doi:10.1017/S1431927617001039
Microsc. Microanal. 23 (Suppl 1), 2017

Application of Temperature Controlled Stage in Atmospheric Scanning Electron Microscopy

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The scanning electron microscope (SEM) is a powerful tool for acquiring surface information of micro and nanostructures under vacuum conditions. Recently, methods for observing samples under atmospheric pressure in a scanning electron microscope (SEM) have been investigated. Hitachi previously released a novel atmospheric SEM (ASEM) technique for observing samples that are present in ambient air conditions and are separated from the electron gun by a membrane [1]. Environment in the specimen chamber can be kept in ambient air conditions while the electron source remains under vacuum (Fig. 1(a)). By using this system, observation of wet, liquid, and even bulk samples is possible. While wet materials are clearly observed at an optimized distance between the membrane and sample surface, typical ASEM images taken in atmosphere have more distortion when compared to conventional, high vacuum SEM images. The reason why ASEM images appear "blurred" is due to the electron beam being scattered by air molecules as shown in Fig. 1(b). To solve this problem, methods have been developed to reduce the electron scattering effect [2, 3]. Here we present an image enhancement algorithm (electron scattering corrector: ES-Corrector) for ASEM image improvement. Blurred images created by scattered electrons can be improved utilizing the ES-Corrector function as demonstrated in figure 2 for leaf surface of Japanese radish collected under atmospheric pressure. The ES-Corrector restored image shows great improvements in clarity and edge sharpness.

The separation membrane may incur water vapor from a wet specimen when the sample is close to the membrane. In these cases, observation of clear images is possible after removal of water by reducing the pressure in chamber via an additional vacuum pump. However, this process poses a risk of changing the shape of a wet sample and therefore to alleviate any potential artifacts, a cooling stage with temperature adjusting controller was utilized. Additionally, the phenomenon of water vapor accumulation on the membrane can also be eliminated by the use of the chilled stage at 1^{o}C. Figures 3 and 4 demonstrate the observation of a cucumber cross section and a frozen carrot. As shown in Fig. 4 it was possible to observe the temporal change of a thawing carrot and confirm differences in the shape of cells between non-frozen and frozen samples.

This newly developed cold stage can be controlled with a temperature range between -20^{o}C and +50^{o}C. Therefore coupling this device with ASEM, a wide range of applications including wet / hydrated specimens and observation of dynamic freeze/thaw investigations can be observed.

References:

[1] Y. Ominami et al., *Microscopy*, **64**, 97-104 (2014).
[2] K. Nguyen, M. Holtz, and D. Muller, Microsc. Microanal. **19** (Suppl **2**) (2013)
[3] Y. Ominami et al., Proc. of SPIE, 9236 923604-1 (2014)

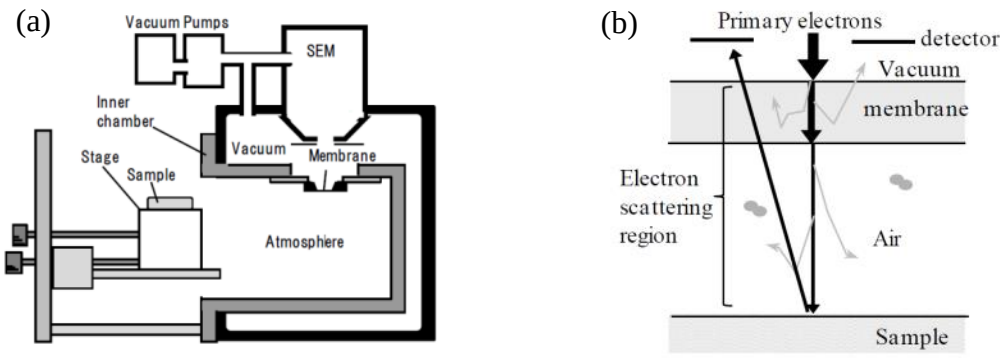

Figure 1. (a) Schematic of our ASEM. (b) Events of primary electrons.

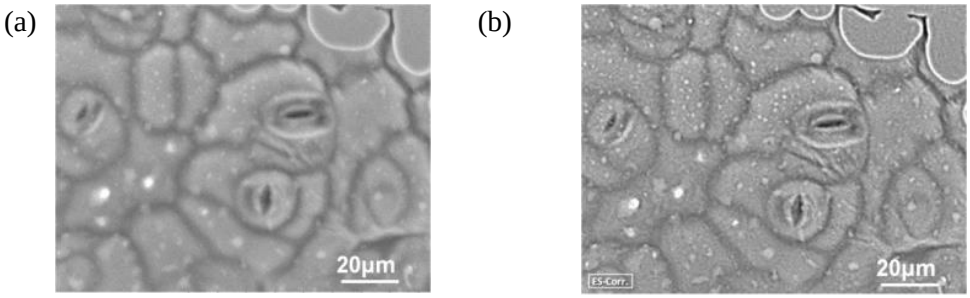

Figure 2. SEM images of leaf surface of Japanese radish. (a) is taken in atmospheric pressure. Images of (b) as restores image using ES-Corrector.

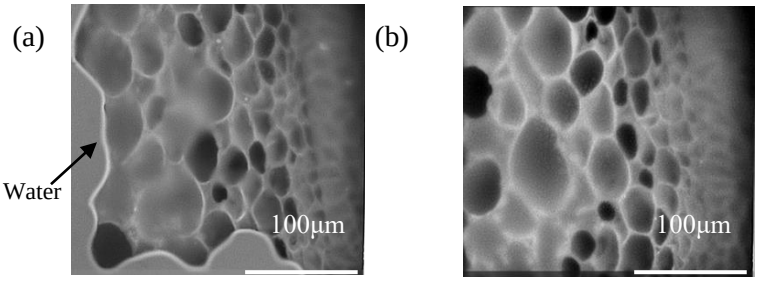

Figure 3. ASEM images of cucumber cross section. (a) is taken in atmospheric pressure at room temperature. (b) is taken in atmospheric pressure using cooling stage (stage temperature is 1ºC).

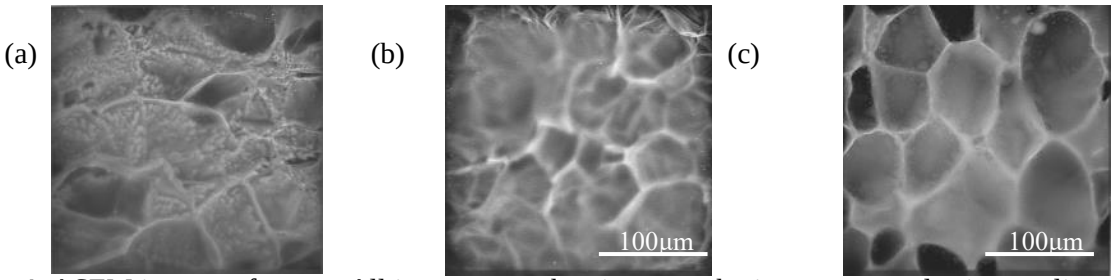

Figure 4. ASEM images of carrot. All images are taken in atmospheric pressure and using cooling stage. (a) is a cross section of the frozen food carrot at -20 ºC. (b) is a thawing carrot of (a) taken at 4 ºC. (c) is a non-frozen carrot taken at 4 ºC.

https://doi.org/10.1017/S1431927617001039 Published online by Cambridge University Press