Measuring Rotational Dynamics with Super-Resolution Imaging

Seongjun Park and John T. King
IBS Center for Soft and Living Matter
UNIST, Ulsan, 689-798, South Korea
E-mail : jtking.ibs@gmail.com

KEY WORDS: Super-resolution microscopy, STED imaging, anisotropy

Measurements of fluorescence anisotropy are powerful tools for studying both biological and material systems, offering fascinating possibilities to study molecule orientation and mobility as wells as processes that affect them [1]. Using femtosecond pulsed excitation with polarization selective time-correlated single-photon counting allows the rotational dynamics of molecular probes to be measured quickly. Changes in the microenvironment, such as solvent viscosity or the size of the molecules, leads to changes in the rotational rate and diffusion, resulting in different anisotropy decay timescales. Given the speed at which anisotropy decays can be experimentally measured it is natural to couple this approach with microscopy for gain spatial-resolution. However, most polarization dependent microscopy is based on a far-field microscope, in which the spatial resolution is limited to the diffraction barrier (> 200 nm) [2]. Therefore, the structural and dynamic variations below the diffraction limit cannot be resolved, making it difficult to understand the heterogeneous dynamic of biology, physics and material science on this nanoscopic scale [2].

Here, we propose the anisotropy-measuring stimulated emission depletion (STED) microscopy. As STED microscopy achieves the resolution below 50 nm, it offers a non-invasive tool for the investigation of the structure and dynamics of a wide variety of soft materials with nanoscale structure and dynamics. By introducing polarization-sensitive detection to a STED microscope, measurements of anisotropy can be possible with < 50 nm spatial resolution. We expect that the proposed system would provide a powerful tool for measuring the orientation of the bio-molecules including fluorophores bound to the membrane that are truly affected by internal viscosities of membranes and lipid composition upon the membrane phase-transition. In addition, as the temperature changes affects the viscosity, thus the rotation diffusion, STED-anisotropy offers an avenue to measure nanoscopic thermal maps.

[1] J. R. Lakowicz, Principles of Fluorescence Spectroscopy, Chapter 10. (Springer, 2011).
[2] K. I. Willig, B. Harke, R. Medda, and S. W. Hell, "STED microscopy with continuous wave beams," Nat. Methods **4**, 915–918 (2007).