

MOBILITY OF SYNAPTIC VESICLES STUDIED BY STED SUPERRESOLUTION MOVIES

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STED microscopy [1] allows imaging of fixed and living samples with a resolution in the range of tens of nanometers. STED microscopy uses visible light which is not harmful to living cells and is not limited to surfaces.

Here, we report studies at high imaging speed [2]. We make high resolution movies at video-rate (28fps) from the inside of living cells. We use localization algorithms to find the objects of interest and particle tracking for the quantitative analysis of their movement. This combination of high spatial and temporal resolution along with image processing was used to study the movement of synaptic vesicles in cultured hippocampal rat neurons. We found their movement restricted within boutons, but faster in nonbouton areas. We observed that a sizable vesicle pool continuously transits through the axons. Perturbing the cytoskeleton slowed down the vesicle movement. Vesicles were found to spend about 16 % of their time immobile in “Hot Spots”.

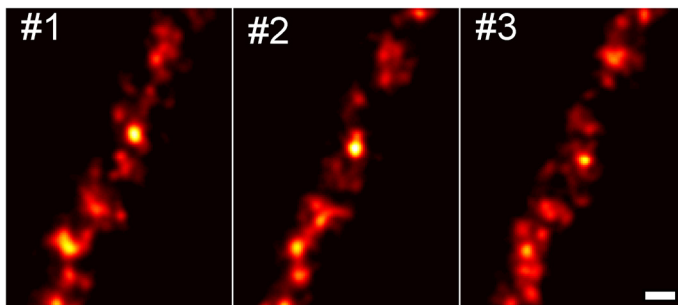


Figure 1: Movement of synaptic vesicles, followed in time. Three frames of a 30s movie. Frame rate 28 fps. Scale bar 250nm

Studying vesicle mobility at different times after endocytosis showed that the mobility was high immediately after endocytosis. After prolonged incubation (hours), the vesicles exhibited limited mobility.

Our studies demonstrate the emerging ability of optical microscopy to investigate not only inanimate but also intracellular physiological processes on the nanoscale in real-time.

[1] S. W. Hell and J. Wichmann, “Breaking the diffraction resolution limit by stimulated emission: stimulated emission depletion fluorescence microscopy”, *Opt. Lett.*, **19**, 780 – 782 (1994)

[2] V. Westphal; S. O. Rizzoli; M. A. Lauterbach; D. Kamin; R. Jahn and S. W. Hell, “Video-rate far-field optical nanoscopy dissects synaptic vesicle movement”, *Science*, **320**, 246 – 249 (2008).