DOCTORAL THESIS

Multifunctional Fluorescent Polymer Dots for Super-resolution Imaging

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Abstract

Conventional optical imaging techniques have encountered fundamental limitations due to the diffraction of light, which constraint the clear visualization of intricate structures and cellular processes. Super-resolution microscopy technologies have revolutionized biological studies by advancing the spatial resolution of optical microscopes to the nanometer scale. However, many currently available fluorescent markers exhibit insufficient brightness and photostability, impeding their ability to extend the use of super-resolution techniques in diverse applications. In this thesis, we demonstrate the development of a series of functional fluorescent polymer dots (Pdots) for different super-resolution imaging modalities. Pdots are widely used in optoelectronic and biological applications because they exhibit extraordinary brightness, robust photostability, and easy-to-modify composition. By varying the polymer species and surface properties, we have developed two types of BODIPY Pdots with narrow fluorescence spectra and pronounced photoblinking properties for two-color high-order super-resolution optical fluctuation imaging (SOFI) applications. Single particle fluorescence brightness of BODIPY Pdots is 6-8 times higher than those of Alexa Fluor dyes. After 8^{th}-order SOFI analysis, the spatial resolution of images of microtubules and mitochondria are enhanced by 5.8-fold and 4.8-fold, respectively, making the best improvements among various fluorescent probes in SOFI studies reported so far. In addition, we have developed three trifunctional MA-Pdots with targeting, anchoring, and fluorescing features for dual-mode super-resolution SOFI and expansion microscopy (ExM) imaging. The covalent anchoring and insensitivity to digestion ensure high labelling efficiency and fluorescence retention of MA-Pdots, alleviating the fluorescence degradation issue in ExM studies. We have demonstrated that MA-Pdots can target different subcellular structures and neuron synapses with high specificity. A surfactant-containing buffer has been developed to enhance the post-expansion fluorescence brightness and fluctuations, which provide sufficient detected photons and photoblinking properties for high-order SOFI applications. The integration of SOFI and ExM allows microtubules to be resolved with a spatial resolution of 30 nm and hollow labelling patterns of mitochondria outer membrane to be observed after three-dimensional analysis. Furthermore, we have developed three types of lifetime-multiplexed Pdots for combinatorial ExM and fluorescence lifetime microscopy imaging (FLIM). These Pdots exhibit overlapping emission spectra but distinct lifetime distributions across a 0.4 ns to 5 ns range. Single particle lifetime multiplex imaging of immunomagnetic beads indicated the potential of the lifetime-multiplexed Pdots for lifetime barcoding, encrypting, and anti-counterfeiting applications. The impressive fluorescence brightness and substantial photon output provided by Pdots facilitate multiplex lifetime imaging in photon-starved expansion microscopy, which has proven to resolve subcellular structures with a spatial resolution of ~49 nm. These results demonstrate that multi-functional Pdots with tunable properties are promising probes for super-resolution microscopy applications.