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Advances in super-resolution imaging technologies JENNIFER LIPPINCOTT-SCHWARTZ, National Institute of Health

Superresolution techniques such as photoactivated localization microscopy (PALM) enable the imaging of fluorescent protein chimeras to reveal the organization of genetically-expressed proteins on the nanoscale with a density of molecules high enough to provide structural context. Various applications of this new technology are now possible. One application is for in cellula pulse-chase analysis to follow protein turnover and diffusion of photoactivated fluorescent proteins. Another approach combines the techniques of PALM and single particle tracking to resolve the dynamics of individual molecules by tracking them in live cells. Called single particle tracking PALM (sptPALM), the technique involves activating, localizing and bleaching many subsets of photoactivated fluorescent protein chimeras in live cells. Spatially-resolved maps of single molecule motions can be obtained by imaging membrane proteins with this technique, providing several orders of magnitude more trajectories per cell than by traditional single particle tracking. By probing distinct subsets of molecules, including Gag and VSVG, sptPALM can provide a powerful means for exploring the origin of spatial and temporal heterogeneities in membranes. Examples such as these will be presented to illustrate the value of super-resolution imaging in providing quantitative insights into protein organization and dynamics at the nanoscale.