope

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The investigations of biological specimens with Helium Ion Microscope (HIM) have gained significant traction over alternative techniques, because of its high spatial resolution (0.25nm)[1] and charge neutralization mechanism. A flood gun is integrated in the chamber and impinges a focused electron beam onto the ion scan field. The positive charges introduced by helium ion beam can be balanced by the negative charge electron beam. This allows of imaging non-conductive samples without needs of metal coating, thus provides less risk of artifacts during sample preparation. It has been reported HIM could reveals rich surface details of wide range of biological samples[2], which have not been achieved by other techniques. Nevertheless, as all the imaging techniques using charged particles, the vacuum environment makes the biological sample preparation to be much more elaborated: the specimens need to be completed dry by chemically altered and dehydrated prior to imaging, which inevitably introduce artifacts and change the morphology of the sample. This therefore presents an obstacle to obtain reliable and faithful imaging.

We overcome this limitation by applying cryogenic techniques to the biological specimen prior to HIM imaging. Instead of chemical dehydration, the biological specimen is rapidly frozen in liquid nitrogen environment to protect from ultra structural changes as well as destroy of cell membrane. This rapid freezing process keeps aqueous environment of biological specimen through amorphous ice formation and maintains the structure similar to the native state under imaging. In our setup, as shown in Figure 1a, the specimen is sandwiched between a pair of gold plate carriers, plunge-froze into Liquid Nitrogen, and finally loaded into the chamber by a cryogenic transfer shuttle (Figure 1b) and broken apart by a tensile fracture holder(Figure 1c) in high vacuum environment (10-7 torr). A cryogenic sample stage is installed in chamber of HIM to preserve the samples in their frozen state during imaging. Initial studies of this novel imaging technique are focusing on Yeast cells. Cryo-fractured yeast gives some information about the cell surface and internal structures. As shown in Figure 2, the yeast cells are embedded in the layer of ice, and the nucleus and exfoliated cell membrane are observed from the fractural surface. Details involved in the sample preparation and cryo-imaging, such as effects of different freezing techniques, ice ablation by high energy ion beam and surface heating by neutralization electron beam are discussed in our presentation.

1Application Note, Carl Zeiss SMT, "Ultra-High Resolution Imaging in ORION®PLUS", PI No. 0220-2008-ENG, Nov. 21, 2008

2 Daniel S. Pickard, et al. oral presentation EIPBN 2009.

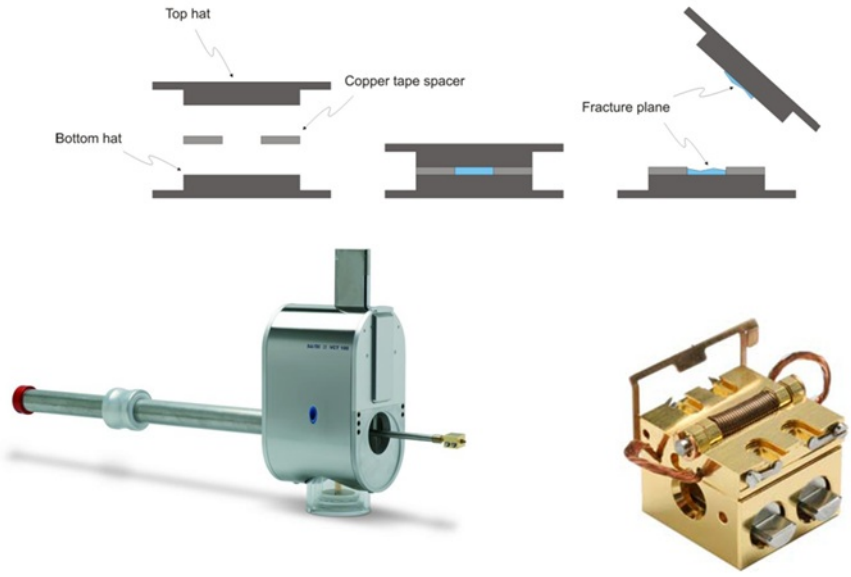

Figure 1. (a) specimen is sandwiched between the 4.5mm diameter gold plate carriers, plunge-froze using Liquid Nitrogen, and finally fractured within the HIM chamber; (b) transfer shuttle (Leica EM VCT100) integrated with (c) tensile specimen holder.

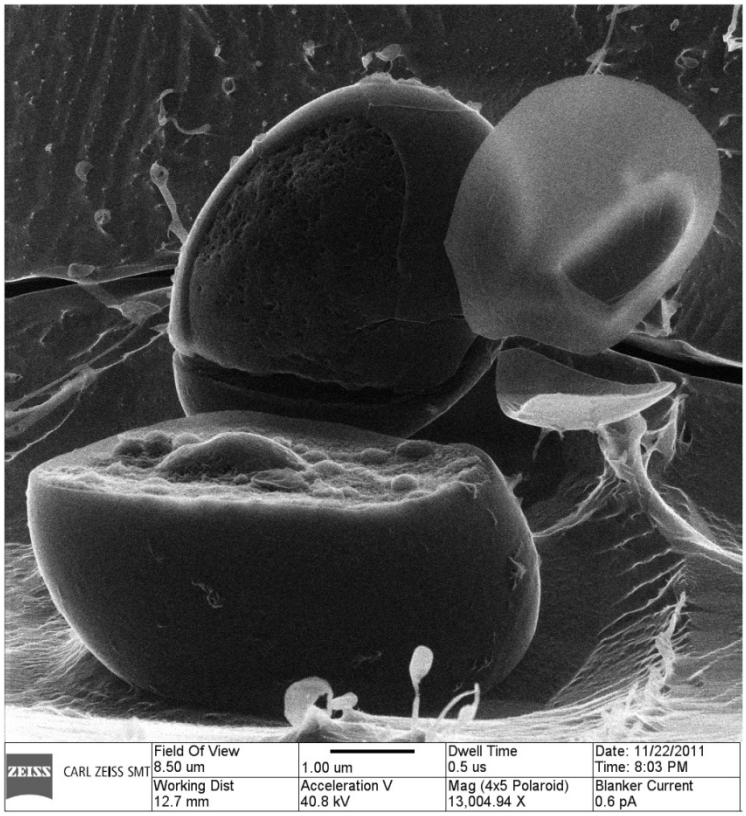

Figure 2. Helium ion microscope image of yeast cell, sample prepared by plunge freezing using Liquid Nitrogen and imaged at temperature of -150ºC.