

Abstract Submitted
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Multiple-particle tracking in three dimensions via temporal focusing two-photon microscopy¹ YU DING, CHUNQIANG LI, University of Texas at el paso — Multiple-particle tracking in three dimensions is of great interest in researches of molecular dynamics and interactions in living cells. The spatial and temporal resolutions of the tracked particles are the key to the development of this technology. Here we present a method of three-dimensional multiple-particle tracking based on temporally focused two-photon microscopy. The z position information of each particle is encoded in the radii of the defocused images. Based on the images, algorithms are developed to reconstruct the 3D (x,y,z) positions of these particles. In our experiment, the long-term spatial localization precision can reach 50 nm in both axial and lateral dimensions with the help of stage system stability. We demonstrate its capability of tracking living cells by videotaping microbes swimming and reconstruct their trajectories in three dimensions. In addition, two-photon dual-color imaging is achieved by simultaneously exciting two types of fluorescent nanospheres mixed with ultrafast laser to demonstrate its potential applications on studies of molecular dynamics and interactions. This method provides a simple wide-field fluorescence imaging approach for deep multiple-particle 3D tracking.

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