19a-D4-7

Determining the diffusion coefficient of fluorescent beads through phasor-FLIM

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FLIM or fluorescence lifetime imaging microscopy [1] traditionally provides a versatile tool for spatially-mapping fluorescence lifetimes and macromolecular interactions [2] through pixel-by-pixel resolution of the excited-state lifetime.

Here, we show that FLIM can also measure lateral motions. In conventional frequency-domain FLIM the phase and modulation of the detected fluorescence are determined by the photophysics of the fluorophore only. However, translational motion on the timescale of FLIM acquisition can significantly perturb apparent phase and modulation values owing to intensity fluctuations. This perturbation can be visualized most conveniently in phasor plot [3, 4].

In FLIM experiment, we focus on motions that cause large intensity fluctuations on the timescale of image acquisition. A simple analytic theory, numerical simulations and measurements on fluorescent beads by means of the phasor plot is defined. Fluctuations due to particle motions increase the number of data points on phasor plot and their area of aggregation, an effect we refer to as phasor broadening. We relate the phasor broadening to diffusion coefficient of beads. The results exhibit a new application of FLIM for detecting and determining translational motions.

References:

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