

# SPECIAL SECTION GUEST EDITORIAL

## Visible Fluorescent Proteins

The discovery of visible fluorescent proteins (VFPs) that emit light from the blue to the red range of the visible spectrum has revolutionized live-cell imaging by fluorescent microscopy. VFPs are now routinely used for studies in cell biology and physiology to detect the functional recruitment, colocalization, and interactions of proteins inside living cells and tissues. Historically, the green fluorescent protein (GFP) was first characterized by Shimomura et al.<sup>1</sup> in 1962 as the accessory protein to the bioluminescent aequorin protein in the jellyfish *Aequorea victoria*. The sequence coding the *Aequorea* GFP was cloned by Prasher et al.<sup>2</sup> in 1992, and then expressed in bacteria and nematode worms by Chalfie et al.<sup>3</sup> in 1994. Later, Miyawaki et al.<sup>4</sup> showed how a biosensor protein based on GFP and calmodulin could be used as a fluorescent indicator for Ca<sup>2+</sup> inside living cells. Since then, a variety of new and improved VFPs have been identified that provide high quantum yields and serve as a potential FRET pair to study protein-protein interactions.<sup>5-9</sup> Further, other VFPs with unique photoactivation or photoconversion properties have been identified and characterized.<sup>8</sup> These new VFPs, which are providing an ever-expanding toolbox of live-cell imaging probes, are reviewed in the introductory article in this special section of the *Journal of Biomedical Optics* (JBO).<sup>9</sup>

The generation of VFPs with improved characteristics has been particularly useful for studies of the protein-protein interactions in living specimens using various microscopy techniques.<sup>7</sup> The combination of different colored VFPs with optical methods that detect Förster (fluorescence) resonance energy transfer (FRET)<sup>7</sup> allow direct measurement of the spatial relationships between proteins in living specimens. This special section on VFPs highlights the use of the FRET methodology with fluorescent proteins for various applications. Here we provide a brief introduction of some of the papers included in this special section of JBO.

A new VFP, the monomeric teal fluorescent protein (mTFP),<sup>10</sup> is shown by Day et al. to be an improved donor protein in FRET applications, especially when using the 458-nm argon laser line as an excitation wavelength. As an alternative to conventional FRET microscopy, fluorescence lifetime imaging microscopy (FLIM) provides a method that is independent of intensity-based measurements to quantify measurements of protein associations in living cells and tissues. A FLIM technique does not require any spectral bleed-through correction and it allows monitoring of multiple protein pair interactions.<sup>7</sup> On the other hand, resonance energy transfer (RET) is also possible between identical fluorophores (cerulean-cerulean) and this is called energy migration RET (EM-RET). Koushik and Vogel describe in their paper the change in lifetime of cerulean that correlates with EM-RET. They suggest that for FLIM-FRET analysis, untagged cyan fluorescent protein (CFP) or cerulean as a donor should be avoided. Hoffmann et al. describe the issues involved with the change in lifetime of cerulean upon irradiation using a streak camera.

Most of the FRET measurements are implemented using VFPs or in combination with exogenous fluorophores or quantum dots in wide-field, confocal, two-photon, spectral imaging, and FLIM microscopy systems. Currently available VFPs-based FRET standards can be used to characterize FRET microscopy systems.<sup>11-13</sup> Li et al. use confocal and FLIM-FRET microscopy to study the spatial relationship of AT1R (angiotensin II type I receptor) with Rab4 and Rab11 during the course of AT1R recycling. Kofoed et al. use CFP and YFP as a FRET pair to study the effect of Er $\alpha$ -Er $\alpha$  (estrogen receptors) interaction during dimerization. Mikhailova et al. demonstrate, using CFP-YFP as a FRET pair, that the TM4 (fourth transmembrane region) may form part of the dimerization between VT2R (arginine vasotocin receptor) and CRHR (corticotrophin releasing hormone receptor). McGrath and Barroso demonstrate that the usage of quantum dots as a donor would simplify FRET data analysis in a biological system such as protein organization in intracellular membranes using a confocal FRET microscopy system. Rogers et al. demonstrate BRET measurements using an electron-multiplying charge-coupled detector (EMCCD) to monitor intracellular calcium at a single cell level. Lessard et al. characterize a far-red fluorescent protein called HcRed, originating from the sea anemone *Heteractis crispa*.

Other techniques such as fluorescence recovery after photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS) are used to study the mobility of protein molecules. Watanabe et al. investigate the dynamics of GFP-histone by two-photon FRAP in tobacco BY-2 cells. They find that the recovery rate in tobacco and HeLa cells are the same. Wu and Berland describe the usage of FCS to monitor protein mobility in living HEK cells. These measurements explain the role of molecular crowding in the intracellular mobility of proteins. Malengo et al. study the dynamics and oligomerization of uPAR (urokinase plasminogen activator receptor) in multiprotein complexes in living cells using FCS. For the active receptor, the diffusion coefficient decreases in monomer-enriched fractions, suggesting that uPAR monomers might be preferentially engaged in multiprotein transmembrane signaling complexes.

FLIM is not only used to study protein-protein interactions in general, but is also suitable for cancer studies. Tregidgo et al. demonstrate that the decrease in lifetime of the GFP molecule is observed in total internal reflection fluorescence (TIRF) microscopy. Ramanujam and Herman describe a FLIM scaling analysis of glucose metabolism in mammary epithelial cells by detrended fluctuation analysis of cerulean fluorescence. They also suggest that acute dependence of cancer cells on glycolysis as compared with normal cells may yield a statistically significant difference in the scaling exponent, thereby providing discrimination between normal and cancer cells *in vitro*. Provenzano et al. describe the usage of exogenous fluorophores (variants of GFPs) and endogenous proteins (tryptophan, NADH, FAD) for breast cancer studies by multiphoton excitation spectral lifetime imaging. They

have developed a novel method to visualize and analyze complex spectral lifetime data sets.

Here we refer to only a few papers, but the readers are encouraged to look at all the references listed in these papers. We thank the authors for their valuable contributions and timely response to all the reviewers' comments. We are also very grateful to the review panel members for their timely response to make this special section possible. The editor (AP) wishes to acknowledge the valuable support provided by the W.M. Keck Center for Cellular Imaging at the University of Virginia.

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