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Correlative Light and Electron Microscopy in Atmosphere

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Correlative light and electron microscopy (CLEM) for observing biological samples has been reported. After optical microscopic observation, electron microscope imaging is performed to clarify nanostructure of the sample. A lot of sample preparation methods to perform CLEM have been developed in order to transfer the biological samples into vacuum condition in electron microscope. We proposed a novel atmospheric SEM (ASEM) technique for observing samples which are present in ambient air conditions **[1]**. In our system, the environment around the sample can be kept in atmosphere conditions. In this presentation, we present CLEM technique using ASEM.

In our CLEM observation process, bulk sample is first observed using optical microscope (**Figure 1(a)**). After that, sample is transferred into ASEM chamber without any sample preparation. One of the major differences of our ASEM from conventional SEMs is the presence of an additional inserted chamber with silicon nitride (SiN) membrane, as shown in **Figure 1(b) and (c)**. To initiate sample loading or exchange, the sample stage is pulled out from the inserted chamber, as shown in **Figure 1(b)**. The sample is mounted on the stage, transferred into the inserted chamber, and placed under the membrane via a side-entry stage, and then SEM observation is performed. Typical atmospheric SEM images taken in atmosphere are more blurred compared to conventional SEM image taken in vacuum condition because electron beam in ASEM is scattered by atmospheric gases. In order to reduce the electron scattering effect, we developed an image enhancement algorism (ES-Corrector) for ASEM **[2]**. By using this algorism, blurring created by scattered electrons in ASEM image can be improved after detection of SEM images as shown in **Figure 2**.

Figure 3 shows optical and fluorescence microscopic images (Fig. 3(a)-(d)) and atmospheric SEM images (Fig. 3(e)-(f)) of pollens of Ericaceae taken in atmospheric pressure (101 kPa). **Figure 4** shows optical and ASEM images of rat organs fixed with neutral buffered formalin. ASEM images shows much more detailed surface structures than optical microscope images. Our CLEM technique using ASEM allows us to simply switch from an optical microscope observation to a SEM observation without any sample preparation. Since ASEM doesn't require evacuation of sample chamber, sample transfer time between optical and electron microscope imaging is dramatically short. The CLEM technique using ASEM has a great chance as a preliminary observation prior to the ordinary SEM observation requiring various sample preparation.

References:

[1] Y. Ominami et al., *Microscopy*, **64**, 97 (2015).
[2] Y. Ominami et al., Proc. of SPIE Vol. 9236 923604-1 (2014).

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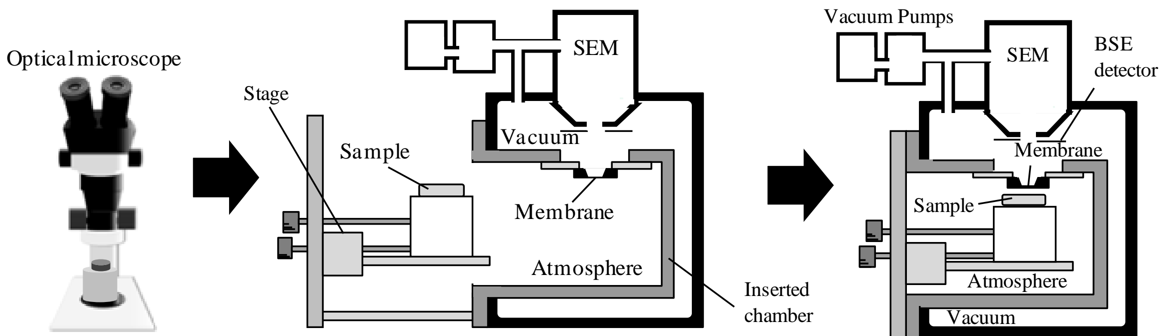

Fig. 1 Observation flow of correlative light and electron microscopy on atmospheric pressure.

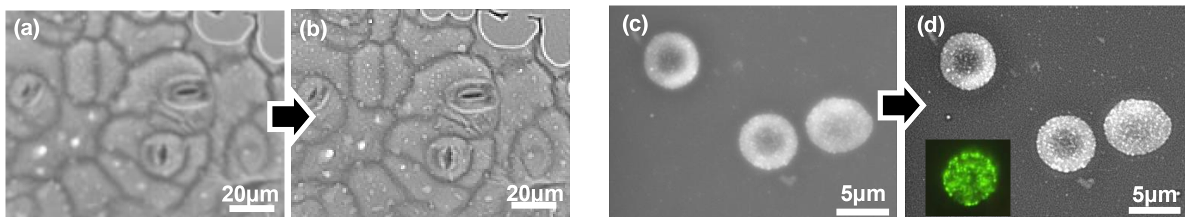

Fig. 2 ASEM images of leaf surface of the Japanese radish and immuno-gold labeling of glycophorin A in rat erythrocytes. (a)(c) Original images, (b)(d) improved images utilizing ES-Corrector. Image in (d) is Immuno-fluorescence using the identical antibody against glycophorin A.

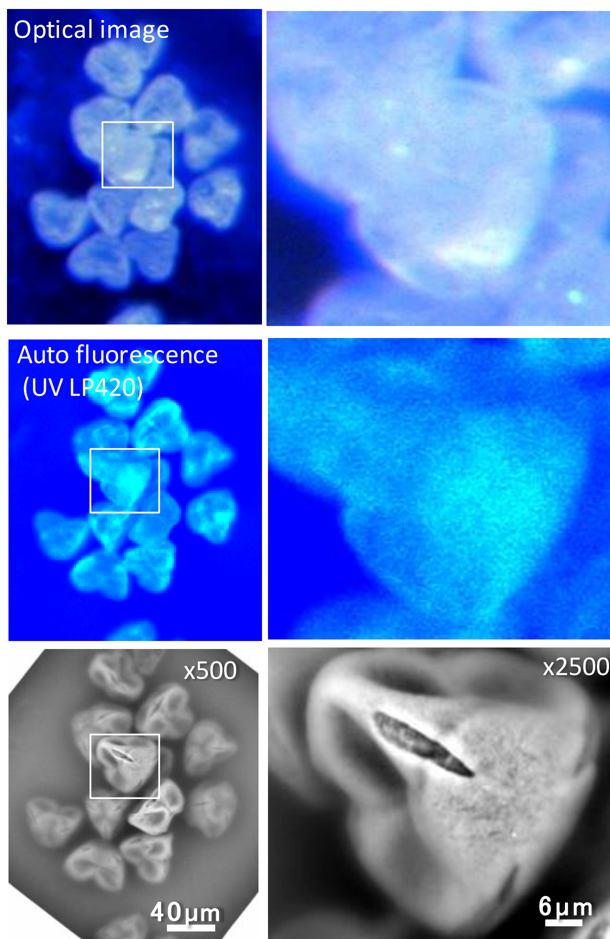

Figure 3 Optical, fluorescence microscopic images and atmospheric SEM images of pollens of Ericaceae taken in atmospheric pressure (101kPa).

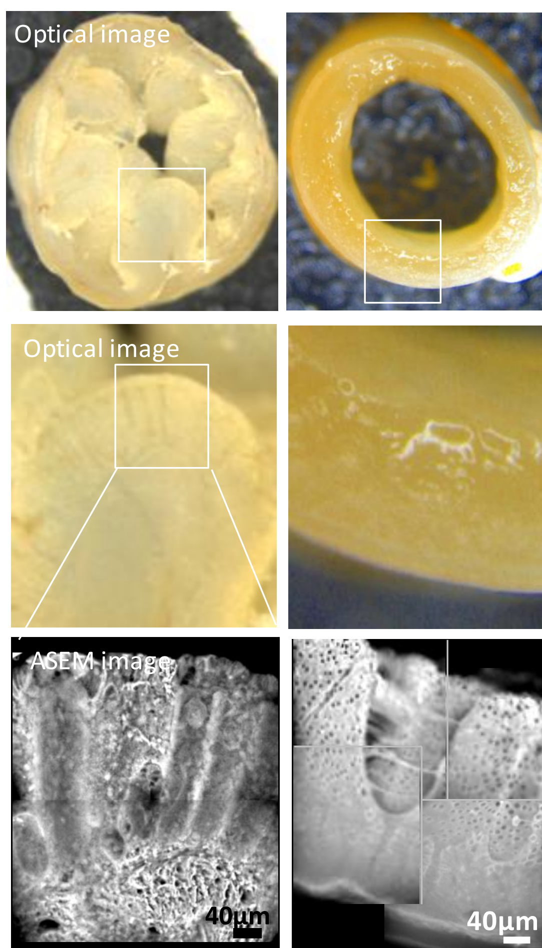

Figure 4. Macro and microscopic images taken using optical microscope and ASEM of (left) large intestine and (right) small intestine of rat organs.

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