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Fluorescent Nano Diamonds for High Spatial Resolution Live Cell Imaging by Direct Electron Beam Excitation

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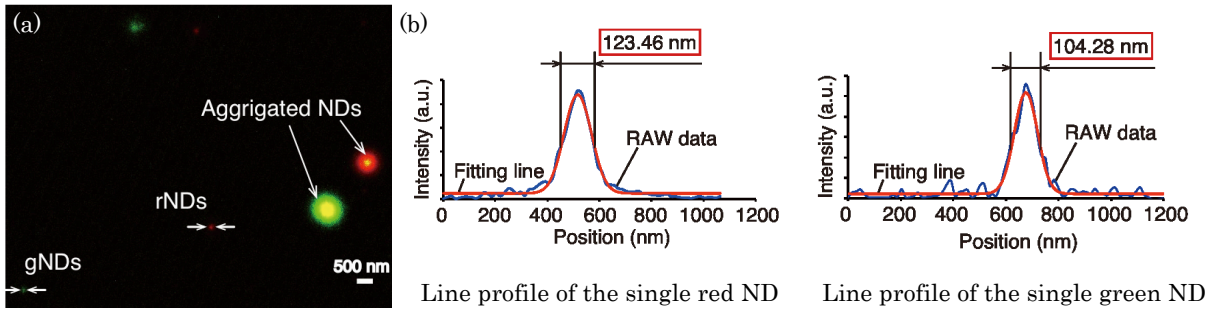
Nano bio-imaging is highly desirable to understand the cellular functions, because these functions are emerged from localization and the dynamic interactions of the cellular molecules in biological cells. Fluorescence microscopes are widely used to follow the processes and dynamics of specific cellular components within living cells. Optical signals include a lot of valuable information about specimens, such as intensity, wavelength and fluorescence life time. However, the spatial resolution is limited by the diffraction to about half the wavelength of the light used.

We have developed a D-EXA (Direct electron-beam excitation assisted optical) microscope [1], in order to achieve visualizing living biological cells with a spatial resolution of a few tens of nanometers. Our microscope enables nano scale resolution, exciting phosphor labeled to the cellular structures with the focused electron beam. Observations of living cells are realized by the electron-transparent film. The film separates vacuum conditions from liquid conditions. Specimens stained with fluorescent materials are put on the thin film, and CL is excited with the electron beam directly through the film. We have demonstrated auto-fluorescence imaging of living HeLa cells and showed the potential for dynamic observation of intracellular structures in living cells [1].

In this research, fluorescent nano diamonds (NDs) are used for labeling specimens. NDs are suitable for bio-imaging in our method due to their characteristics, such as chemical stability, photostability, low or noncytotoxicity, strong CL emission, and wavelength selectability [2]. It can be applied for multi-staining procedures. First, we observed CL from NDs and investigated on the spectrum of the CL. Figure 1 shows CL images of 100 nm green and red emission NDs in water-based solution. Simultaneous multicolor imaging and the 100 nm spatial resolution were demonstrated as shown in the line profile of Fig. 1(b). We also observed NDs uptake in intact HeLa cells without slicing specimens, and NDs attached along the membrane of cells with high spatial resolution.

The D-EXA microscope with phosphors clarifies fine structures of live cells in water-based solution. It is expected that our microscope with multistaining techniques becomes a promising nanoscopy technique to reveal the various cellular functions



Line profile of the single red ND Line profile of the single green ND

Fig. 1: Observation results of 100 nm green and red NDs.
(a) A CL image of green and red NDs in water solution. (b) Line profiles of single green and red NDs.

References

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