Abstract

Selective Fluorescence and Fluorescence-free Single-Molecule Detection Techniques & Applications

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A novel multi-functional optical single-molecule imaging system was developed based on four dimensional (4D) real-time confocal microscopy, multi-color total internal reflection fluorescence (TIRF) microscopy, dark filed microscopy, and Nomarski differential interference contrast (DIC) microscopy on an epifluorescence microscope platform. The micro-cell incubator was also combined with the imaging system for long time monitoring in live single cell. The 4D images indicated a combination of 3D image and multiple spatial or time images of specimen obtained with 10-nm spatial interval. Optical sectioning was accomplished with a *z*-motor, which made the sequential layered sections to obtain 4D information. The integrated optical molecular imaging system showed excellent detection sensitivity and 3D spatial resolutions (i.e., *x*-*y*, *z*-axis = 20, 30 nm) without moving the single cell sample at the single-molecule level. Others detection techniques such as enhanced dark field illumination, total internal reflection scattering (TIRS), and super-resolution (SR) microscopy were investigated at the single-molecule level. Those are expected tools to provide crucial information and essential approaches for understanding various characterizations, dynamics of single-biomolecules and nanoparticles in live single cell as well as the molecular interactions at the single-molecule level.