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Sub-diffraction Position Determination with Four Laser Diodes for Tracking/Trapping a Single Molecule JAMES A. GERMANN, BRIAN K. CANFIELD, JASON K. KING, LLOYD M. DAVIS, University of Tennessee Space Institute — Prolonged observation of single biological molecules by overcoming diffusion can reveal interesting new properties. Observation times may be increased by physically confining a particle, but this often leads to interactions that affect molecular properties. Another way of increasing observation time is to trap a single molecule in solution three-dimensionally. However, optimal trapping of single particles relies on rapid determination of particle position for feedback to counteract Brownian diffusion. In our experiment, a tetrahedral region with foci located at the vertices is created by combining four modulated 635 nm laser diodes with three beam splitters. Fluorescence is measured with a single-photon avalanche diode and separated into bins corresponding to each excitation focus. A maximum-likelihood estimation algorithm is used to determine particle position with sub-diffraction precision in real time. To test the tracking capability of the four-focus setup, fluorescently labeled latex beads were tracked in an aqueous glycerol solution. Two setups, a piezoelectric stage and a three-dimensional electrokinetic trap, are being implemented to maintain a single fluorescent latex bead in the middle of the tetrahedral region.

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