Development of Rapid and Accurate CLEM System and Application for Tissues and Cells of Plant and Animal

Dr. Kiminori TOYOOKA
Mass Spectrometry and Microscopy Unit, Technology Platform Division,
RIKEN Center for Sustainable Research Science (CSRS), Japan

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, I developed a CLEM system (named MirrorCLEM) with Hitachi High-tech 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) or transmission electron microscopy (TEM). We developed original jigs for observing samples mounted on coverslips or slide glass under an FE-SEM, as well as the software for quickly and accurately observing the same position of fluorescence microscopy in an 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 (LSM) 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 LSM. And this system was capable of displaying an overlay of the LSM and FE-SEM images in real time. Moreover, we developed MirrorCLEM system for TEM. Fluorescence of ultra-thin sections on an EM grid was detected by the high-sensitive detector on LSM, and the position was taken correlative images by TEM-MirrorCLEM. We are now trying to take correlation with fluorescence lifetime imaging and FE-SEM. Here I will give a presentation about development and improvement of MirrorCLEM system, sample preparation for CLEM, and its application for tissues and cells of plant and animal.