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A Simple System for Long-Term 3D Tracking of Quantum Dot Probes in Live Cells BRIAN LONG, TANIA VU, Oregon Health and Science University — The intracellular signaling of G-protein coupled receptors is governed in part by their location and transport on the cell surface and within the cytosol. Bright and relatively photostable fluorescent nanocrystal quantum dot (QD) probes are well-suited for investigation of the spatial dynamics of membrane receptor proteins, including their membrane diffusion, internalization, and intracellular transport. A major obstacle to obtaining long-term information on receptor dynamics with microscopy is that QD probes can only be tracked for as long as they remain within the depth of field of the microscope. We have implemented a simple and flexible 3D tracking system that requires only an epifluorescence microscope, computer control of a piezo-driven stage and an EMCCD camera. We demonstrate 100-200 nm z-position accuracy over a 10 micron depth for 10s of minutes, with temporal resolution of 7.2s per (x,y,z) coordinate. These capabilities allow measurement of QD probe positions for durations relevant to the long-term signaling dynamics of membrane receptors. We will present the application of this system to measuring the spatial dynamics of QD-membrane receptor probes for long durations in live cells.

Brian Long
Oregon Health and Science University

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