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Towards multicolor correlative light and cathodoluminescence imaging with using upconversion nanophosphors

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1. Introduction

Correlative light and electron microscopy (CLEM) is one attractive method of observing biological specimens because it combines the advantages of both light microscopy (LM) and electron microscopy (EM) [1]. In LM, specimens are fully hydrated, and molecular species are distinguished by using probes of different colors. EM provides both high spatial resolution images superior to those obtained with LM and highly detailed structural information of cellular components. The combination of LM and EM gives much more information than either method alone, which helps us to analyze cellular function in more detail. However, it is still difficult to distinguish the molecular species with EM images. Quantum dots (QDs) provide information about the biomolecular species in not only LM but also EM [2]. In this method, cells are treated with immuno-staining using QDs, and the target proteins are distinguished by the size of the QDs in EM.

We have previously investigated correlative imaging with rare-earth doped Y_2O_3 nanophosphors that emit light via both light and electron-beam excitation, where the light emission induced by an electron beam is called cathodoluminescence (CL) [3]. Because of the electron beam excitation, the spatial resolution of CL microscopy matches to 10 nm order. It will be possible to distinguish molecular species by the color of CL without discrimination of the size of the probes such as QDs.

We propose a new correlative imaging method using a combination of upconversion (UC) fluorescence excited by near-infrared (NIR) light and CL using UC nanophosphors as probes. UC is a process in which lower energy, longer wavelength excitation light is transduced to higher energy, shorter wavelength emission light [4]. So far, in LM observation for CLEM, ultraviolet (UV) or visible light has been used for excitation. However, UV and visible light have limited ability to observe deep tissue regions due to absorption, scattering, and autofluorescence. On the other hand, NIR light does not suffer from these problems. We investigated the UC and CL spectra of Y_2O_3:Tm, Yb and Y_2O_3:Ho, Yb nanophosphors and imaged the phosphors inside HeLa cells with UC and CL.

2. Experiments

Y_2O_3:Tm, Yb and Y_2O_3:Ho, Yb UC nanophosphors were synthesized by using sol-gel method. The molar concentration of each rare-earth ion were written as $(Y_{0.975}Tm_{0.005}Yb_{0.02})_2O_3$, and $(Y_{0.9}Ho_{0.05}Yb_{0.05})_2O_3$. The UC spectra of these nanophosphors were measured under 980 nm NIR laser excitation. Y_2O_3:Tm, Yb nanophosphors emit blue, red, and NIR UC fluorescence. Y_2O_3:Ho, Yb nanophosphors emit green and red UC fluorescence. Fig. 1 shows a part of UC spectrum of the both nanophosphors. These emission bands are not overlap with each other. CL spectrum of each nanophosphor is similar to the UC spectrum because the emission spectra are derived from doped rare-earth ions. These results indicate color imaging is possible in LM and CL microscopy. We will demonstrate the color LM and CL imaging of cultured cells with the UC nanophosphors.

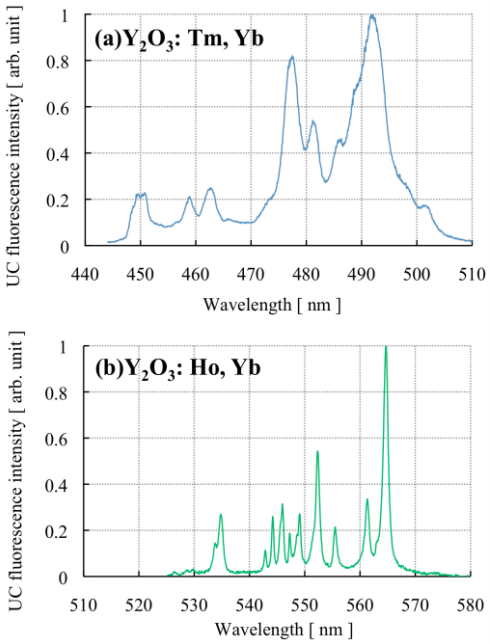

Fig. 1: UC spectra of (a) Y_2O_3:Tm, Yb and (b) Y_2O_3:Ho, Yb.

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Rerferences

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