3D SINGLE MOLECULE TRACKING WITH MULTIFOCAL PLANE MICROSCOPY REVEALS NEW CELLULAR PROCESSES IN THICK CELLULAR SPECIMENS

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Single molecule microscopy is a powerful tool to study heterogeneous processes in live cells. However, 3D single molecule imaging of intracellular trafficking events in a thick sample such as an epithelial-cell monolayer poses several technical challenges. Current 3D single molecule tracking approaches are not well suited for studying the intracellular trafficking pathways due to saturation. Using multifocal plane microscopy (MUM)\textsuperscript{1-3} in conjunction with a novel MUM localization and tracking algorithm MUMLA\textsuperscript{3}, we have demonstrated fast 3D single molecule tracking in a \textasciitilde10 micron thick live cell sample\textsuperscript{4}. Using this approach, we have studied the 3D dynamics of single transferrin (Tf) molecules in a polarized epithelial cell monolayer.

The use of MUM led to the unexpected discovery of a novel cellular process, intercellular transfer, which involves the rapid exchange of Tf molecules between two adjacent cells in the monolayer\textsuperscript{4}. We also report 3D single molecule tracking of endocytosis and exocytosis at the lateral plasma membrane of cells in the monolayer\textsuperscript{4}. This lateral membrane has been notoriously difficult to image with other cellular imaging modalities. A detailed characterization of these events based on the temporal and 3D intracellular spatial behavior of Tf molecules has been made. The methods and approaches used in this study have broad applicability to investigate 3D trafficking pathways in other cell systems and models.


