Mapping Local Solvent Mixing in Microfluidics with Two-photon STED-Anisotropy Imaging

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

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Fluorescence anisotropy based measurements are powerful tools for studying the rotational time of biological and material systems, which can be related to the local viscosity around a chromophore. Extending this methodology to super-resolved fluorescence anisotropy imaging, with < 200 nm spatial resolution and sub-second temporal resolution, offers fascinating possibilities to study molecular dynamics in heterogeneous, non-equilibrium systems [1].

Here, we propose the two-photon STED-anisotropy microscopy to measure the viscosity profiles in soft matter systems. The technique is demonstrated on an example system of practical importance: the transverse broadening of two miscible liquids mixing in a microfluidic channel [2]. To date, measurements of transverse broadening rely on confocal imaging that cannot distinguish chromophore diffusion from solvent mixing, which is easily distinguished simultaneously with fluorescence anisotropy imaging. We expect that the proposed system can provide a useful tool for studying the dynamics of bio-molecules, in vivo temperature mapping, and non-equilibrium polymer physics with high spatial and temporal resolution and at great depth.

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[2] Whitesides and Howard Stone Experimental and theoretical scaling laws for transverse diffusive broadening in two-phase laminar flows in microchannels," Appl. Phys. Lett. **76**, 2376 (2000).