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Editorial

Fluorescent Probes for Live Cell Imaging

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1. Introduction

The discovery of green fluorescent protein (GFP) and the chemical labeling with fluorescent dyes as versatile tools for live cell imaging, has sparked a revolution in life science and biomedical research. As the fluorescent probes toolbox and imaging platforms continue to evolve, more sophisticated, non-invasive, and information-rich imaging experiments are made possible in more and more biological systems. Inspired by the versatility of these approaches, in addressing the important questions of many different areas, we put together a special issue dedicated to the ongoing endeavors in developing and applying fluorescent probes for live cell imaging.

In this special issue, a number of reviews and original research papers report on the state-of-the-art practices in this field, covering all aspects of live cell imaging including probe design, sample preparation, and labeling, application, instrumentation and data processing.

2. The Special Issue

The special issues included two excellent original articles and four reviews that showcase the use of fluorescent probes, in reporting different biological targets, while proposing new challenges that the field is still facing.

Wilson et al. explored the use of fluorescent intensity, of enhanced GFP (EGFP), for the quantification of the cell number in *Pseudomonas aeruginosa* bacterial cell suspension and biofilm [1]. They found a linear relationship between PA14/EGFP bacteria cell count and the fluorescence intensity, at 514 nm. Existing methods for quantification of planktonic and biofilm bacterial counts relied on plate counting of colony-forming units, which are laborious and time-consuming. The use of EGFP fluorescence as a proxy marker of bacterial counts affords a convenient and cost-effective way of monitoring bacteria cell accumulation.

Sylvia et al. reported the rational design and photophysical characterization of spiropyran-based chemosensors for magnesium (Mg^{2+}), a divalent cation important in mammalian cell function [2]. The sensor showed up to a two-fold increase in fluorescence upon interaction with Mg^{2+} and had a three-fold higher dissociation constant for Ca^{2+}, as compared to the Kd value for Mg^{2+}. The sensor reversibly bound Mg^{2+} and had improved photostability, as compared to the non-photoswitchable Rhodamine B fluorophore. The authors tested the chemosensor in a suspended core optical fiber, which represented a first step in the development of lighted-controlled, reversible dip-sensor for Mg^{2+}. Because Mg^{2+} deficiency has been linked to various pathological conditions, the development of probes for Mg^{2+} will play important roles in its detection, and functional studies in pathophysiological settings.

Galas et al. put together a comprehensive review that analyzed all three essential key elements for successful live cell imaging, i.e., the "Probe-Sample-Instrument" (PSI) triad [3]. The synthesis and photophysical properties of probes, strategies for probe labeling in various biological samples, and the choice of appropriate microscopy techniques were highlighted as the key aspects for live cell imaging

studies. The detailed analysis together with examples of applications should provide an important guide for researchers of any level who are considering such experiments.

Lavogina et al. reviewed the design, structure, and application of different classes of protein kinase probes that report on kinase activation in human live cells [4]. While examining their mechanism of actions, the authors raised crucial awareness of minimizing the interference in these biological systems caused by the introduction of these probes, which represents an important yet often underappreciated aspect of live cell imaging.

Bucevičius et al. reviewed the history and properties of Hoechst dyes—which have been widely used as probes—for staining DNA in the nucleus of cells [5]. Capitalizing on the remarkable affinity, and specificity, of Hoechst dyes towards DNA, the Hoechst moiety has been conjugated to other molecules, for a wide variety of applications. These hybrid molecules become powerful agents in sensing, targeting, and detection of events in the nucleus, bringing the old Hoechst dye a new life in the modern biological research.

Finally, Chen et al. reviewed fluorescent protein-based biosensors for imaging Ca^{2+}, voltage, and synaptic activity, which have become powerful and indispensable tools for modern neuroscience research [6]. Throughout the development of these tools, it is apparent that fluorescent proteins played a key role. By examination of the design and principle of the existing tools, they also proposed areas where future developments are needed.

To conclude, the reviews and papers published in this Special Issue highlighted the power and the potential of combing fluorescent probes with live cell imaging, in addressing questions that are challenging, otherwise, with other approaches. With new probes and instruments continue to emerge, it is envisioned that the future of this field will be glowing even brighter.

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