Tracking single quantum dots in three dimensions: Following receptor traffic and membrane topology

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Proposal Title: Probing the 3D nano-environment of live cells molecule by molecule (D. Lidke)

Research Achievement:

The detection of single molecules by laser induced fluorescence has become a powerful tool for the characterization and measurement of biological processes. For example, single molecule microscopy has been used to monitor individual enzymatic turnovers[1], directly observe the hand over hand motion of individual motor proteins with near nanometer precision[2], and has been used to visualize the diffusion and transport of individual lipids and receptors on live cells[3]. Much has been learned from these single-molecule studies that had been obscured in conventional ensemble microscopy, such as the direct observation of unexpected modes of travel around domain structure within live cell membranes[3].

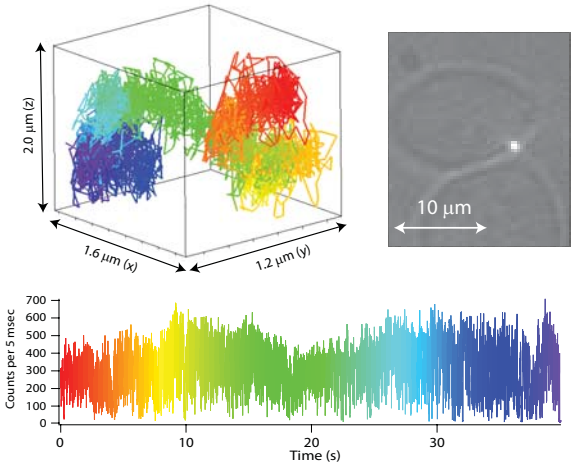

Figure 1 Top Left: 3D trajectory of a quantum dot labeled IgE-FcεRI on the side of a rat mast cell. **Top Right:** Image of this receptor on the cell obtained while 3D tracking. **Bottom:** Counts measured during the 40 second trajectory. Rainbow color scheme used to denote passage of time. Adapted from [7].

Note that in the examples cited above, the motion of the molecule under investigation was limited to zero[1], one[2], or two dimensions[3]. We point out perhaps the obvious: *most aspects of life, including intracellular signaling and trafficking, are inherently 3-dimensional.* However, tracking a single fluorescent molecule or a single quantum dot traveling through 3 dimensional space is a difficult (and until recently) unsolved technical problem. A small number of advanced 3D tracking methods based upon closed-loop feedback have been recently developed by a handful of research groups (Reviewed in[4]). Our approach[5-7] uses a unique spatial filter geometry and active feedback to always keep a molecule (or quantum dot) in the center of the field of view of a confocal microscope. This system can follow individual quantum dots over an extended X, Y, and Z range (tens of microns) at a spatial precision of ~50 nm obtained during 5 milliseconds of observation. We note our 3D tracking methods and algorithms are quite insensitive to a large homogeneous background, making them useful in "dirty" environments such as cells[6]. This microscope enables following the 3D transport and function of individual fluorescently labeled biomolecules (proteins, DNA, or RNA) inside living cells with near 100 picosecond temporal resolution for durations up to minutes.

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Here, facilitated with a CINT user proposal from experts in cellular signaling (Diane Lidke and Bridget Wilson, University of New Mexico), we are using our 3D tracking microscope to directly observe the three dimensional spatio-temporal dynamics of quantum dot labeled IgE-FcεRI, an important signaling molecule for the allergic response. Our 3D tracking results are consistent with prior observations of 2D diffusion of this receptor[8], and also capture dynamic z-motion on both the cell wall and on the apical membrane. Figure 1 shows one such trajectory of an individual IgE-FcεRI taken on the side of a rat mast cell. A rainbow color scheme has been applied to denote the passage of time. Both corralled diffusion and dynamic, directed motion (green period) are observed in this one 40 second trajectory.

We highlight the fact we record the arrival time of every photon detected during the trajectory with ~100 picosecond timing resolution.[6] This time-resolved photon stream can be used to determine changes in the emission lifetime as a function of position and positively identify single quantum dots via photon-pair correlations (photon anti-bunching)[6]. We further note that recording individual photon arrival times gives our microscope ~ 9 orders of magnitude superior temporal resolution than a conventional CCD-based microscope, bridging the decades of time between fast biomolecular conformational fluctuations[9] and cellular signaling processes[7].

Future Work: While our initial investigations have focused on membrane topology and dynamics, we are working towards following further steps in IgE-FcεRI signaling cascade, including its down-regulation via receptor-mediated endocytosis.

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Publications:

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