Towards far-field fluorescence nanoscopy

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A distinct advantage of far-field light microscopy is the non-destructive imaging of the interior of biological specimens. An obvious disadvantage is the limited spatial resolution, which for more than a century has been paradigmatic. The concept of stimulated emission depletion (STED-) microscopy and the related concept of Ground-State-Depletion (GSD) Microscopy allow for a fundamental breaking of the diffraction barrier. The physics of breaking the diffraction barrier is not just the ‘depletion’ of the excited or the ground state, rather it is the saturation of the depletion. Saturation disentangles the final size of the fluorescent spot from the diffraction limited spot size of the focal light beams involved. The passband of the optical transfer function of the system is automatically enlarged. Saturation signifies a nonlinear intensity relationship, but importantly, in both cases the non-linearity is based on processes that are per se linear: 1-photon absorption and stimulated emission.

It is a fundamental insight that, in these concepts the spatial resolution can be increased to a few nanometers. The optical passband can be enlarged accordingly. This is in stark contrast to confocal and two-photon fluorescence microscope whose passband also surpasses Abbe’s barrier, but at most by a factor of two. There is no way, not even in theory, by which this passband could be enlarged further. By contrast, in STED- GSD or related saturation-based concepts the passband can be increased, in principle at will, by increasing the level of saturation. Therefore, while confocal and two-photon microscopes are inevitably diffraction-limited, STED- or GSD-microscopy are not. As a result they really break the limits set by diffraction.

Besides discussing fundamental insights and physical-technical challenges on breaking the diffraction barrier we give an overview on recent progress on improving the spatial resolution in far-field microscopy.

3. V. Westphal, C.M. Blanca, M. Dyba, L. Kastrup, and S.W. Hell, unpublished.
4. L. Kastrup and S.W. Hell, unpublished.