

Localization microscopy coming of age: From concepts to biological impact

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Super-resolution fluorescence imaging by single-molecule photoactivation or photoswitching and position determination (*localization microscopy*) has the potential to fundamentally revolutionize our understanding of how cellular function is encoded at the molecular level. Among all powerful high-resolution imaging techniques introduced in recent years localization microscopy excels at it delivers single-molecule information about the distribution and, adequate controls presupposed, even absolute numbers of proteins present in subcellular compartments. This provides insights into biological systems at a level we are used to think about and model biological interactions. We briefly introduce basic requirements of localization microscopy, its potential use for quantitative molecular imaging, discuss present obstacles and ways to bypass them, and give future prospects how the refined understanding of photoswitching mechanisms can pave the way to further increase the spatial resolution of localization microscopy approaching real molecular dimensions.