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Characterization of 4-foci pulse-interleaved two-photon fluorescence confocal microscope for particle tracking and trapping JAMES GERMAN, ALEXANDER TEREKHOV, GUOQING SHEN, LLOYD DAVIS, University of Tennessee Space Institute — Single fluorescent biomolecules may be readily detected in a confocal fluorescence microscope as they are transported through a tightly focused laser beam. For many applications, two-photon excitation by femtosecond laser pulses provides a key advantage in that the sample excitation is confined to the center of the focal spot. We are examining means to extend the confocal microscope to provide information on the position and trajectory of particles as they traverse the probe volume. We have set up a femtosecond Ti:Sapphire laser, dispersion pre-compensation optics, and double Michelson interferometer to create four beams of temporally interleaved pulses, which enter a confocal microscope so as to produce four foci centered at the vertices of a tetrahedron. We report experiments to adjust and measure the irradiance profile produced by each beam, and the net irradiance from the combination. A 3-D piezoelectric nano-translation stage under LabView control is used to translate a target through the laser foci. Fluorescence, or scattered light, may be imaged to a camera, or to a single-photon counting module. We explain how time-resolved photon counting may be used with maximum-likelihood analysis to determine the position of a particle as it is translated through the probe volume.

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