Resolving single-molecule FRET under stimulated emission depletion by using STED-MFIS microscopy

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Stimulated Emission Depletion (STED) microscopy [1] and Multiparameter Fluorescence Image Spectroscopy (MFIS) [2,3] were combined to selectively measure and characterize biomolecular systems on surfaces and in living cells with molecular resolution. While MFIS allows for detailed spectroscopic analysis and provides Ångström resolution via Förster Resonance Energy Transfer (FRET), STED microscopy overcomes the diffraction limit and localizes molecules with a resolution down to 30 nm. Thus, macromolecules can be localized with nanometer accuracy (STED), while monitoring their structure with Ångström resolution (FRET). The fruitful combination of both techniques is demonstrated in a benchmark study using double dye labeled DNA duplexes as spectroscopic ruler. Systematic distance variation within a FRET pair up to the practical resolution limits of STED allows us to map the localization of macromolecules with high precision and to resolve their inter- and intramolecular structural and dynamic features. Simultaneously different procedures to determine the resolution are tested. Finally, the applicability in cellular imaging is demonstrated , allowing to unravel processes in living cells down to the single-molecule level.

[1] Hell, Stefan W., and Jan Wichmann. "Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy." *Optics letters* 19.11 (1994): 780-782.

[2] Kudryavtsev, Volodymyr, et al. "Monitoring dynamic systems with multiparameter fluorescence imaging." *Analytical and bioanalytical chemistry* 387.1 (2007): 71-82.

[3] Weidtkamp-Peters, Stefanie, et al. "Multiparameter fluorescence image spectroscopy to study molecular interactions." *Photochemical & Photobiological Sciences* 8.4 (2009): 470-480.