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**3D single molecule tracking in thick cellular specimens using multifocal plane microscopy**[1] SRIPAD RAM, E. SALLY WARD, University of Texas Southwestern Medical Center, RAIMUND J. OBER, University of Texas at Dallas — One of the major challenges in single molecule microscopy concerns 3D tracking of single molecules in cellular specimens. This has been a major impediment to study many fundamental cellular processes, such as protein transport across thick cellular specimens (e.g. a cell-monolayer). Here we show that multifocal plane microscopy (MUM), an imaging modality developed by our group, provides the much needed solution to this longstanding problem. While MUM was previously used for 3D single molecule tracking at shallow depths ($\sim$ 1 micron) in live-cells [1], the question arises if MUM can also live up to the significant challenge of tracking single molecules in thick samples. Here by substantially expanding the capabilities of MUM, we demonstrate 3D tracking of quantum-dot labeled molecules in a $\sim$10 micron thick cell monolayer. In this way we have reconstructed the complete 3D intracellular trafficking itinerary of single molecules at high spatial and temporal precision in a thick cell-sample.

[1] Biophys J., 2008, 95:6025-6043.

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