

Conference 7185: Single Molecule Spectroscopy and Imaging II

nanoprobes for targeting of actin filaments in different cell types including lung, oral, skin and breast cancer. We proposed two approaches to verify specificity of the actin labeling with gold nanoparticles using fluorescent nanoprobes in live cells and standard immunochemistry method to stain fixed cells. Future development of this approach for visualization of intracellular interactions between biomolecules allow us to delineate growth factor activated signaling networks that can lead to improved strategies for cancer prevention and therapy.

References.

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7185-24, Session 7

Recent advances in photon coincidence measurements for photon antibunching and full correlation analysis

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Photon Coincidence Analysis is nowadays a widely used technique to study fluorescence intensity fluctuations, taking place on a timescale from seconds down to picoseconds. Photon bursts in the microsecond regime are e.g. used to study diffusion properties via Fluorescence Correlation Spectroscopy (FCS). Photon bunching in the microsecond regime allows to study fast conformational changes as well as internal photophysics like singulett-triplet transitions. Interphoton delay times in the ns regime carry information about the fluorescence lifetime and can also be used to characterise molecular rotation. Down in the picosecond regime, photon antibunching is used to quantify a small number of emitters and especially to proof the existence of a single emitting dye molecule.

All of these methods can be carried out with the single molecule sensitive confocal fluorescence microscope MicroTime 200 and are based on time-correlated single photon counting (TCSPC). We developed a generalized approach to store the individual photon arrival time information with ps accuracy on a timescale up to hours which allows to study all mentioned phenomena in a single measurement (Full Correlation Analysis). Using the new HydraHarp 400 TCSPC unit we can now acquire photon information in 4 completely independent detection channels. We will present the straightforward experimental concept as well as typical results and recent application examples.

7185-25, Session 7

High-speed low-cost correlator for single molecule fluorescence correlation spectroscopy

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Fluorescence correlation spectroscopy (FCS) has been extensively applied to study the kinetics and photophysics of molecules as well as interactions between molecules by extracting information from the fluctuation of signals. In particular, single molecule applications of FCS promise the greatest amounts of information. Ideally, one would like to carry out FCS in real-time, however, due to the time-consuming nature of the correlation process, performing the correlation in real-time is totally nontrivial. Generally an expensive hardware correlator or a TCSPC board is required for this purpose. Recently highly-efficient algorithms based on multi-tau method have been proposed to build up a software correlator.

In this work, we set forth an innovative algorithm capable of realizing the real-time correlation, without turning to the multi-tau method. This algorithm

takes advantage of the low count rate generally existing in the FCS experiments, directly using the time interval between each photon its adjacent photon to efficiently update the correlation function. Based on this efficiency, it is possible to build a low-cost software correlator with just an ordinary counter board. We practically demonstrate the feasibility by setting up this correlator to measure the diffusion motion of rhodamine 6G in water using FCS. The algorithm was validated by duplicating the signal from the photon detector and sending it to both the ordinary counter board with our software correlator and a commercial correlator simultaneously. The perfect coincidence of the correlation curves from these two correlators and the real-time display of the correlation function indicate the validity and practicability of our approach.

7185-26, Session 7

Femtosecond pulse shaping for single molecule measurements

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Shaped optical pulses have demonstrated great utility for the study and control of quantum coherences [1-5] in molecules leading to e.g. selective bond-breaking [6], controlled energy transfer [7] and isomerisation [8]. Further, shaped pulses have been successfully employed in multiphoton spectroscopy[9] and microscopy[10]. Expanding the application field of shaped optical pulses to the nanoscale, in particular to single molecule spectroscopy, prospects highly interesting insight into complex (bio-) systems.

Pulse-shaping techniques induce spatio-temporal distortions that can lead to a different time evolution of the spectrum at different positions in the beam, with consequent implications for e.g. quantum control and pump-probe experiments. Clearly, this spatio-temporal coupling can have a profound effect on measurements on the nanoscale, where the subjects of investigation are smaller than diffraction limited foci.

Here, we present a novel pulse shaping scheme that avoids spatio-temporal distortions, and therefore allows accurate measurements at the nano-scale. This pulse shaping technique is used to control the excitation probability of single molecules by shaping pulses on the femtosecond to picoseconds timescale. Unprecedented information about the ultrafast dynamics and spectral properties of the single molecules is obtained. Measurements on single biomolecules can be envisaged that would provide unique insight into the role of coherences in biological processes.

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