Intracellular Protein trafficking represents a fundamental process that is important for the normal functioning of the cell. The study of protein transport merits the use of single molecule imaging approaches, as it enables the analysis of the individual transport pathways that is not feasible through conventional imaging techniques. However, 3D single molecule tracking poses several challenges, especially in thick samples such as a 10 µm thick cell monolayer. Current approaches to 3D single molecule tracking are not well suited for imaging the trafficking pathways in a cell monolayer due to technical limitations such as restricted imaging depth, poor temporal resolution, and the ability to track only a few molecules at one time.

Here we show that multifocal plane microscopy (MUM) [1-3] can overcome these challenges. MUM offers several advantages in that it not only allows fast 3D tracking of several molecules at the same time, but it also supports the imaging of the cellular environment with which the single molecule interacts. The latter is especially crucial for the reconstruction of the 3D itinerary of the single molecule. While MUM has been previously used for 3D single molecule tracking across shallow depths (1 micron) in live cells [3], the question arises if MUM can also live up to the significant challenge of tracking single molecules in a thick sample. By using a 4-plane MUM setup in conjunction with a novel multicolor imaging approach, we demonstrate 3D tracking of quantum-dot labeled single molecules in a ~10 micron thick live-cell monolayer. This enabled us to image exocytosis and endocytosis at the lateral plasma membrane, which has not been possible so far, and is important for understanding protein transport across cellular barriers.

