# INTEGRATION OF TIME-RESOLVED FLUORESCENCE TECHNIQUES FOR CONFOCAL LASER SCANNING MICROSCOPES

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Confocal laser scanning microscopes (CLSM) are an essential tool in biological and biomedical research. Their functionality can be further enhanced by adding time-resolved data acquisition capabilities, enabling Fluorescence Lifetime Imaging (FLIM) and Fluorescence (Lifetime) Correlation Spectroscopy (F(L)CS) down to the single molecule level.

One of the results obtained with such a system shows how distance measurements on a nanometer scale can be improved applying FLIM-FRET. The quantitative evaluation of FRET measurements is in principle straight forward, if all FRET molecules are labelled with donor and acceptor molecules. Incomplete labelling, however, is one of the most prominent sources of error for the determination of the FRET efficiency. The presented results show how FLIM FRET measurements and an analysis based on a two-exponential decay model can be used to separate complete from incomplete labelled molecules. The result of such an analysis yields not only the lifetime distribution of FLIM FRET images, but also the distribution of complete to incomplete FRET molecules. Furthermore, FRET efficiencies and distances of the complete FRET molecules only can be derived, avoiding the errors introduced by the incomplete labelling. The results allow to distinguish easily between different FRET efficiencies and variations in the amount of complete to incomplete FRET molecules inside living cells.
The complete analysis is performed using an easy-to-use scripting language of the data analysis software “SymPhoTime”. All results are generated as false color images and histograms.