FLUORESCENCE LIFETIME IMAGING USING PHASOR ANALYSIS

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KEY WORDS: two-photon microscopy, fluorescence lifetime imaging, time-correlated single photon counting, in vitro, NADPH oxidase activation, phasor analysis

Fluorescence lifetime imaging microscopy (FLIM) is a technique to generate images which contrast is obtained by the excited-state lifetime τ of fluorescent molecule instead of intensity and emission spectrum. Typical lifetimes are in the range of a few nanoseconds. In contrast to conventional microscopy techniques FLIM helps to identify fluorophores with similar emission spectra, but different lifetimes. Due to this property it is possible to image biochemical processes with microscopic resolution within living cells or even living organisms. Here we expose our phasor strategy to determinate the fluorescence lifetime measured *in vitro/ in vivo* by time correlated single photon counting device (TCSPC).

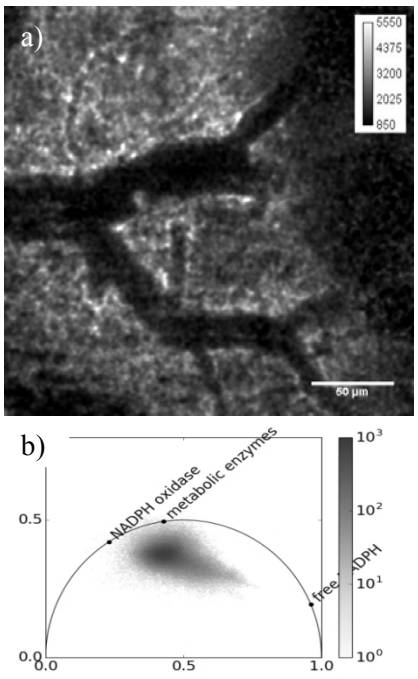

Fig 1: NADPH oxidase activation *in vivo* in mouse brain a) phasor analyzed FLIM image, lifetimes in ns b) corresponding phasor plot

In time domain FLIM measurement the determination of lifetime is based on fitting the histogram of photon delay exponentially whereat the decay constant is the lifetime of fluorophore. In the ideal case only a single species of fluorophores populated the observed excitation volume the temporal decay in time-domain measurement is mono-exponential. But most samples are heterogeneous, which means that the decay contains two or even more lifetime components and complicates the analysis of lifetime, especially if the number of lifetime components is unknown.

For that case the phasor approach [1] is a promising analysis method. Here the lifetime data are measured in time-domain but transferred to a virtual phase domain by calculating the discrete Fourier Transformation numerically. The sum of all contained fluorescence lifetimes can be calculated from the normalized real and imaginary result of that transfer. In case of a mono-exponential decay plotting those results will give a position named phasor of a half-circle (r = 0.5, centrum at (0.5/0)). If the excitations volume contains two fluorescent species, this position in the plot will lie along the straight line connecting the phasors of pure components.

[1] Digman, Michelle A., et al. "The phasor approach to fluorescence lifetime imaging analysis." *Biophysical journal* 94.2 (2008): L14-L16.