Phasor S-FLIM: a change of paradigm

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Fluorescence Lifetime Imaging Microscopy (FLIM) and spectral imaging are two broadly applied methods for increasing dimensionality in microscopy. However, their combination is typically inefficient and slow in terms of acquisition and processing. By integrating technological and computational advances, we built a robust and unbiased Spectral FLIM (S-FLIM) system. Our method combines true parallel multichannel Digital Frequency Domain (DFD) electronics with a multidimensional phasor approach to extract detailed and precise information about the photophysics of fluorescent specimens at optical resolution, named Phasor S-FLIM. To show the flexibility of the Phasor S-FLIM technology and its applications to the biological and biomedical field, we address four common, yet challenging, problems, such as the blind unmixing of spectral and lifetime signatures from multiple unknown species, the unbiased bleedthrough- and background-free Föster Resonance Energy Transfer (FRET) analysis of biosensors, the photophysical characterization of environment-sensitive probes in living cells and parallel 4-colors FLIM imaging in tumor spheroids.