Confocal, 3D tracking of individual quantum dot labeled signaling molecules on live cells

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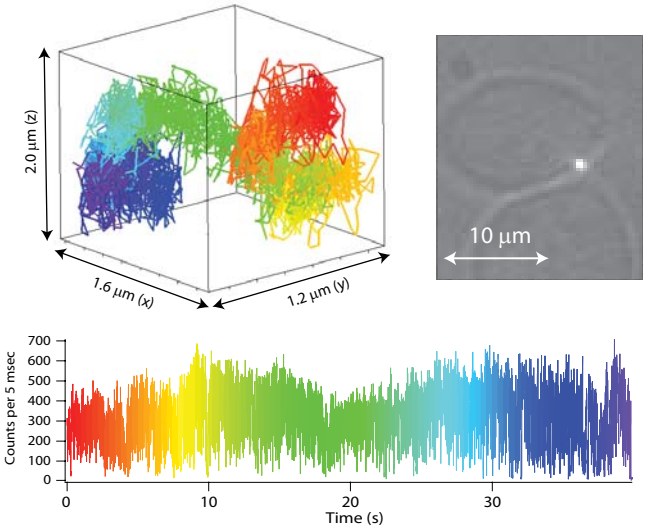
We have constructed a new confocal fluorescence-microscope that uses active feed-back and a unique spatial filter geometry to follow individual fluorescent quantum dots as they diffuse throughout 3 dimensional space at rates faster than most intracellular transport processes (~microns/second). This system can follow individual molecular motion over an extended X, Y, and Z range (tens of microns), enabling one to study the transport of individual fluorescently labeled biomolecules (proteins, DNA, or RNA) performing their functions inside living cells. Our preliminary investigations in this area are focused on following the spatial dynamics of the IgE receptor Fc(epsilon)RI on rat mast cells, an important signaling molecule for the allergic response. We find the types of motion of this receptor on the surface are highly heterogeneous (broad range of diffusion coefficients, corralled diffusion, and periods of directed motion), with substantial and measurable excursions in all three spatial dimensions (X, Y, and Z).

I. Summary

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 study individual molecular conformation and has been used to visualize the diffusion and transport of individual lipids and receptors on live cell membranes[1]. Much has been learned from these single-molecule studies that had been obscured in ensemble measurements of the same processes, such as the observation of unexpected modes of travel around domain structure within the cellular membrane[1].

Note that in the example cited above, the motion of the molecule under investigation was limited to two dimensions[1]. We point out perhaps the obvious: most aspects of life, including intracellular signaling and trafficking, are inherently 3-dimensional. However, tracking 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 3D molecular tracking methods have been recently developed by a handful of research groups (Reviewed in[2]). Our approach[3-5] 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. We note our 3D tracking methods and algorithms are quite insensitive to a large homogeneous background[4] and have been successfully applied to follow 3D traffic of IgE-Fc(epsilon)RI in live cell studies.[5]

The figure below shows one such trajectory of an individual IgE-Fc(epsilon)RI taken on the side of a rat mast cell. A rainbow color scheme has been applied to denote the passage of time. (Figure adapted from [5]). Our 3D tracking results are consistent with prior observations of 2D diffusion of this receptor[6], and also capture dynamic z-motion.



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