

# FLUORESCENCE NANOSCOPY THROUGH REVERSIBLE OPTICALLY SATURABLE TRANSITIONS

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

We discuss the principle of breaking the diffraction barrier through reversible saturable optical transitions. This principle was first proposed in the mid 1990's in the form of Stimulated Emission Depletion (STED)[1] and Ground State Depletion (GSD) microscopy[2] and recently also in a related concept[3]. In all cases, the diffraction barrier is broken by a saturated depletion of the ground or the excited state of the fluorophore. The saturation level defines the size of the ultrasharp focal spot and the concomitantly enlarged bandwidth of the optical transfer function (OTF). We show that the resolution can be approximated by  $\Delta x = \lambda / (\pi n \sqrt{I/I_{\text{sat}}}) = \lambda / (\pi n \sqrt{\zeta})$ , whereby  $I_{\text{sat}}$  is the characteristic intensity required for saturating the transition, and  $I$  denotes the intensity applied[4]. Hence the quest for nanoscale resolution boils down to maximizing the saturation factor  $\zeta = I/I_{\text{sat}}$ , which means increasing  $I$ , and if this is not possible, lowering  $I_{\text{sat}}$  [4-6]!

We give first 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, the nonlinearity produced by saturation does *not* rely on the joint action of multiple photons, but stems from the population kinetics of the fluorophore states. Hence transitions that are easy to saturate (i.e. with low  $I_{\text{sat}}$ ), allow huge  $\zeta$  at low intensities.

In consequence, in 1995 we have proposed as a further option to STED, the saturation of the triplet state[2], which reduces  $I_{\text{sat}}$  by  $\sim 10^3$ , and also the 'switching' between conformational fluorophore states[6,7], which gives another factor of  $10^3$ . We have proposed that such saturable switches are encountered in photochromic compounds[7] and photoswitchable GFP-like proteins such as *asFP*[4,6], which should render nanoscale resolution with the ultralow intensities of a lamp.

Finally, we show that a slightly modified version of our concept may serve as an alternative to the current X-ray and synchrotron efforts in nanolithography[6] with the potential of providing material (nano)structures of any size and density with visible focused light.

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