

SPECIES-SELECTIVE NANOSCALE IMAGING IN FIXED AND LIVING CELLS

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Far-field fluorescence microscopy is a non-invasive and very sensitive analysis technique, allowing for the disclosure of complex biological systems. However, its applicability is often blocked due to two reasons. 1) Diffraction prevents that in far-field images one may resolve alike objects closer together than about 200nm. 2) Co-localization of various biological molecules requires the institution of several markers differing in at least one spectroscopic property such as colour, which may challenge large experimental complexity. We present far-field imaging with nanoscale resolution and simultaneous simple but precise allocation of different markers. Nanoscopic imaging is based on photoswitching of single-emitters and the determination of their positions with sub-diffraction resolution on a continuously running camera. Observing fluorescence on two different channels detecting, for example, different wavelength ranges or different polarization directions allows separating several markers based on their spectroscopic single-molecule signature, while using a single laser source only. We demonstrate far-field images of fixed and living cells with down to 10-20 nm spatial resolution, highlighting the co-localization of up to four different structures. The presented approach represents a new class of functional imaging that may shed light on still unresolved biological problems.