NANOSCOPIC IMAGING OF BIOMOLECULES AND CELLS WITH STORM

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Optical microscopy is one of the most widely used imaging methods in biological research. Several advantages, such as the exquisite molecular specificity, the relatively fast time resolution and the noninvasive imaging nature, make light microscopy a particularly powerful tool for cell, tissue and animal imaging. However, the spatial resolution of far-field optical microscopy, classically limited by the diffraction of light to $\sim$300 nm, is substantially larger than typical molecular length scales in cells, leaving many biological problems beyond the reach of light microscopy. To increase the image resolution, we have developed a new form of high resolution light microscopy, stochastic optical reconstruction microscopy (STORM). STORM uses photo-switchable fluorescent probes to temporally separate the otherwise spatially overlapping images of individual molecules, allowing the construction of high-resolution images. The imaging process includes multiple imaging cycles. In each cycle, only a fraction of the fluorophores are switched on, such that each of the active fluorophores is optically resolvable from the rest. This allows the position of these fluorophores to be determined with nanometer accuracy. Over the course of many activation cycles, the positions of numerous fluorophores are determined and used to construct a super-resolution image. Using this concept, we have achieved three-dimensional fluorescence imaging of molecular complexes and cells with $\sim$20 nm lateral and $\sim$50 nm axial resolutions. Furthermore, we have discovered a family of photo-switchable probes with distinct spectral properties and demonstrated multicolor super-resolution imaging. This new form of fluorescence microscopy allows molecular interactions in cells and cell-cell interactions in tissues to be imaged at the nanometer scale.