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Combined High-Speed Single Particle Tracking of Membrane Proteins and Super-resolution of Membrane-Associated Structures

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Abstract

Many experiments have shown that the diffusive motion of lipids and membrane proteins are slower on the cell surface than those in artificial lipid bilayers or blebs. One hypothesis that may partially explain this mystery is the effect of the cytoskeleton structures on the protein dynamics. A model proposed by Kusumi [1] is the Fence-Picket Model which describes the cell membrane as a set of compartment regions, each ~ 10 to 200 nm in size, created by direct or indirect interaction of lipids and proteins with actin filaments just below the membrane. To test this hypothesis, we have assembled a high-speed single particle tracking microscope and use a hybrid tracking and super-resolution approach on the same cell. We labeled the high-affinity Fc□RI receptor in Rat Basophilic Leukemia (RBL) cells and tracked these transmembrane proteins at up to 1000 frames per second. The cells were fixed immediately after tracking and further labeled for super-resolution imaging of actin filaments and other membrane-associated components were collected. For best correlation of tracking and super-resolution, we refined a fixation protocol to prevent morphology changes during the fixation process that often go unnoticed. Bright field images allow re-alignment of cell with about ~ 10 nm precision. This sequential approach allows use of far-red dyes for tracking and super-resolution, ameliorating chromatic aberrations. We will present the results of this high-speed tracking within the context of actin and other membrane associated proteins imaged with ~ 20 nm resolution.

References

[1]. Ritchie, K.; Iino, R.; Fujiwara, T.; Murase, K.; Kusumi, A. The fence and picket structure of the plasma membrane of live cells as revealed by single molecule techniques (Review). Mol. Membr. Biol. 2003, 20, 13−18.