Focused Ion Beam for Biological Studies

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Purpose: In biology, transmission electron microscopy (TEM) can explore the ultrafine structure of the cross-section of samples. Instead, scanning electron microscopy (SEM) is for observing the sample surface. In semiconductor field, focused ion beam (FIB) and SEM are performed together to mill and observe chip interior. According to previous literatures, the attempts to apply the FIB/SEM dual system to mill/section biological samples usually resulted in poor sectional image. To improve the image resolution and contrast for further biological investigations, we conducted this study.

Methods: We developed a series of sample holders, each with different semiconductor substrate and pattern, through micro-processing such as e-beam lithography and PECVD. We utilized spinach chloroplasts and retinal pigment epithelium cells (ARPE-19) as the milling targets. The biological specimens were pre-fixed with glutaraldehyde and post-fixed with OsO_4 (O-T-O-T-O method). Then the specimens were vacuum-dried separately. The chloroplasts and ARPE-19 cells were subsequently milled by Gallium ion with a FEI nova-600i FIB/SEM dual system in different parameters. The cross-section was observed with SEM.

Results: With the sample holders and the milling methods we developed, the ultrafine structures of the chloroplasts and ARPE-19 cells could be disclosed in details. The SEM image resolution and contrast approximated the TEM image of usual biological samples. (Figure 1, Figure 2)

Conclusion: FIB/SEM dual system may play a role in biological studies, as exploring the ultrafine structure of the cross-section of chloroplasts and ARPE-19 cells by FIB milling and SEM observation.

Figure 1. Subcellular organelles of APRE-19 are revealed by FIB milling and SEM photography. (50,000X)

Figure 2. Mitochondria structures of ARPE-19 are revealed by FIB milling and SEM photography. (75,000X)