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Application of FIB technique to 3D observation of resin embedded biological tissues

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A dedicated FIB system with variable operating voltages and high ion beam current has been developed and used in the TEM investigation of various materials. In addition, the technique allowing the extraction of a sample of micrometers in size(i.e. a micro-sampling technique) from millimeter-sized material has been developed[1-2]. The instruments and the technique have made TEM sample preparation quicker and easier, and are now applied to various soft materials including those with a very low-melting point, such as toners or even paraffin embedded materials. However, the FIB technique has not been applied to the TEM sample preparation of biological tissues. This is mainly because the cost of sample preparation is higher than that of the conventional ultramicrotome technique; however, the size of the sample is much smaller than that prepared by the conventional method. Recently, a technique for observing three-dimensional (3D) structures of nanomaterials has been developed. In this technique, the sample is shaped into a pillar and mounted on the tip of a needle-stub, and the observation of the sample is carried out using a FIB-STEM (TEM) compatible sample rotation holder. Since the technique requires the extraction of a piece of sample from a specific site and the shaping of the extracted sample into a pillar, the FIB milling with micro-sampling technique is essential. Here, we describe the use of the technique for 3D observation of a specific site in a resin embedded biological tissue. Figure 1 schematically shows the sample preparation of resin embedded cells. A part of the sample is FIB milled to the thickness of 15μm(a-b) and observed by STEM to find a cell to be investigated(c). After the additional FIB milling remaining the site(e), The sample is transferred to the STEM for 3D observation of a fine structure of the specific site (f). Stereoscopic observation of a bright field STEM observation of a resin embedded *Candida tropicalis* cell in a 4x3μm pillar sample is shown in Fig.2. Figure 3 shows high angle annular dark field (HAADF) images of a resin embedded *Candida tropicalis* cell in a 0.3x0.3μm pillar sample. Fine structures of the cell such as cell wall and mitochondorion are clearly and three dimensionally demonstrated.

References

[1]. T. Ohnishi et al., Int. Symposium for Testing and Failure Anal.(1999) 449-453
[2]. T. Kamino et al., Microsc. Microanal. **6** (2000) 510-511

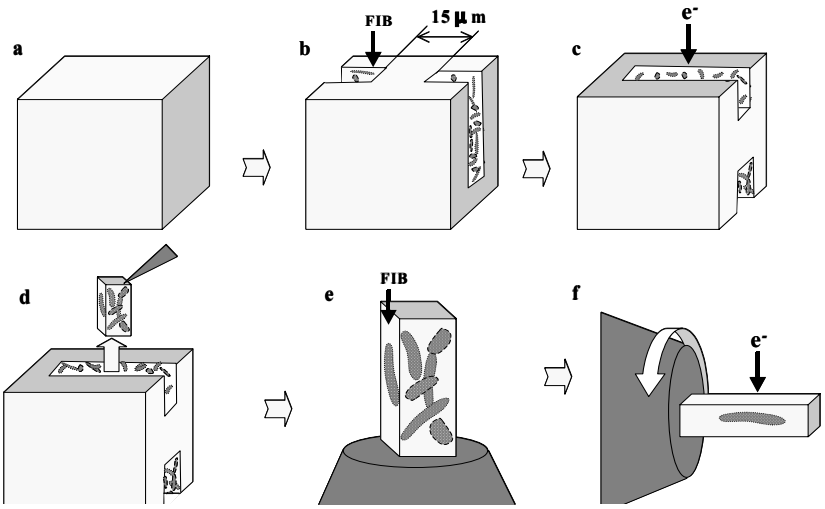

Fig.1 Procedure for sample preparation for 3D structural observation of a specific site in a resin embedded biological tissue.

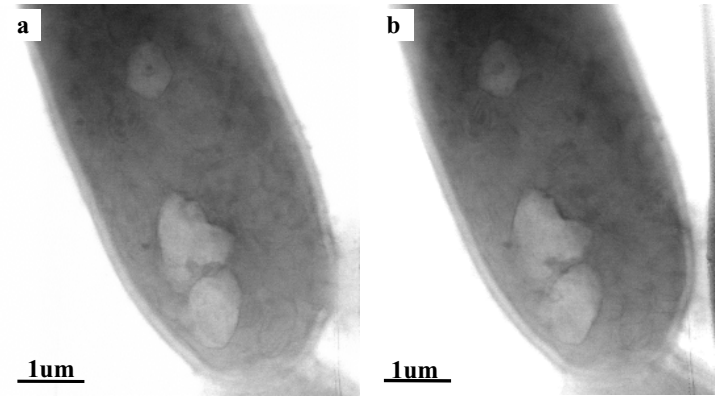

Fig.2 Stereoscopic observation of a resin embedded *Candida tropicalis* cell in a 4 x 3µm pillar shaped sample.

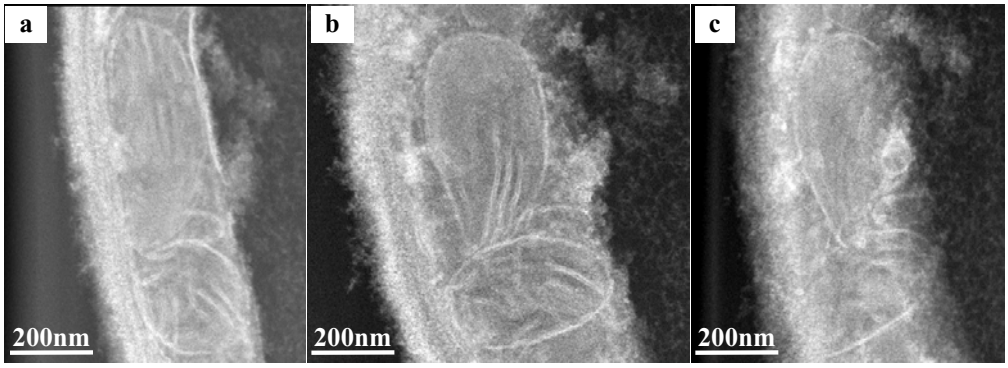

Fig.3 HAADF-STEM images of a resin embedded *Candida tropicalis* cell in a 0.3 x0.3µm pillar shaped sample observed from three different directions (a: 0º, b: 50º and c: 100º).

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