Development of rapid and accurate correlative light and electron microscopy for fluorescence-labeled organelles using light microscopes and FE-SEM

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Electron microscopy (EM) excels at capturing limited region such as cells and organelles with high resolution, but it is difficult to observe a wide area like organ and tissue, and it is not easy to determine the localization of molecules. The other hand, light microscopy (LM) is good at capturing a wide region and determination of molecules labeled with fluorescence and dyes. In order to capture a wide range of intracellular biological phenomena in organs and tissues with high resolution, it is important to use the technologies by combining EM and LM. Correlative light and electron microscopy (CLEM) gives an answer to these limitations by allowing for the detection of EM-level structure in addition to fluorescence or light information in the same region of interest. To resolve this problem, we developed a CLEM system (named MirrorCLEM) which is a system for observing the ultrastructure of organelles and successfully visualized the fluorescently labeled organelles by using a combination of fluorescence microscopy and field-emission scanning electron microscopy (FE-SEM). We developed an original jig for observing samples mounted on cover slips or slide glass under a FE-SEM, as well as the software for quickly and accurately observing the same position of fluorescence microscopy in a FE-SEM. With this system, the adhesive cultured mouse cells or plastic sections of plant tissues embedded in-resin can be observed under a laser scanning confocal microscope (LSCM) from low magnifications to magnifications that are high enough to clearly observe the ultra-structure of interest. After taking alignment, the FE-SEM stage could be correlated to the target position in the lower magnification image which is observed with the LSCM. And this system was capable of displaying an overlay of the LSCM and FE-SEM images in real time. In this poster, we will give a presentation about sample preparation, improved MirrorCLEM system, and its application.