Timothy M Ragan, Peter T C So, and Enrico Gratton.
3-D particle tracking using two-photon excitation: probing the cytoplasmic structure.
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Abstract
Particle tracking techniques have been especially fruitful in the investigation of the cellular plasma membrane. Previous results (Luby-Phelps et al. 1986) indicate that the cytoplasm, like the plasma membrane, has a structure comprised of many compartments. Particle tracking would be the ideal technique to explore these questions. Unfortunately, all the particle tracking techniques developed so far have been limited to 2 or 3-D systems with a restricted axial range and frequency response. To address this problem, we developed a 3-D particle tracking system built around a microscope that uses the inherent 3-D localization of two-photon excitation. We use an x-y scanner and piezo z-stage to quickly scan the sub-micron two-photon excitation spot over the region of interest to build a 3-D image, and, with an automatic feedback mechanism, we reposition the scan region to follow the fluorescent particle and construct a 3-D trajectory. Our system has an axial range of over 50 μm and frequency response of about 30 Hz. We discuss the technical aspects of the instrument (such as frequency response and vibration sensitivity) that affect the choice of systems that can be studied with this technique. We also present results on the motion of small particles in the cytoplasm. For comparison, we present results on the motion of these same particles in a 3-D matrix of polyacrylamide gel, a well characterized system where the average pore size can be systematically varied, and we present results about the cytoplasmic structure that affect the motion of particles, such as average ... [truncated at 250 words]