

## 4D electron microscopy with a millisecond temporal resolution

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Neuronal synapses undergo rapid alternations in morphology and protein distributions to process and integrate information. However, it is difficult to study because synapses are extremely small and many of the processes occur on a millisecond time scale. To visualize synaptic architecture, the resolution of electron microscopy is necessary. However, electron microscopy only captures a static image of a cell. To overcome this problem, we have developed a technique, flash-and-freeze, that couples optogenetic stimulation of neurons with rapid high-pressure freezing. In this technique, a brief pulse of light induces neuronal activity, leading to the transmitter release via fusion of vesicles at synaptic terminals. These neurons are then frozen after defined time intervals of a few milliseconds to seconds. By controlling the time interval between the stimulation and the freeze, snapshots of synaptic events can be captured at a millisecond temporal resolution. Using this technique, we are studying how vesicles are recycled at synaptic terminals. I will discuss our discovery of the novel endocytic pathway, ultrafast endocytosis, and current understanding on its molecular mechanisms.