

Fluorescence Lifetime FRET for Detection of Dimerization of Transcription Factor Proteins Using an Upgrade Kit for Confocal Microscopes

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Confocal Laser Scanning Microscopes (LSMs) are widely used tools in biochemistry, cell biology and other related sciences today. The capabilities of the instruments can be greatly enhanced by adding time as another dimension of information. In that way new measurement modes like FLIM, lifetime-FRET and FCS become feasible. The upgrade is easily done using picosecond diode lasers along with time-correlated single photon counting (TCSPC) electronics and detectors for data acquisition. Key to the new capabilities is a special measurement mode, which stores the full photon information in a generalized format. The pulsed diode lasers are integrated in a special laser coupling unit that allows the combination with the continuous-wave (cw) lasers of the LSM. In this way it is possible to easily change from cw lasers for typical LSM experiments to pulsed lasers used for FLIM measurements independent of the chosen wavelength.

The power of this approach will be shown for time-resolved FCS experiments and lifetime-FRET measurements of the interaction of proteins in their native environment. The example demonstrates the detection of dimer formation of transcription factor proteins in the nucleus of living cells. Due to the dimer formation the fluorescence of the donor FRET molecule is quenched leading to a decrease in fluorescence lifetime. In this example the fluorescence lifetime is used for FRET analysis and therefore avoids all problems associated with intensity based FRET analysis. The example is essentially a FLIM-FRET measurement, which images the FRET efficiency and therefore visualizes directly the proximity of the donor and the acceptor molecule.

(Sample courtesy of Ye Chen and Ammasi Periasamy, Keck Center for Cellular Imaging, University of Virginia).

FCS experiments can not only be used to determine diffusion coefficients, but also allow to determine the concentration of the fluorophore. However, scattered light (Rayleigh, Raman) always contributes to the measured signal and hampers the accurate determination of the concentration easily by one order of magnitude. This presentation demonstrates how lifetime assisted FCS overcomes this limitations, because it can easily discriminate the scattered light contribution from the fluorescence decay. In that way more accurate concentration measurements can be performed.