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Imaging Nanoscale Protein Distributions in Live Cell Membranes with Fluorescence Photoactivation Localization Microscopy (FPALM)

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Resolution in light microscopy is limited by diffraction, yet structures exist on sub-diffraction length scales which are of great interest to the fields of biology and nanoscience. Recent developments in laser scanning microscopy, single molecule photophysics, and fluorescence spectroscopy are beginning to allow far-field optical methods to circumvent the diffraction barrier. Fluorescence photoactivation localization microscopy (FPALM) [1] images distributions of large numbers of (10^4 to 10^6) single photoactivatable fluorescent molecules, to generate a position map of those molecules with near-molecular ($\sim$20-30 nm) localization-based resolution. Molecules are initially trapped in an inactive (non-fluorescent) state, but illumination with brief pulses of 405 nm light photoactivates a stochastic subset of the inactive molecules. Photoactivated molecules are then illuminated by a second laser, and their fluorescence is imaged using a high-sensitivity camera at approximately five to ten frames per second. Molecules appear as diffraction-limited spots, which are then localized before photobleaching removes them from view. Additional molecules are photoactivated, imaged, and bleached, in a repetitive process that builds up data from thousands of molecules. Positions of localized molecules are plotted, weighted by their intensities, to form a super-resolution image of the sample. We present FPALM images of living and fixed fibroblast cells transfected with photoactivatable green fluorescent protein (PA-GFP), and of annealed crystalline and glass surfaces coated with PA-GFP. FPALM provides a means to image structures by far-field fluorescence microscopy with demonstrated resolution of $<$30 nm.

[1] Hess, S. T., Girirajan, T. P. & Mason, M. D. "Ultra-high resolution imaging by fluorescence photoactivation localization microscopy." *Biophys. J 91*, 4258-72 (2006).