COLOR IMAGING OF FLUORESCENT NANO DIAMONDS IN INTACT CELLS BY DIRECT ELECTRON BEAM EXCITATION

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Cathodoluminescence microscopy is a powerful tool to reveal the distribution of specific cellular structures with nano meter scale resolution. However, it is difficult to observe living cells, because these imaging techniques require vacuum conditions and various sample preparations, such as thin slicing, staining with metal species and freezing the specimens.

We have developed a D-EXA (Direct electron-beam excitation assisted optical) microscope [1], in order to observe dynamic motions of the specific cellular molecules with high spatial resolution, using immunostaining methods. Specimens stained with fluorescent materials are put on a thin film, and cathodoluminescence (CL) is excited with focused electron beam through the film. Since the film separates vacuum conditions from liquid conditions, live cells are observed in water-solutions without any treatments, as in the electron microscopy. We have demonstrated auto-fluorescence imaging of living HeLa cells and showed the potential for live cell imaging [1].

In this research, fluorescent nano diamonds (NDs) are used for labeling specimens, due to their chemical stability, photostability and low or noncytotoxicity. Furthermore, they can be linked to biomolecules, and the emission of NDs is selectable by the formation of the luminescent centers [2], so they can be applied for multistaining procedures.

First, we observed green and red emission NDs with our system, and confirmed that NDs emit CL. Figure 1 shows CL images of green and red emission NDs under atmospheric pressure. Green and red emission NDs are mixed and observed with high spatial resolution. Each ND can be distinguished by the difference of the emission wavelength.

It is also demonstrated that NDs taken into cellular granules of intact HeLa cells can be imaged, without slicing specimens. The D-EXA microscope has a potential to image dynamic motions of specific granules with immunostaining techniques.

References: