3D Single Molecule Tracking in Live Cells with High Spatial and Temporal Resolution: Endocytosis and Exocytosis

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Single molecule tracking in three dimensions (3D) in a live cell environment promises to reveal important new insights into cell biological mechanisms. However, single molecule experiments are currently severely limited by a lack of appropriate methodology that allows for tracking in 3D with high temporal and spatial resolution. As a result, important biological processes remain largely unexplored. For example, there is a lack of data concerning intracellular trafficking pathways in 3D of exocytosing receptors prior to exocytosis and the pathways of endocytosed receptors or ligands post endocytosis. In [1] we introduced a novel imaging modality, multifocal plane microscopy (MUM) for the study of subcellular dynamics. Here we show that MUM provides a powerful approach with which single molecules can be tracked in 3D in live cells. MUM allows for the simultaneous imaging at different focal planes, thereby ensuring that trajectories can be imaged continuously at high temporal resolution. Importantly, this approach overcomes the very limited resolution of a conventional microscope along the optical axis and opens the way for high resolution 3D single molecule tracking within a live cell environment. In the current study, we use MUM to reveal complex intracellular pathways that could not be imaged with classical approaches. In particular we track quantum dot labeled immunoglobulin (IgG) molecules from the sorting endosome to exocytosis [2] and from the plasma membrane, through endocytosis to a sorting endosome.