High-Resolution Light Microscope Reveals the Fundamental Mechanisms of Nerve Communication

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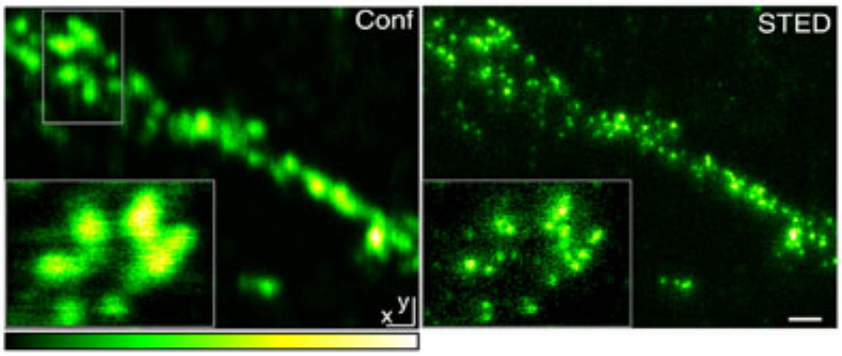


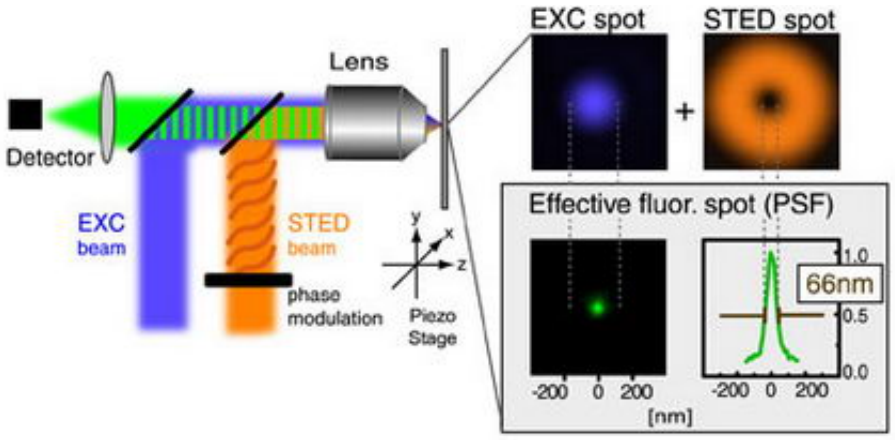
Resolution increase with STED microscopy applied to synaptic vesicles: Conventional (confocal) microscopy is not able to resolve single vesicles in the synapse of a nerve cell, in contrast to STED microscopy. Image: Max Planck Institute for Biophysical Chemistry

The development of STED microscopy has allowed researchers at the Max-Planck Institute for Biophysical Chemistry to image, for the first time, proteins from single synaptic vesicles, answering long-standing questions of neurocommunication (Nature, 13th April 2006). In a simultaneous publication (Science Express, 13th April 2006), STED microscopy revealed the spatial distribution of the bruchpilot protein and aided neurobiologists from the European Neuroscience Institute and the University of Würzburg in understanding the protein's central role in the formation of active synaptic zones.

STED microscopy radically distinguishes itself from conventional far-field light microscopy in the fact that its resolution is no longer fundamentally limited by the wavelength of light used. Using STED, nanoscale optical studies are now possible inside cells.

Since its discovery in the 17th century, the light microscope has been the key to new biological and medical discoveries. Light, however, propagating as a wave, is subject to the phenomenon of diffraction, whose resolution-limiting effects were first described by Ernst Abbe in 1873. Abbe observed that structures which were closer to each other than ~200nm could not be visually separated when observed using visible light; when viewed through the optical microscope they are perceived as a blurred, single entity. Abbe's realization of the resolution limitation of the optical microscope was long thought to be a unalterable law of far-field light imaging. Achieving higher resolution required the use of an electron microscope.

Despite that fact that electron beams can be more tightly focused, it is often difficult to efficiently label the proteins of a cell to render them visible with an electron microscope. Moreover, electron beams are only able to penetrate the first several micrometers of a biological sample. For these reasons, among others, despite using electron microscopy for high-resolution cell imaging, many questions of nerve function remained unanswered. In contrast, using fluorescent molecules as markers, one can specifically label individual proteins with high efficiency, rendering them visible with the conventional fluorescent microscope. Unfortunately the high resolution required to separate nanoscale structures was lacking due to the diffraction barrier.



STED microscopy: The excitation light beam (EXC beam, in blue) is steered by a mirror through the objective lens, and due to diffraction is focused to a spot ca. 200 nm in diameter on the sample. The excitation light excites fluorescent markers which tag molecules of interest (e.g. proteins) in the sample. The markers are excited to a higher energy state, from which they emit light of a longer wavelength (via fluorescence decay) when they return to the ground state. By scanning this blue excitation spot over the sample (the cell) and recording the emitted fluorescent light with a computer, one can form an image of the sample. The smaller the excitation spot is, the higher the resolution of the microscope. However, due to diffraction, the excitation spot cannot be made smaller than ~200 nm by focusing with a lens. The trick with STED microscopy is that one uses a second beam (STED beam, in orange) to quench the fluorescent markers before they fluoresce. Because the STED beam is doughnut-shaped and centered over the excitation spot, one is able to preferentially quench the markers at the outer edge of the excitation spot and not those in the center. The result is a smaller effective fluorescence spot (green), here reduced to a diameter of ~66 nm. By making the STED doughnut very intense, it is in principle possible to shrink the fluorescent spot to molecular size, thus attaining molecular resolution - an exciting goal for the near future. Image: Max Planck Institute for Biophysical Chemistry

In recent years researchers in the department of NanoBiophotonics at the MPI for Biophysical Chemistry in Göttingen have been able to break the

Abbe resolution limit of far-field optical microscopy, as applied to fluorescent imaging, using a technique known as Stimulated Emission Depletion (STED) microscopy. The STED microscope used to obtain data for both publications is able to attain a resolution of 50-70 nm; the original fluorescent spot, roughly 200 nm in diameter, is reduced in surface area within the imaging plane by roughly an order of magnitude using the STED technique.

This resolution was sufficient for researchers from the neurobiology department to visualize, for the first time, individual synaptic vesicles - more precisely, to visualize the protein synaptotagmin, which is embedded in the membranes of individual vesicles. Vesicles are membrane 'bubbles' roughly 40 nm in diameter filled with neurotransmitters, which transport chemical messenger molecules to synapses, the contact points between nerve cells, enabling nerve signals to pass between cells. Their contents are released at the synapse when the vesicle membranes fuse with the membrane of the nerve cell. Previously it was unclear whether the proteins sticking in the vesicle membrane (e.g. synaptotagmin) spread out over the cell membrane after the fusion event or they remained together, localized in the membrane patch which previously formed the vesicle. With the aid of STED microscopy the researchers in Göttingen were able to show that the synaptotagmin molecules of a single vesicle remain together after fusion. The membrane of the nerve cell thus behaves in an 'economical' fashion: the vesicle proteins released onto the membrane of the nerve cell can be collectively reabsorbed to form another vesicle.

Neural vesicles do not fuse with the cell membrane with equal probability at all locations along the synapse junction, but preferentially at so-called 'active zones.' The bruchpilot protein discovered in fruit flies plays a decisive roll in the formation of these active zones. This is explained in the Science publication by Kittel et al. With STED imaging the scientists discovered that the bruchpilot protein is distributed in rings

of ca. 150nm diameter, forming the active zones. In these areas it appears that bruchpilot establishes the proximity between the calcium channels and the vesicles enabling the efficient release of neurotransmitters.

These studies demonstrate for the first time that resolution below a half-wavelength of visible light is no longer reserved for the electron microscope when observing cells. As demonstrated by recently completed research (please see the press release from XXth of May, 2005), the resolution of STED microscopy can be further increased, in principle to reach molecular scales. The STED microscope has opened a new chapter in the story of light microscopy, one in which the fundamental questions of biological processes at the nanoscale can potentially be answered with focused light.

Source: Max Planck Institute for Biophysical Chemistry