

**PROBING BROWNIAN ROTATIONAL MOTION
BY FLUORESCENCE CORRELATION SPECTROSCOPY
IN THE NANOSECOND TIME RANGE**

P. Sandeep and A. Volkmer

3rd Institute of Physics, University of Stuttgart, 70550 Stuttgart, Germany

E-mail: a.volkmer@physik.uni-stuttgart.de

KEY WORDS: Brownian rotational diffusion, fluorescence correlation spectroscopy, confocal fluorescence microscopy, time-tagged time-correlated single-photon counting.

By monitoring the Brownian motion of a fluorescent biomolecule of interest in free solution, information regarding its hydrodynamic volume as well as its solvent properties (i.e. viscosity) is obtained. While the translational diffusion of a fluorescent biomolecule, typically occurring on the micro- to millisecond time scale, is conveniently obtained from a conventional fluorescence correlation spectroscopy (FCS) experiment, the more sensitive Brownian rotational dynamics of the molecule, typically occurring on the pico- and nanosecond time scale, is generally obtained from the measurement of its time-resolved fluorescence anisotropy upon pulsed excitation. The application of the latter technique, however, is inherently limited to the measurement of rotational correlation times not exceeding the fluorescence lifetime of the fluorophore. Hence, the accurate measurement of the rotational diffusion of a biological macromolecule, which is typically in the order of tens of nanoseconds, is not feasible.

Based on recent advances in time-tagged time-correlated single photon counting technology [1] in combination with confocal fluorescence microscopy, we demonstrate polarization-sensitive FCS extended to the pico- and nanosecond time range. This is achieved by investigating the fluorescence by means of registering distinct photon arrival times with picosecond time resolution followed by an analysis in terms of their second-order correlation function. This approach not only allows us to probe the Brownian rotational diffusion of a biomolecule in free solution at any time scale between a few picoseconds and the translational diffusion time without the need for pulsed excitation, but also enables us to simultaneously measure both the translational and rotational diffusion of a biological macromolecule within a sub-femtoliter sample volume. By taking into account the photo-physical properties of the fluorophore, i.e. the fluorescence lifetime and triplet kinetics, we present a detailed experimental characterization of the Brownian rotational motion contributing to the recorded fluorescence intensity correlation function in comparison with its exact theoretical treatment [2,3].

[1] S. Felekyan et al., *Rev. Sci. Instrum.*, 76 (2005) 083104.

[2] M. Ehrenberg and R. Rigler, *Chem. Phys.*, 4 (1974) 390.

[3] S.R. Aragon and R. Pecora, *Biopolymers*, 14 (1975) 119.