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Technology watch

LESSENING LIMITATIONS

Although the recycling of synaptic vesicles has been studied for over three decades, it is not clear what happens after the vesicles fuse with the plasma membrane. Do the vesicle components stay together to facilitate efficient recycling, or do they diffuse in the plasma membrane? This question has been difficult to answer because the vesicles are too small (~40 nm) and too closely packed to be resolved by the available fluorescence microscopes. However, in *Nature*, Hell and colleagues now describe how — using stimulated emission depletion (STED) — they have broken the diffraction limit of fluorescence microscopy to allow synaptic-vesicle recycling to be monitored at a nanoscale resolution.

In a STED microscope, the excitation beam is overlapped with a doughnut-shaped beam that can de-excite fluorophores by stimulated emission. Aligning the two beams correctly ensures that fluorescence is only allowed in the central area of the excitation spot. Using this approach, the authors reduced the focal spot area by about an order of magnitude below the diffraction limit. This allowed the visualization of individual vesicles in the synapse and enabled them to show that at least some vesicle components remain together during recycling. So, this microscopy technique, "…despite using regular objective lenses and visible light, is no longer limited by diffraction."

REFERENCE Willig, K. I. *et al.* STED microscopy reveals that synaptotagmin remains clustered after synaptic vesicle exocytosis. *Nature* **440**, 935–939 (2006)

CELLS IN 3D

Growing evidence indicates that microenvironment studies that use adherent two-dimensional cultures might not be appropriate for many cell types because the presentation of extracellular signals in a three-dimensional (3D) context affects the cellular response. Bhatia and colleagues have therefore created a method that allows researchers to precisely organize cells by using external physical forces and by trapping the newly formed 3D structures in a biocompatible, stimuli-sensitive hydrogel.

In *Nature Methods*, the authors describe how they could rapidly form reproducible 3D multicellular structures in a photopolymerizable hydrogel by using dielectrophoretic forces; the forces were used to localize the cells and ultraviolet light was then used to crosslink the hydrogel and trap the newly formed structures. They were able to produce and study tissues with varying microstructures, and the method could be effectively used to pattern any cell type, including specialized cells. They established the biological importance of this technique by showing that the 3D microorganization of bovine chondrocytes markedly affects their behaviour. In the authors' view, approaches like this are needed "…to define the structure–function relationships of multicellular systems to realize the full potential of living cells, including stem cells, as therapeutic entities."

REFERENCE Albrecht, D. R. *et al.* Probing the role of multicellular organization in three-dimensional microenvironments. *Nature Methods* **3**, 369–375 (2006)