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Far-Field Fluorescence Nanoscopy

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The resolution of a far-field optical microscopy is usually limited to $d = \lambda/(2\, n \sin\alpha) \quad > 200$ nm, with $n \sin\alpha$ denoting the numerical aperture of the lens and λ the wavelength of light. While the diffraction barrier has prompted the invention of electron, scanning probe, and x-ray microscopy, the 3D-imaging of the interior of (live) cells requires the use of focused visible light. I will discuss new developments of optical microscopy that I anticipate to have a lasting impact on our understanding of living matter. Emphasis will be placed on physical concepts that have overcome the diffraction barrier in far-field fluorescence microscopy. To set the scene for future directions, I will show that all these concepts share a common strategy: exploiting selected states and transitions of the fluorescent marker to neutralize the limiting role of diffraction. The first viable concept of this kind was Stimulated Emission Depletion (STED) microscopy where the spot diameter follows$d \approx \lambda \Big/ \left(2\, n \sin\alpha \sqrt{1 + I/I_s}\right)$; $I/I_s$is a measure of the strength with which the molecule is send from the fluorescent state to the dark ground state. For $I/I_s \to \infty$ it follows that $d \to 0$, meaning that the resolution that can, in principle, be molecular. The concept underlying STED microscopy can be expanded by employing other transitions that shuffle the molecule between a dark and a bright state, such as (i) shelving the fluorophore in a dark triplet state, and (ii) photoswitching between a 'fluorescence activated' and a 'fluorescence deactivated' conformational state. Examples for the latter include photochromic organic compounds, and fluorescent proteins which undergo a cis-trans photoisomerizations. Photoswitching provides ultrahigh resolution at ultralow light levels. Switching can be performed in an ensemble or individually in which case the image is assembled molecule by molecule at high resolution. By providing molecular markers with the appropriate transitions, synthetic organic chemistry and protein biotechnology plays a key role in this endeavor. Besides being a fascinating development in physics, far-field optical "nanoscopy" is highly relevant to the life sciences. In fact, it has already been a key to answering important questions in biology [1, 2]. Due to its simplicity and improving performance, I expect far-field optical nanoscopy to enter virtually every cell biology laboratory in the near future.

[1] S. W. Hell, Far-field optical nanoscopy, *Science* 316 (2007) 1153.
[2] Westphal, V., S. O. Rizzoli, M. A. Lauterbach, D. Kamin, R. Jahn, S. W. Hell, Video-Rate Far-Field Optical Nanoscopy Dissects Synaptic Vesicle Movement, Science (2008) DOI: 10.1126/science.1154228.