

Single molecule spectroscopy on the nanoscale with focused visible light

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Because it combines molecular specificity with the use of visible light, which penetrates deep into living tissue fluorescence far-field microscopy allows imaging and functional analysis inside complex biological systems. Unfortunately, the diffraction barrier determines a minimal size of approximately 200nm for the focal spot when focusing light using far-field techniques. Both fluorescence imaging and fluorescence fluctuation analysis are hampered by this because they cannot be used to analyze features or dynamic processes that take place on length scales shorter than the diffraction limit. Fluctuation analysis, which is a single-molecule technique, is thus also limited to samples where the dye concentration is low enough to ensure the presence of only a few emitters in the diffraction limited detection volume.

In imaging, photoswitching dye molecules between at least two distinguishable molecular states such as a 'bright' and a 'dark' state has allowed breaking of the diffraction barrier by sequential readout of sub-diffraction spatial information. For example, the first such technique, STED microscopy, uses stimulated emission to squeeze the detection volume.

Combining switching techniques with fluorescence fluctuation spectroscopy and other single molecule techniques is similarly promising as their application to imaging: Switching can be used to either scale the concentration of visible dyes or the detection volume. This allows the application of single molecule techniques at almost arbitrary concentrations and the analysis of ultra-fast dynamic behavior on length-scales of a few tens of nanometers.

References:

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