SINGLE MOLECULE MICROSCOPY: FROM NANODIAMONDS TO NANOMANIPULATION

Benedikt Kraemer 1, Felix Koberling 1, Marcelle Koenig 1, Volker Buschmann 1, Michael Wahl 1, Olaf Schulz 2, Robert Ros 2 and Rainer Erdmann 1

1 PicoQuant GmbH, Rudower Chaussee 29, 12489 Berlin, Germany, info@picoquant.com
2 Arizona State University, Tempe, AZ 85287-1504, USA, robert.ros@asu.edu

KEY WORDS: Single Molecule Spectroscopy, AFM, High Resolution Imaging, Fluorescence, FLIM, confocal, quenching, nanodiamond

Single molecule based techniques made their way from studies of biological dynamics and conformations, towards DNA sequencing and ultra high resolution imaging. From the very beginning, confocal microscopy was the workhorse due to its versatility and straightforward multiparameter detection capability.

Photon coincidence measurements (antibunching) started as the ultimate proof for the existence of a single emitter but nowadays also decipher the true number of immobilised emitters in the sub-micron observation volume. We use this technique to characterise a new and promising class a luminescent labels, NV defect centers in single nanodiamonds, where knowledge about the number of independent emitters in a single nanodiamond is the prerequisite to understand the complex fluorescence decay behaviour.

The combination of atomic force microscopy (AFM) with single-molecule-sensitive confocal fluorescence microscopy enables a fascinating insight into the structure, dynamics and interactions of single biomolecules and their assemblies. Sub ensemble single fluorophore counting becomes possible as well as the observation of sub-diffraction imaging features.

Adding an AFM tip to the confocal observation volume allows to complement the optically acquired information with topographic imaging. In addition, nanophotonic effects, such as fluorescence quenching or enhancement due to the AFM tip, are used to increase the optical resolution beyond the diffraction limit, thus allowing to identify different fluorescence labels within e.g. a macromolecular complex. Silicon tip induced single molecule quenching could be demonstrated on individual organic fluorophores [1].

References: