Coordinate-targeted fluorescence nanoscopy with multiple off-states

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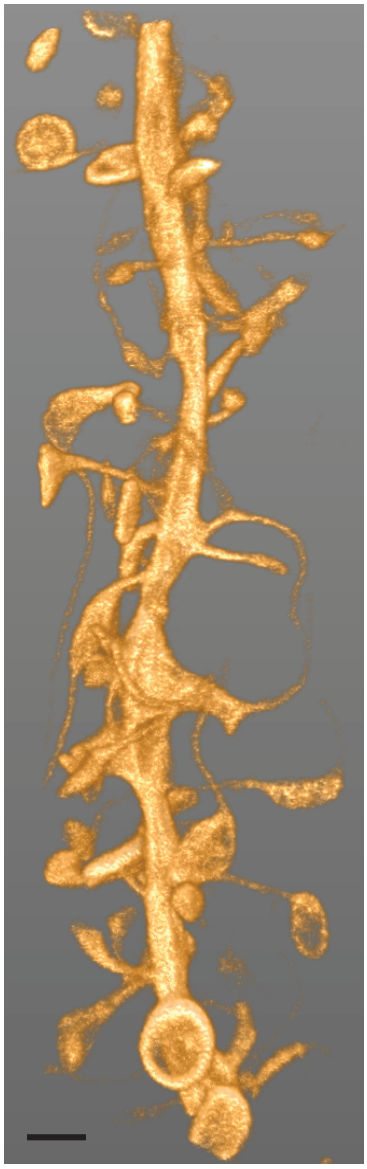

Far-field superresolution microscopy or nanoscopy techniques "super-resolve" features residing closer than the diffraction-limit by transiently preparing fluorophores in distinguishable (typically on- and off-) states and reading them out sequentially. In coordinate-targeted superresolution modalities, such as stimulated emission depletion (STED) microscopy, this state difference is created by patterns of light, driving for instance all molecules to the off-state except for those residing at intensity minima. For high resolution, strong spatial confinement of the on-state is required. However, this also subjects fluorophores at intensity maxima to excess light intensities and photobleaching. In addition, as spatial confinement of the on-state is increased, state contrast between designated on- and off-regions has to be improved, too.

To address these issues, we introduced the concept of using multiple off-state transitions for coordinate-targeted nanoscopy [1]. Applied to STED microscopy, transfer of fluorophores to a second, inert off-state allowed protecting fluorophores in high intensity regions. Using reversible photoswitching as second off-transition led to a realization that we dubbed "protected STED". Our approach improved repeated imaging capability and at the same time enhanced resolution and contrast through a synergistic effect of multiple off-transitions on molecular state contrast. This allowed e.g. decoding the structure of dendrites studded with dendritic spines in living brain tissue with 3D diffraction-unlimited resolution, as shown in the image on the left (scale bar: 1 μm, taken from Ref. [1]).

References:

[1] Danzl, Sidenstein *et al.*, 2016, *Nature Photonics* 10:122.