

Nanoscale Thermal Imaging of Cell using Fluorescence in Near-field Region

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Nanoscale resolution imaging of cell metabolism is expected to visualize a single-cellular activity and to lead to an innovative drug development. However, the conventional method using an optical microscope cannot achieve a nano-scale resolution because of a diffraction-limit. This research is intended to reveal a metabolic activity on the surface of cells non-invasively using near-field fluorescence with high resolution. We have developed a novel local temperature measurement method using fluorescence near-field optics thermal nanoscopy (Fluor-NOTN). In order to achieve nanoscale spatial resolution, near-field light generated in the proximity of a small aperture was utilized. The sample surface-modified fluorophores are locally excited by near-field light, and fluorescence is detected by the small aperture. Fluorescence lifetime, which contains the information of the sample temperature, is then measured. In addition, an oxygen and ion concentration that are related to a cellular activity can be measured by a fluorescence lifetime sensing. In this report, a new scheme for fluorescence lifetime imaging system has been proposed in order to measure a temperature distribution of sample with nano-scale resolution. By controlling the probe-sample distance and the optical systems sequentially, the simultaneous detection of the fluorescence lifetime, fluorescence intensity and the topography of the sample can be achieved. Because the collected signal of fluorescence in near-field is extremely weak, a time correlated single photon counting method is utilized in order to gain a measurement sensitivity. Moreover, high-precision optical alignment system is proposed to suppress the optical noise. This system can improve the efficiency of collecting signal by automated alignment system that track a signal spot of fluorescence in near-field. Using our imaging system, we have visualized the cell adhesion and measure a distribution of fluorescence lifetime on the cell membrane.