

Molecule-scale resolution and dynamics in fluorescence microscopy

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I will discuss MINFLUX [1-4], a recent molecular localization and superresolution method that has reached Angström localization precision and resolution of the size of a fluorophore molecule. MINFLUX and the related MINSTED concept [5,6] are being established for routine applications in cell and molecular biology, structural biology and neuroscience. Relying on much fewer fluorescence photons than the widely used camera-based localization methods, these techniques are poised to characterize dynamic processes of single proteins, as demonstrated by tracking the nanometer conformational changes of the motor proteins kinesin-1 [7] and dynein in living cells [8]. MINFLUX has also been demonstrated to measure intramolecular distances with Angström precision, providing a precise and reliable alternative to FRET [9]. Harnessing confocal detection, MINFLUX also provides nanometer-range resolution deeper down in layers of cells and (mildly) scattering tissue [10]. Finally, I will show an arguably surprising ability of MINFLUX to separate individual identical fluorophores without sequential ON/OFF switching or activation of fluorescence. Thus, the simultaneous, uninterrupted, nanometer-scale tracking and imaging of multiple, identical (same-color) fluorophores becomes possible for the first time [11]. This novel superresolution principle should allow MINFLUX to reveal the conformational changes of individual proteins in their native environment.

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