Breaking Abbe's barrier: fluorescence microscopy with diffraction-unlimited resolution

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In 1873, Ernst Abbe discovered that the resolution of focusing ('far-field') optical microscopy is limited to ~ 200 nm which has been the practical resolution limit ever since. In this lecture we discuss concepts that, by establishing certain molecular states in subdiffraction regions, neutralize the resolution-limiting role of diffraction in fluorescence microscopy. The first viable concept of this kind, Stimulated Emission Depletion (STED) microscopy[1], establishes a fluorescent molecular state in a nanosized area using a focal intensity distribution featuring a zero. The doughnut confines the fluorescent state near its central zero in such a way, that the effective fluorescence spot (point spread function) can be arbitrarily reduced in size[1-4]. The concept underlying STED microscopy can be expanded by employing other molecular transitions that switch fluorescence emission: (i) shelving the fluorophore in a metastable triplet state[3,5], and (ii) photoswitching optically bistable markers between a 'fluorescence on' and a 'fluorescence off' conformational state[2]. Examples for the latter include photoswitchable organic compounds and fluorescent proteins which undergo a photoinduced cis-trans isomerization or cyclization reaction. Due to their optical bistability, the diffraction barrier can be broken at low intensity values. By providing appropriate bistable molecular markers, organic chemistry and protein biotechnology play a key role in overcoming the diffraction barrier[2]. Finally, we discuss recent work showing that far-field 'nanoscopy' solves fundamental problems in biology[7].

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