

## **Super-resolution imaging of intracellular fluorescent proteins using PhotoActivated Localization Microscopy (PALM)**

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In conventional biological imaging, diffraction places a limit on the minimal  $xy$  distance that two marked objects can be discerned. Consequently, resolution of target proteins within cells is typically coarser by two orders of magnitude than the molecular scale at which the proteins are spatially distributed. Here, we introduce a method capable of optically resolving selected subsets of fluorescently-tagged proteins within cells at mean separations of  $<50$  nanometers, overcoming the diffraction barrier in fluorescence microscopy. Termed PhotoActivated Localization Microscopy (PALM), the method involves serial photoactivation and subsequent photobleaching of numerous sparse subsets of photoactivated fluorescent protein molecules. Individual molecules are localized at near molecular resolution by determining their centers of fluorescent emission via a statistical fit of their point-spread-function. The position information from all subsets is then assembled into a super-resolution image, in which individual fluorescent molecules are isolated at high molecular densities (up to  $10^5$  molecules/ $\mu\text{m}^2$ ). We present PALM imaging of intracellular structures (including lysosome, Golgi apparatus and mitochondria) in cryo-prepared thin sections as well as PALM imaging of molecules near the cell surface (such as vinculin and actin) in fixed cells. Finally, correlative PALM/transmission electron microscopy images are presented of a mitochondrial marker protein, illustrating the feasibility of relating the nanometer scale distribution of a specified protein at high molecular density (i.e., 5500 molecules per mitochondria) to cellular ultrastructure.

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