An introduction into Fluorescence Lifetime Imaging and Correlation Spectroscopy

Volker Buschmann, Benedikt Krämer, Matthias Patting, Felix Koberling, and Rainer Erdmann

PicoQuant GmbH, Rudower Chaussee 29, 12489 Berlin, Germany
www.picoquant.com, info@picoquant.com

KEY WORDS: Fluorescence Lifetime Imaging (FLIM), Förster Resonance Energy Transfer (FRET), Fluorescence (Lifetime) Correlation Spectroscopy (F(L)CS), Time-correlated Single Photon Counting (TCSPC), Confocal Laser Scanning Microscopy (cLSM)

The upgrade of confocal LSM towards time-resolved methods allows for new methods like FLIM, FRET and FCS, and contains pulsed lasers, sensitive photon counting detectors and time-correlated single photon counting electronics.

The fluorescence lifetime depends on the fluorophore environment (e.g. polarity, pH, temperature, ion concentration), thus enabling to sense the local environment inside cells and tissues. Furthermore, FLIM is applied to discriminate multiple labels and to eliminate signal artefacts (e.g. autofluorescence). With FLIM, the autofluorescence itself is used to identify certain components within plant tissues.

FRET enables to determine intra- and intermolecular interactions in vitro and in vivo. Furthermore, FRET sensors monitor environmental conditions in cells like pH. FRET measurements inside cells are significantly improved by FLIM since changes in the donor fluorescence lifetime are monitored that are concentration independent. Thus, FLIM-FRET can reveal sub-populations and allows to determine the fraction of free compared to bound donor molecules within a complex.

FCS is a correlation analysis measuring the temporal fluctuations of the fluorescence intensity caused by single fluorescent molecules entering or leaving the observation volume (or changing their fluorescence properties). Thus, this technique can measure diffusion behavior, interaction and absolute concentrations of labelled molecules within cells and reveals the photophysics of fluorophores.