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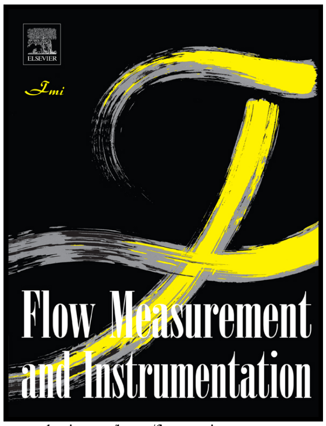

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Three-Dimensional Particle Tracking in Microfluidic Channel Flow Using In and Out of Focus Diffraction

Bushra Tasadduq[1], Gonghao Wang[1], Mohamed El Banani[1], Wenbin Mao[1], Wilbur Lam[2], Alexander Alexeev[1], and Todd Sulchek[1,2]

[1] *Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta GA, 30332, USA*

[2] *Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta GA, 30332, USA*

ABSTRACT

Three-dimensional particle tracking is important to accurately understand the motion of particles within complex flow fields. We show that three-dimensional trajectories of particles within microfluidic flow can be extracted from two-dimensional bright field video microscopy. The method utilizes the defocusing that occurs as particles move out of the objective focal plane when viewed through a high numerical aperture objective lens. A fast and simple algorithm based on cross correlation to a set of reference images taken at prescribed amounts of defocus is used to extract out-of-plane particle position. In-plane particle position is determined through center point detection and therefore the particle position in all three dimensions can be constructed at each time point. Particle trajectories at high flow velocity of greater than 2 mm/s can be tracked by utilizing a high speed camera to obtain unblurred images. Three dimensional computational fluid simulations are used to validate the particle tracking methods.

Keywords: Particle tracking, microfluidics.

1. Introduction:

Conventional video microscopy depicts particle motion projected onto a plane defined by the focus of the objective. For many applications, visualization of motion in the third dimension is required to fully understand the trajectory of particles. Three-dimensional tracking particularly benefits visualizing particle motion in complex flow fields such as that in biomedical devices (Wu, Di Carlo et al. 2008, Tsai, Kita et al. 2012, Wang, Mao et al. 2013, Wang, Crawford et al. 2015) and physiological systems. Several methods can be used to track particles in three dimensions. Laser Doppler velocimetry (Weinberg 1969) utilizes the wavelength shifts due to particle motion within the path of multiple lasers to reconstruct particle motion. Particle image velocimetry (PIV) measures the position and velocity of particles by pulsing a laser and observing the illuminated locations with microscopy (Melling 1997). Extending this technique, two cameras can be positioned along two Cartesian axes to track particles along all three dimensions (Maas, Gruen et al. 1993, Cheung, Ng et al. 2005). Such systems can be complex to implement, however, and require careful alignment of the optical components which results in reduced observational volume that is limited by the optical depth of field in two dimensions. Recently, a single camera combined with multiple objectives was used to track particles in three-

Corresponding author
E-mail address: todd.sulchek@me.gatech.edu
Phone number: 650-954-4592