Spectrally resolved fluorescence lifetime and FRET measurements

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We present two different approaches that allow multi-wavelength fluorescence lifetime measurements in the time domain in conjunction with a confocal laser scanning microscope and a pulsed excitation source. One technique is based on a streak camera system, the other technique is based on a time-correlated-single-photon-counting (TCSPC) approach. When applied to Förster resonance energy transfer (FRET) measurements, these setups are capable to record time-resolved fluorescence decays for the donor and the acceptor simultaneously.

Lifetime-based FRET measurements can provide useful information about the interacting partners which is not accessible via intensity-based measurements. Moreover, only lifetime based FRET measurements allow to test if a kinetic model is consistent with the data. Under the assumption that only a part of the donor molecules interacts with an acceptor and that associated donor-acceptor pairs are separated by a single fixed distance a biexponential function can be used to describe the donor fluorescence decay adequately. From the parameters recovered by a biexponential fit one can determine the efficiency of energy transfer and get an estimate of the fraction of bound and unbound donor molecules. It should be the aim of any lifetime-based FRET measurement to determine these parameters as accurately as possible.

Conventional lifetime imaging techniques, however, record only the donor fluorescence decay in the part of the donor emission spectrum that does not overlap with the acceptor fluorescence spectrum. This approach has one disadvantage: When FRET efficiency is high, donor fluorescence is quenched effectively and donor fluorescence signals are weak. In this case, it can be difficult to analyze reliably the donor fluorescence decay. We show how this problem can be overcome by multiwavelength lifetime measurements. Instead of a single fluorescence decay curve a fluorescence decay surface can be reconstructed from the respective data sets (Fig. 1) and subjected to a global analysis. In this way the parameters of the fit can be determined more accurately.