LIVE CELL IMAGING BY DIRECT EXCITATION OF FOCUSED ELECTRON BEAM

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The development of nanoimaging techniques for specimens in liquid conditions is highly required in various applications such as analysis of colloidal solutions, observation of crystal growth and self-assembly process and so on. Especially, imaging of biological specimens with high resolution is crucial for a deeper understanding of cell functions. We have developed a D-EXA (Direct electron-beam excitation assisted optical) microscope \cite{1,2} to achieve nanometer-scale resolution. Our method is the combination of a scanning electron microscope and a fluorescence microscope and it can be applied for observation of specimens in liquid conditions.

In our method, electron beam is focused into the specimens in liquid, in order to excite cathodoluminescence directly. High spatial resolution of a few tens of nanometers is realized since electron beam is well focused in the specimens, even if some of the electrons are scattered in the the specimens. Images are reconstructed from the cathodoluminescence intensity detected with raster scanning of the electron beam. Specimens are placed in liquid on the thin film and observed, since the film separates vacuum condition, which is required for the propagation of the electron beam, from the liquid condition around the specimens.

We observed live cells under culture solution and the dynamic movements of granules in the cells without any treatments, such as fixation and drying, in order to confirm the potential for live cell imaging using the D-EXA microscope. Figure 1A shows the autofluorescence image of MARCO-expressing CHO cells in culture solution acquired with the D-EXA microscope, and Fig. 1B shows the phase contrast microscope image. The shape of the cell and granules were clearly recognized in Fig. 1A. It is demonstrated that the D-EXA microscope is a useful tool for bioimaging under physiological conditions.