Title
A method for distance measurement between fluorescent particles in the 10-200 nm range

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Abstract

We introduce a method to measure relative distances of fluorescent particles of different color immobilized on a quartz surface in a two-photon scanning fluorescence microscope, with two channel photon counting detection. The method is sensitive in the 10-200 nm range, filling the gap between fluorescence resonant energy transfer and far field light microscopy. Instead of raster scanning of an image, excitation beam is moved periodically in a circular orbit with a radius of the size of the point spread function (300 nm), in order to achieve maximum sensitivity in the radial direction. Fluorescence intensity varies periodically with the scanning frequency (500 Hz). Fast Fourier transform of the fluorescence signal during one or more orbit gives the modulation of the first harmonic, which depends upon the radial distance of the particle from the center of scanning. The coordinates of the center of mass of particles are calculated from the modulation and phase, simultaneously in the two channels and relative distance of the particles is calculated. Accuracy of the distance measurement is determined by the total number of photons detected. Experiments demonstrating the advantages of the method were performed on green and red fluorescent spheres of different size. Supported by the NIH , PHS 5 P41 RR03155.