

**The Leica TCS SMD series:
A new platform for single molecule detection and analysis techniques**

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Molecular interactions, such as protein complex formation, protein DNA interactions or ligand-receptor binding are of utmost significance for quantitative biology. The specific identification of interacting molecules and quantification of binding parameters are important for the development of predictive models. Such models will deepen our insights into living cells on a biological, chemical, and physical level.

Further understanding and quantitative characterization of such basic processes can be obtained by the examination of single molecules. Well established methods in this field are Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Lifetime Imaging Microscopy (FLIM). The combination of FCS with fluorescence lifetime, Fluorescence Lifetime Correlation Spectroscopy (FLCS) and gated FCS, allow reducing cross talk and improving signal quality. Spectral imaging combined with FLIM leads to a new depth of data analysis and interpretation. The goal is to maximize the extracted information inherent to fluorescence.

We introduce the TCS SMD series, which integrate hard- and software from PicoQuant GmbH (Berlin, Germany) with our high end confocal system TCS SP5. This series constitutes a flexible platform for a variety of single molecule detection demands, particularly of FCS and FLIM. The complete data acquisition is controlled by one single software. Straightforward application wizards guarantee an easy handling and allow automated recording of FLIM volume stacks and FLIM lambda stacks for spectral and time resolved imaging. FCS time series conducted at data points predefined in three dimensions allow automated measurements of diffusion parameters in living cells.

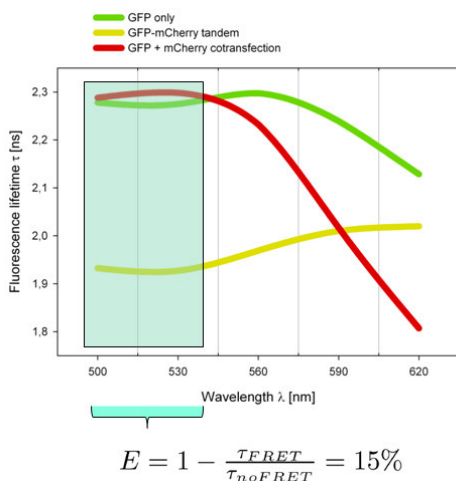


Figure 1: Spectrally optimized computation of FRET efficiency from spectral FLIM image stacks.

Samples: GFP (donor), GFP-mCherry tandem (FRET pair), and GFP and mCherry (FRET negative control) transfected into HeLa cells
Courtesy: M. Weiss, J. Szymanski, DKFZ, Heidelberg, Germany

Excitation: 470 nm, 40 MHz repetition frequency

The acquisition of a spectral FLIM stack allows optimizing the spectral range used for computation of FRET efficiency.