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Label-free cell organelle imaging by D-EXA microscopy

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Dynamic cell organelle imaging is essential for deep-understanding of biological processes, because cellular functions emerges as a result of localization and dynamic interaction of molecules. Recently several concepts of super-resolution optical microscopy are developed and have been contributed to the field of cellular biology.

We have developed the D-EXA (Direct electron-beam excitation assisted optical) microscope, that is a super-resolution fluorescence microscope with nano-meter scale spatial resolution [1]. In this micros-copy, endogenous substances or fluorescent labels connected to biomolecules in cells are directly ex-cited with focused electro beam to acquire cathodoluminescence (CL) images. Thus a spatial resolution of a few tens of nanometers can be potentially achieved. Live cell imaging is also possible by using an electron-transparent film substrate to separate the liquid environment from vacuum for electron beam propagation. Specimens are directly irradiated with electron beam through the substrate. We have demonstrated dual-color observation of fluorescent nano diamonds (FNDs) incorporated into HeLa cells. The distribution of FNDs is clearly imaged in HeLa cells with high spatial resolution [2].

Auto-fluorescence imaging was also demonstrated in fixed and living cells. The D-EXA microscope enables to observe auto-fluorescence of cellular components without any staining processes. Fig. 1 (a) and (b) show a phase contrast image and corresponding auto-fluorescence intensity image with pseu-do-color of the fixed HeLa cell [3]. Cells were fixed with a 1 % glutaraldehyde solution, and observed in phosphate buffered saline (PBS) solution. As shown in fig. (b), intracellular fine particles and the cell outline were clearly observed with bright contrast. These particles correspond to intracellular granules observed in phase contrast image (a), as indicated with arrows. Dynamic behaviors of intracellular granules were observed with time-lapse imaging in living HeLa cells maintained in culture medium. Although long-term observation is difficult due to huge damage caused by electron beam bombard-ment, the potential for live cell imaging was strongly demonstrated. Auto-fluorescence imaging was also performed in various types of cell lines, and organelles, such as a nucleus, nucleolus, mito-chondria, and intracellular granules, were obviously recognized in culture medium without any proce-dures. These results show that the D-EXA microscope has the potential for label-free organelle imaging in living cell with high spatial resolution.

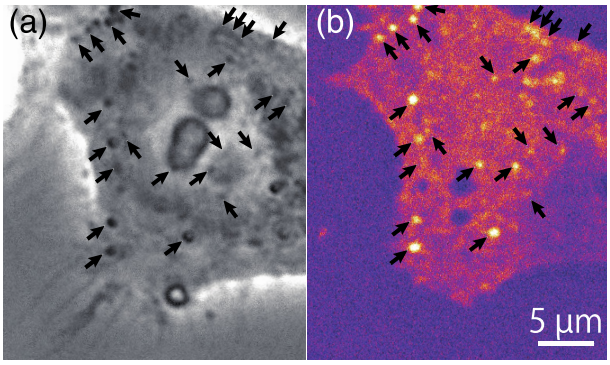

Fig. 1: Observation results of intracellular granules in fixed HeLa cell in PBS solution. (a) Phase contrast image. (b) Autofluorescence image acquired with the D-EXA microscope.

References

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