

**Single Particle Tracking Microscopy of Fast Molecular Diffusion in Membranes:  
Single Molecule Localization at 25 Microsec. Timescales and 17 nm Resolution**

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Visualizing the motion of individual molecules, protein and lipid, in the plasma membrane of live cells presents special problems. Of key importance in imaging diffusive motion is that the time and length scales are coupled through the diffusion coefficient. As such, to observe smaller length scale features in a molecules motion, higher time resolution imaging is required. Further, since the time scales as the square of the length, for a thousand-fold increase in spatial resolution, a million-fold reduction in the time scale is required. The diffusion coefficient of lipid in a synthetic bilayer vesicle is 5-10  $\mu\text{m}^2/\text{s}$ , thus, for a 20 nm resolution,  $\sim 20 \mu\text{s}/\text{frame}$  is required. I will discuss our implementation of high-speed single particle tracking, where we have achieved the tracking of a lipid, labeled with a 40 nm diameter colloidal gold particle, in a live cell membrane at a frame rate of 25  $\mu\text{s}/\text{frame}$  and resolution of 17 nm [1]. It will be shown that this resolution is required to observe the fine scale structure present in the plasma membrane. This structure may be critical to cellular processes such as signal localization [2] and membrane polarization [3].

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