

Far beyond Abbe's barrier

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Since its discovery by Abbe in 1873, the diffraction barrier has received a lot of attention. However, the subdiffraction microscopy concepts of the mid 20th century remained either too vague or subject to unrealistic physical conditions. Consequently, until recently, all far-field fluorescence microscopes remained conceptually and practically diffraction-limited.

In this contribution, we discuss the principle of breaking the diffraction barrier through reversible saturable optical (fluorescence) transitions (RESOLFT). This principle was put forward in the form of Stimulated Emission Depletion (STED) [1,3] and Ground State Depletion (GSD) microscopy [2, 3] in the mid 1990's. In all cases, the diffraction barrier is broken by a saturated transition (depletion) between two states of a marker, whereby the transition is effected with an intensity distribution featuring one or more intensity minima (zero). The saturation level defines the size of the ultrasharp focal spot and/or the concomitantly enlarged bandwidth of the optical transfer function (OTF). We show that in a RESOLFT concept the resolution can be approximated by $\Delta d = \lambda / (\pi n \sqrt{I/I_{\text{sat}}})$, whereby I_{sat} is the characteristic intensity required for saturating the transition, and I denotes the intensity applied [4]. If the minima are produced by focusing optics with a numerical aperture $n \sin \alpha$, the minimal distance at which two identical objects can be discerned can be approximated by

$$\Delta d \approx \frac{\lambda}{2n \sin \alpha \sqrt{1 + I/I_{\text{sat}}}}$$

which can be regarded as an extension of Abbe's equation on the optical microscopy resolution [4,8]. The diffraction-unlimited nature of the RESOLFT family of concepts is reflected by the fact that the minimal resolvable distance can be continuously decreased by increasing $\zeta = I/I_{\text{sat}}$ [1-6]. Hence the quest for nanoscale resolution comes down to maximizing $\zeta = I/I_{\text{sat}}$. This is possible by increasing I or by lowering I_{sat} [4,8,9].

We give evidence of STED-microscopy displaying PSF of 10-20 nm FWHM, corresponding to a 15-fold enlargement of the OTF over Abbe's barrier. The success of STED stems from the fact that the saturation of the single-photon transition of stimulated emission provides strong nonlinearities at comparatively low intensities. The reason for that is simple but critical: Unlike in multiphoton events, saturation is *not* effected by the joint action of multiple photons, but stems from the population of the fluorophore states [4,8].

Therefore, transitions that are easy to saturate (i.e. low I_{sat}), allow huge ζ at low intensities. Examples include the saturation of the triplet state [2,3], which reduces I_{sat} by $\sim 10^3$ as compared to STED. Of similar interest is the 'switching' between conformational fluorophore states [6-9], which gives a factor of $>10^6$. Suitable candidates for saturable switches are encountered in photochromic compounds [6-9] and photoswitchable GFP-like proteins [4,6,9], which should ultimately give nanoscale resolution at intensities provided by a lamp.

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