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Three-dimensional position determination of nanoparticles using a two-photon microscope JAMES GERMANN, LLOYD DAVIS, BRIAN CANFIELD, ALEXANDER TEREKHOV, University of Tennessee Space Institute-Center for Laser Applications — We are developing a means to extend the two-photon microscope to enable three-dimensional sub-diffraction measurement of the position and trajectory of a single nanoparticle as it traverses the probe volume. By use of a Ti-Sapphire laser and a double-Mach-Zehnder interferometer configuration, four laser beams with temporally interleaved pulses are created. These are tightly focused by a 1.2-NA water-immersion microscope objective to four overlapping volumes centered at slightly offset points arranged in a tetrahedron. Fluorescence from the four-focus probe volume is then collected onto a single-photon avalanche diode and the photon time stamps are recorded. Time-resolved photon detection with maximum-likelihood analysis is thereby used to determine the position of the nanoparticle from the relative intensities of fluorescence from each of the four foci. We present measurements of the profile of the four-focus configuration and results from calibration experiments obtained by translating a single gold nanodot or a fluorescent nanobead through the probe volume. Application of the position determination to single-particle trapping is also briefly discussed.

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