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**IT-7-P-1610 Observation method of cross-sectioned cells by cryo-scanning electron microscopy**

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Protein and cellular structures have been visualized in a close-to-native state by cryo-transmission electron microscopy (cryo-TEM). In many cases for cryo-TEM cells are so thick that we have to prepare cryo-ultrathin sections. In such case compression of cryo-sections must be taken into consideration. The compression makes the image complicated because it occurs inhomogeneously. Cells and organelles are compressed whereas small rigid complexes such as ribosomes and microtubules have been reported to resist compression.

On the other hand freeze-fractured cells and tissues have been examined by cryo-scanning electron microscopy (cryo-SEM). However this method is limited because observation objects are only randomly fractured surface.

In order to visualize non-distorted cross-sectioned cells, we focused on the block surface after cryo-sectioning, and imaged it by cryo-SEM. Budding yeast was pelleted, high-pressure frozen and cryo-sectioned. The sections were imaged by cryo-TEM while the block was imaged by cryo-SEM. As a result, ultrastructure such as ribosomes and invaginated plasma membranes as well as organelles were clearly visualized by cryo-TEM, however vesicle structure such as whole cells, nuclei and vacuoles were ellipsoidal in the same direction (Fig. 1a). They were obviously compressed along the cutting direction. Meanwhile the block was transferred to cryo-SEM and observed. We could image cells and organelles without any staining or coating. In the cryo-SEM images yeast was oval in shape, and nuclei and vacuoles were circle in shape (Fig. 1b). They are consistent with the fluorescently-labeled images by light microscopy.

Furthermore we showed an example of repetitive cryo-sectioning and observation by cryo-SEM. A piece of diaphragm was cryo-sectioned in the direction parallel to sheet-like structure of diaphragm and observed by cryo-SEM. On the sectioned face near the surface of isolated diaphragm connective tissue was clearly observed (Fig. 2a). In order to observe structure beneath the connective tissue, the block observed by cryo-SEM was returned to the cryo-ultra-microtome using a cryo-transfer system. After the block was cryo-sectioned again, sectioned surface was observed by cryo-SEM again. As shown in Fig. 2b, sectioned muscle cells appeared. Repetitive sectioning and observing would be helpful to find objects localized in a limited area.

In this study it was shown that non-compressed cross-sectioned hydrated cellular and tissue architectures are clearly visualized by cryo-SEM.

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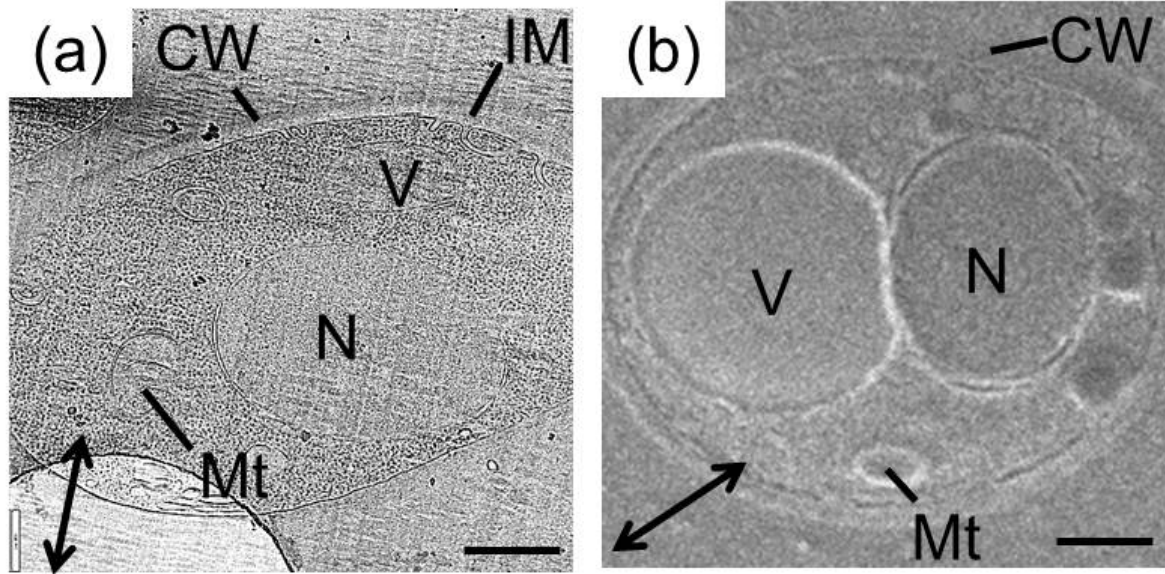


Fig. 1: Comparative observation by cryo-SEM and cryo-TEM. (a) Cryo-section of budding yeast observed by cryo-TEM. (b) Cryo-sectioned surface of budding yeast observed by cryo-SEM. Arrows: cutting direction, CW: cell wall, N: nucleus, V: vacuole, Mt: mitochondrion, IM: invaginated plasma membrane, Bars=500 nm.

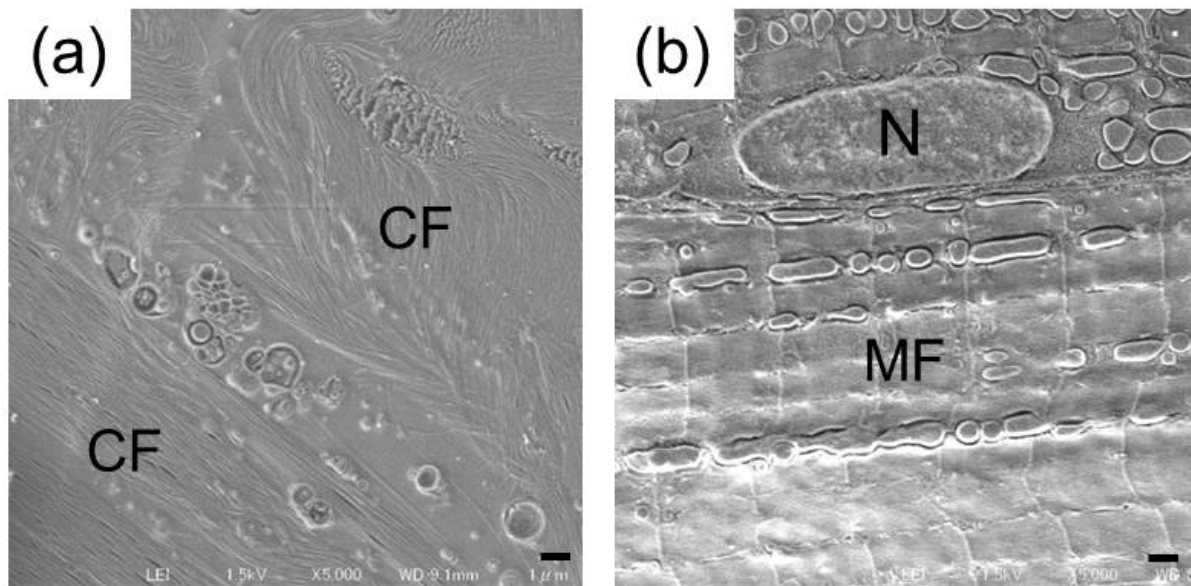


Fig. 2: Repetitive sectioning and observation of a block surface of diagram. A piece of diagram was cryo-sectioned and observed by cryo-SEM repeatedly. (a) Sectioned diagram near the surface of the frozen block. (b) Sectioned diagram inside the frozen block. CF: collagen fibers, N: nucleus, MF: muscle fibers, Bars=1  $\mu$ m.