

## RECENT TECHNICAL DEVELOPMENTS IN TIME-RESOLVED MICROSCOPY FOR DIFFUSION STUDIES DOWN TO THE SINGLE MOLECULE LEVEL

P. Kapusta, U. Ortmann, S. Orthaus, B. Krämer, M. König, V. Buschmann, F. Koberling, A. Bülter, and R. Erdmann

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

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Modern time-resolved measurements permit to follow fluorescence dynamics from sub-nanosecond range up to fluctuations in the second range and beyond. The underlying universal technique (Time-Tagged Time-Resolved (TTTR) recording) allows to synchronizing the data acquisition with external hardware such as scanners and to record simultaneously timing as well as intensity, spectral and spatial information on a single photon basis. By exploiting the full information content of such measurement data, classical intensity based analysis schemes like FCS can be improved by sorting and weighting the detected photons. This talk will illustrate some of the principles and new methods such as:

- **Fluorescence Lifetime Correlation Spectroscopy (FLCS)**, which allows in a superior fashion to suppress common parasitic contributions like Raman scattering and detector afterpulsing [1]. The technique also enables to study diffusion properties of different species which just differ in their fluorescence lifetime without the need for multicolour labeling.
- **Two-Focus FCS (2fFCS)**, in which orthogonally polarized Pulsed-Interleaved Excitation (PIE) is used to build up a robust dual foci geometry with a well known focal distance. This intrinsic length scale can now be used to study diffusion in solution and allows to overcome various uncertainties like relying on the size and shape of the confocal volume in single focus FCS. It also dramatically improves the accuracy in determining absolute diffusion coefficients [2].
- Combination of a sample scanning **Atomic Force Microscope (AFM)** with a single-molecule-sensitive confocal fluorescence microscope in order to perform measurements of force and topography on a single particle whilst simultaneously monitoring its response using fluorescent probes
- Combination of single molecule sensitive confocal microscopy with single molecule sensitive spectrometers

References:

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