siFLIM: dedicated single-image Fluorescence Lifetime IMaging for fast read-out of cell signaling

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Förster(Fluorescence) Resonance Energy Transfer (FRET) has become a powerful tool to study protein-protein interactions and signal transduction in living cells. FRET is commonly read out either by detecting the ratio of the donor and acceptor intensities (sensitized emission) or by detecting the excited state lifetime of the donor, which decreases with increasing FRET (Fluorescence Lifetime IMaging or FLIM). FLIM is robust, immune to bleaching and inherently quantitative, but typically rather slow and photon-inefficient. Moreover, FLIM detection typically requires several images to be collected from the cells, leading to potential artifacts in lifetime when FRET changes rapidly, for example, during fast transients in metabolite concentrations, and when vesicles move within the cell.

Using a new generation of cameras capable of collecting two phase images simultaneously, we developed a fast and artifact-immune technique to obtain lifetime images in just a single image. This approach, which we termed single-image FLIM (siFLIM), dramatically increases speed and photon efficiency of fluorescence lifetime detection[1]. We show that in time-lapse experiments, lifetimes calculated from siFLIM closely match those calculated from conventional 12-phase FLIM experiments, even though siFLIM requires much less photons.

To match the power of siFLIM, we also developed a series of dedicated FRET sensors tailored for FLIM detection. These sensors employ mTurquoise2[2] as a donor, and they have dark variants of YFP as acceptors. We sandwiched the cAMP-binding protein EPAC between the mTurquoise2 donor and a tandem of two monomeric dark Venus acceptors to generate a superior cAMP sensor[3], and also generated a novel dark-acceptor sensor to report activity of Gαq in living cells. We show that with these FLIM sensors, siFLIM is fast enough to follow rapid cell signaling events at video rate.

[1] Raspe et al, siFLIM: Single Image Frequency-Domain FLIM provides fast and photon-efficient lifetime data; submitted. [2] Goedhart et al, Nat Commun. 2012; [3] Klarenbeek et al, 4th-generation cAMP FRET sensors, PLoS One, 2015.