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A novel method for 3-D particle tracking in biological systems.

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Abstract

We describe a novel method to track fluorescent particles in 3-D, applicable to the study of motion of fluorescent molecules in cells or other biological systems. In this method, the laser beam of a two-photon excitation microscope moves in a circular path with radius of half the width of the point spread function (PSF). When the fluorescent particle is located within the scanning radius of the laser, the precise position of the particle in the x-y plane can be determined by its fluorescence intensity distribution along the circular scanning path. A z-nanopositioner on the objective allows us to change the laser focus at two different planes located half the width of the PSF apart. The difference of the fluorescence intensity in the two planes is used to calculate the z-position of the fluorescent particle. With a fast feedback mechanism, the position of the laser beam is directed to the center of the fluorescent particle, thus the laser follows the particle movement in 3-D. Calibration experiments showed that this new method allows the tracking of particles with a time resolution of 64 ms in 3D and 16 ms in 2D. The standard deviation for the position of the particle was 20 nm in either the x, y or z axis. As an example of the applicability of this method to biological systems, we studied the dynamics of fluorescent particles in cells. Supported by the NIH, PHS 5 P41-RRO3155, and by UIUC.