Super-resolution STED microscopy and its application in neuroscience

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Fluorescence microscopy is a widely used technique, especially in biology. It combines staining specificity with relatively simple imaging capabilities. Especially if applied in the far-field it is almost non-invasive and therefore ideal to study protein assemblies or dynamics in living cells, tissues or animals. Until recently it was widely accepted that far-field optical microscopes cannot visualize details closer than about half the wavelength of light. Therefore, electron microscopy is needed to reveal structural details at exceptionally high resolution, down to the molecular level. EM, though, lacks the ability to image dynamic changes of the same morphological structures; temporal information is only gathered via comparative studies prepared at different time-points. However, to understand how and why the sub-structure of cells changes, and what functional consequence this change induces, we need to visualize cells or even whole, intact living organism over extended periods of time, i.e. in longitudinal studies. However, given the poor optical resolution small sub-cellular structures have still not been accurately assessed by standard light microscopy techniques available due to the diffraction limited resolution of far-field light microscopy being ~200-300 nm.

With the 2014 Nobel Prize in Chemistry 'for the development of superresolved fluorescence microscopy' for Betzig, Hell and Moerner, a novel family of light microscopy techniques became widely recognized, which surpass the limited resolution of light microscopy: The general terms 'superresolution' microscopy or 'nanoscopy' encompass several techniques, which can be divided in coordinate-targeted approaches (e.g. stimulated emission depletion microscopy (STED), reversible saturable optical fluorescent transition microscopy (RESOLFT)), and coordinate-stochastic approaches (e.g. stochastic optical reconstruction microscopy (STORM), and photoactivated localization microscopy (PALM), etc.). Amongst these techniques, STED microscopy stands out for its fast recording speed; its inherent 3D sectioning capability; the potential to image deep within tissue; and the possibility of exploiting a vast array of commercially available fluorescent dyes including standard fluorescent proteins, such as GFP, YFP or red-emitting fluorescent proteins. Moreover, the recorded image is a linear response of the number of fluorophores without any mathematical processing being involved. Therefore, STED microscopy is ideally suited for imaging within tissue and as such for imaging and quantifying morphological changes in the living mouse brain.

I will present applications of STED microscopy to image neuronal structures in the brain of living mice. We image the cerebral cortex of a living mouse through a glass window, so that we can observe the dynamics of dendritic spines in the molecular layer of the visual cortex. We had superresolved actin and its morphological changes in the cortex of an anaesthetized mouse, which was the first STED microscopy of a dendritic sub-structure in a living mouse. Recently, my group has superresolved the sub-structure of the post-synaptic density protein PSD95 in the anaesthetised mouse and recorded morphological changes over 6 hours.

These results show that STED nanoscopy is a highly suitable tool for research in neuroscience which can play a substantial role in the study of learning and memory.