

BREAKING ABBE'S BARRIER: DIFFRACTION-UNLIMITED RESOLUTION IN FAR-FIELD MICROSCOPY

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In 1873, Ernst Abbe discovered that the resolution of focusing ('far-field') optical microscopy is limited to $d = \lambda / (2n \sin \alpha) > 200$ 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, in the life sciences 80% of all microscopy studies are still performed with lens-based (fluorescence) microscopy. The reason is that the 3D-imaging of the interior of (live) cells requires the use of focused visible light. Hence, besides being a fascinating physics endeavor, the development of a far-field light microscope with nanoscale resolution would facilitate observing the molecular processes of life.

In this lecture, I will discuss novel physical concepts that radically break the diffraction barrier in focusing fluorescence microscopy. They share a common strategy: exploiting selected molecular transitions of the fluorescent marker to neutralize the limiting role of diffraction¹.

The first viable concept of this kind was Stimulated Emission Depletion (STED) microscopy². In its simplest variant, STED microscopy uses a focused beam for fluorescence excitation, along with a red-shifted doughnut-shaped beam for subsequent quenching of fluorescent molecules by stimulated emission. Placing the doughnut-beam on top of its excitation counterpart in the focal plane confines the fluorescence near its central intensity-zero where stimulated emission is absent. The higher the doughnut intensity, the stronger is the confinement. In fact, the spot diameter follows $d \approx \lambda / (2n \sin \alpha \sqrt{1 + I/I_s})$, with I denoting the intensity of the quenching (doughnut) beam and I_s giving the value at which fluorescence is reduced to $1/e$. Without the doughnut ($I=0$) we have Abbe's equation, whereas for $I/I_s \rightarrow \infty$ it follows that $d \rightarrow 0$, meaning that the fluorescence spot can be arbitrarily reduced in size^{3,4}. Scanning this subdiffraction spot across the specimen delivers images with a subdiffraction spatial resolution that can, in principle, be molecular! Thus, the resolution of a STED microscope is no longer limited by λ , but on the perfection of its implementation. We will demonstrate a resolution down to $\lambda/45 \approx 15$ -20 nm with nanoparticles and biological samples, i.e., 10-12 times below the diffraction barrier⁵.

The concept underlying STED microscopy can be expanded by employing other molecular transitions that control or switch fluorescence emission, such as (i) shelving the fluorophore in a metastable triplet state^{4,6}, and (ii) photoswitching (optically bistable) marker molecules between a 'fluorescence on' and a 'fluorescence off' conformational state³. Examples for the latter include photochromic organic compounds, and fluorescent proteins which undergo a photoinduced cis-trans isomerization or cyclization reaction. Due to their optical bistability/metastability, these molecules entail comparatively low values I_s , meaning that the diffraction barrier can be broken at low I . A complementary approach is to switch the marker molecules individually and assemble the image molecule by molecule. By providing molecular markers with the appropriate transitions, synthetic organic chemistry and protein biotechnology plays a key role in overcoming the diffraction barrier.

Finally, I discuss more recent work of my group showing that the advent of far-field 'nanoscopy' has already solved fundamental problems in (neuro)biology, such as the fate of synaptic vesicle proteins after synaptic transmission⁷. Besides, the emerging far-field 'optical nanoscopy' also has the potential to advance nanolithography, the colloidal sciences, and to help elucidate the self-assembly of nanosized materials.

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² S. W. Hell and J. Wichmann, Opt. Lett. **19** (11), 780 (1994).

³ S.W. Hell, Nature Biotechnol. **21** (11), 1347 (2003).

⁴ S. W. Hell, in *Topics in Fluorescence Spectroscopy*, ed. by J.R. Lakowicz (Plenum, NY, 1997), 5, pp. 361.

⁵ M. Dyba and S.W. Hell, Phys. Rev. Lett. **88**, 163901 (2002); V. Westphal and S.W. Hell, Phys. Rev. Lett. **94**, 143903 (2005); G. Donnert, et al., Proc Natl Acad Sci **103**, 11440 (2006).

⁶ S. W. Hell and M. Kroug, Appl. Phys. B **60**, 495 (1995).

⁷ K. I. Willig, S. O. Rizzoli, V. Westphal et al., Nature **440** (7086), 935 (2006).