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Single Emitter Localization using a Four-focus Confocal Fluorescence Microscope JAMES A. GERMANN, BRIAN K. CANFIELD, LLOYD M. DAVIS, University of Tennessee Space Institute — We demonstrate that four spatially separated and temporally pulsed laser foci can be used to detect and localize a single fluorescent emitter to below the diffraction limit in a confocal microscope. Optical excitation is accomplished using LabVIEW Real-Time to control sequential pulsing of four laser diodes. The beams are coupled collinearly through three beam splitters and focused in a custom confocal microscope. The individual foci are positioned at the vertices of a micron-sized tetrahedron, which establish a Cartesian coordinate system. Fluorescence photons are counted by a single-photon avalanche diode and time-gated based on the pulse excitation sequence. Emitter location is estimated from the count rates generated at the four foci using Maximum Likelihood techniques. Preliminary results for tracking a fluorescently labeled nanoparticle in an aqueous/glycerol solution with a piezoelectric stage are presented. Future research with the four-focus microscope will concentrate on trapping a single fluorescent molecule.

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