Published in final edited form as:

Meas Sci Technol. 2008 ; 19(9): 095403–. doi:10.1088/0957-0233/19/9/095403.

The μ PIVOT: an integrated particle image velocimeter and optical tweezers instrument for microenvironment investigations

N Nève¹, J K Lingwood¹, J Zimmerman¹, S S Kohles^{1,2}, and D C Tretheway¹

¹ Department of Mechanical & Materials Engineering, Portland State University, PO Box 751, Portland, OR 97207-0751, USA

² Department of Surgery, Oregon Health & Science University, Portland, OR 97239, USA

Abstract

A novel instrument to manipulate and characterize the mechanical environment in and around microscale objects in a fluidic environment has been developed by integrating two laser-based techniques: micron-resolution particle image velocimetry (μ PIV) and optical tweezers (OT). This instrument, the μ PIVOT, enables a new realm of microscale studies, yet still maintains the individual capabilities of each optical technique. This was demonstrated with individual measurements of optical trap stiffness (~ 70 pN μm^{-1} for a 20 μm polystyrene sphere and a linear relationship between trap stiffness and laser power) and fluid velocities within 436 nm of a microchannel wall. The integrated device was validated by comparing computational flow predictions to the measured velocity profile around a trapped particle in either a uniform flow or an imposed, gravity-driven microchannel flow ($R^2 = 0.988$, RMS error = 13.04 $\mu\text{m s}^{-1}$). Interaction between both techniques is shown to be negligible for 15 μm to 35 μm diameter trapped particles subjected to fluid velocities from 50 $\mu\text{m s}^{-1}$ to 500 $\mu\text{m s}^{-1}$ even at the highest laser power (1.45 W). The integrated techniques will provide a unique perspective toward understanding microscale phenomena including single-cell biomechanics, non-Newtonian fluid mechanics and single particle or particle–particle hydrodynamics.

Keywords

optical tweezers; μ PIV; particle hydrodynamics; cell biomechanics; microfluidics; laser-based techniques

1. Introduction

In the field of fluid mechanics, the hydrodynamic interaction between particles contributes significantly to the rheological properties of many suspensions (Zubarev and Kats 1989) regardless if the suspending fluid is Newtonian or non-Newtonian. While the theoretical framework for particle–particle interactions in Newtonian fluids is well established, theoretical analysis of particle–particle interactions in non-Newtonian fluids is limited due to difficulties in establishing a proper constitutive equation for non-Newtonian fluids. To develop computational models of non-Newtonian fluid suspensions capable of predicting bulk flow properties, detailed knowledge of particle–particle interactions is required not only to validate existing models but also to provide insight into the physical mechanism required for further model development. The direct study of single particle or particle–particle interactions will resolve the hydrodynamic forces between suspended particles in fluids, aid the development

of Newtonian and non-Newtonian fluid suspension models, and provide experimental validation of existing models.

In the field of bioengineering, the study of single-cell biomechanics offers a focused approach and valuable tool for understanding and potentially controlling mechanotransduction, the cell's biological response to its *in vivo* mechanical environment (Kohles *et al* 2007). Specific knowledge of individual cellular biomechanics could elucidate disease etiologies and prioritize the biomechanical factors most critical to stimulating regenerative processes (Kamm and Kaazempur-Mofrad 2004). As there is no consensus on the mechanical signals that are optimally effective in modulating cell function, much is left to be studied. The first steps toward understanding the phenomenon of mechanotransduction involve manipulating and characterizing the mechanical environment in and directly around single cells.

With recent advances in microfluidics and further development of advanced imaging and measurement techniques, exploring microscale phenomena and elucidating the physics of microscale processes is now possible. The objective of this work was to develop an instrument capable of (1) manipulating an isolated particle or cell in a microfluidic environment without mechanical attachment, (2) imaging the isolated particle/cell, (3) characterizing the influential microfluidic environment, and (4) quantifying applied stresses and induced strains. To achieve this, two laser-based techniques were integrated, optical tweezers (OT) and micron-resolution particle image velocimetry (μ PIV) within a single microscope. With optical tweezers, a single particle or cell may be trapped and manipulated in a fluid environment without physical attachment to a surface. With μ PIV, the fluid motion within microfluidic devices and around suspended particles may be characterized with submicron resolution. The integrated instrument (the μ PIVOT) satisfies the present objectives and enables a new experimental method to explore fluid-particle interactions at the microscale.

This work details the μ PIVOT integration, validates the μ PIVOT by comparing computational velocity predictions to the measured velocity profile around a trapped particle in either an imposed microchannel flow or a uniform flow, discusses possible limitations of the integrated optical techniques, and highlights applications enabled by the device. The validation demonstrates the first reported full field velocity measurements around a freely suspended particle in either an imposed microchannel flow or a uniform flow.

2. Laser techniques

2.1. Optical tweezers

Optical tweezers or optical trapping (OT) is an infrared ($\lambda = 1064$ nm) laser-based technique capable of suspending and manipulating micron-sized objects with nanometer position detection and applied forces on the order of pico-Newtons (Ashkin and Dziedzic 1987, Lang *et al* 2002). For a detailed review of optical tweezers, see Svoboda and Block (1994) and Neuman and Block (2004). In brief, an optical trap is produced by passing a laser beam through a high numerical aperture objective lens and focusing it to its diffraction-limited spot. For particles larger in dimension than the wavelength of the trapping laser (particle diameters in this work), a ray optics approach indicates that individual rays of light are refracted as they pass through the trapped object. This change in direction and the associated momentum imparts an equal and opposite force on the object. Without additional imposed forces, an object is 'trapped' at the focal point (Ashkin 1992). Actual trap behavior and trapping force are generally calibrated experimentally as the trapping force depends on the particle type, laser power, particle shape, particle size and fluid media. An optical trap is most effective when the trapped particle size is on the order of the wavelength of the optical tweezers laser (Svoboda and Block 1994).

In general, an optical trap is modeled as a linear mechanical spring with the trap force $F_{\text{trap}} = k * \Delta x$, where Δx is the particle displacement from the trap center (actual focal point) and k is the trap stiffness (Visscher *et al* 1996). This linear approximation is only valid for small particle displacements. To calculate the trap stiffness, the trap force is equated to a known applied force. Four methods to calculate trap stiffness are the drag force method, the nonlinear lateral escape force method, the equipartition method and the power spectrum method (Svoboda and Block 1994, Simmons *et al* 1996, Visscher *et al* 1996). The simplest of these approaches is the drag force method, where the static trap force is equated to the drag force. This is particularly appropriate for this work and is the primary method employed as the drag force is well defined for a rigid solid sphere. For highly non-spherical and/or biological objects, the drag force method alone may not be sufficient, therefore additional trap calibration methods may be necessary. From low Reynolds number (Re) hydrodynamics, the drag force for a stationary sphere in a moving fluid between two infinite parallel plates (a good approximation for the high aspect ratio microchannels in this experimental study) is given by (Happel and Brenner 1983)

$$F_{\text{drag}} = \frac{6\pi\mu av}{1 - 1.004\left(\frac{a}{l}\right) + 0.418\left(\frac{a}{l}\right)^3 + 0.21\left(\frac{a}{l}\right)^4 - 0.169\left(\frac{a}{l}\right)^5} \quad (1)$$

where a is the radius of the spherical particle, l is the half height of the channel, μ is the fluid viscosity, and v is the fluid velocity experienced by the sphere. This modified Stokes drag accounts for particle–wall effects associated with the finite plate separation. The trap stiffness is then calculated by linearly fitting a range of drag force (F_{drag}) versus displacement data (Δx) (the difference between the particle position when the particle is trapped without flow and trapped with flow) and determining the slope.

Researchers have applied OT to study kinesin motor motility (Asbury *et al* 2003), manipulate biological structures (Svoboda and Block 1994) and order suspended particles (Polin *et al* 2005). Knöner *et al* (2005) characterized the velocity near a spinning object by suspending a $1\text{ }\mu\text{m}$ particle with OT, then tracking the particle motion after trap cessation in a particle tracking technique. Leonardo *et al* (2006) extended this technique to multi-point measurement with holographic OT. While these techniques are capable of measuring velocities throughout a three-dimensional (3D) microchannel, the pointwise nature of the technique limits simultaneous velocity measurements to a few (a reported maximum of eight points measured simultaneously) locations within the field of view. This may not provide sufficient detail to accurately determine the fluid stresses surrounding a suspended object. In addition, the resolution of the velocity measurement is limited by the trapped particle size, which is greater than a micron ($1\text{ }\mu\text{m}$ for Knöner *et al* (2005) and $1.1\text{ }\mu\text{m}$ for Leonardo *et al* (2006)).

2.2. Micron resolution particle image velocimetry

Micron-resolution particle image velocimetry (μPIV) is a two-dimensional (2D) full field velocity measurement technique (Santiago *et al* 1998) capable of resolving velocity fields to within 436 nm of a microchannel wall (Tretheway and Meinhart 2002). Velocity measurements are obtained by seeding the flow domain with fluorescent nanoparticles, volume illuminating the region of interest with pulses from two frequency doubled Nd:YAG lasers at the nanoparticle excitation wavelength, and imaging the emitted light by selectively removing the excitation wavelength with an epifluorescent filter. The lasers are synchronized with a charge coupled device (CCD) camera such that the camera captures the emitted particle light from each laser pulse on consecutive image frames. The resulting image pairs are cross-correlated and ensemble averaged (for steady or phase locked time periodic flows) to increase the nanoparticle signal-to-noise ratio (Meinhart *et al* 2000). Note, only particles within the depth of focus of the objective lens contribute significantly to the correlation, therefore the velocity measurement is effectively confined to within a single plane. A three-dimensional (3D)

velocity field may be obtained by scanning layer-wise image planes through the fluid sample and combining the multiple planar velocity fields by applying the continuity equation.

3. Device design

The two laser-based techniques, μ PIV and OT, were integrated to form the μ PIVOT. The OT allows the suspension and manipulation of microspheres or cells, and μ PIV provides imaging and velocity measurements from which fluid induced stresses may be determined. The distinct wavelengths of each laser allow the integration of both techniques through a single high numerical aperture lens positioned within an inverted microscope (TE2000E, Nikon, Tokyo, Japan). Figure 1 provides a schematic of the designed integrated device.

3.1. The OT system

The OT system was constructed on an optical table (Newport R4000, Irvine, CA) with laminar flow isolation (Stabilizer I-2000 Series, Newport). A 1064 nm wavelength laser (BL-106C, J series, Spectraphysics, Mountain View, CA) provides the trapping light source. The laser beam is initially expanded through a 2 $\times$ beam expander (BE02X-C, Thorlabs, Newton, NJ) to ensure that the back aperture of the microscope objective is overfilled which enhances trapping efficiency (Svoboda and Block 1994). Following the beam expander, a safety shutter (SH-10, Electro-Optical Products Corp., Glendale, NY) linked to a foot pedal (Treadlite II T-91-D, Linemaster Switch Corp., Woodstock, CT) is inserted to block the laser beam when necessary. The expanded beam is directed by a mirror (10QM20HM.15, Newport) into a quartz half-wave plate (10RP12-34, Newport) that rotates the direction of polarization. When at 45° the half-wave plate generates equal magnitude *s* and *p* polarization components of the beam. Passing this beam through a polarizing cube beamsplitter (05BC16PC.9, Newport) yields two independent and equally intense traps (*s* component of the beam for one, and *p* component for the other) at the specimen. The resulting two beams are directed by separate mirrors (same as above) through plano-convex lenses (BK7, LA1433-C, Thorlabs) positioned on vertical and linear stages (MVN80 and UMR8.25, Newport) which provide a 3D translation range of 25 mm $\times$ 25 mm $\times$ 12.5 mm per beam. These movable lenses form the front half of a 1:1 telescope. Coupled with a fixed back lens mounted in the lower optical port of the microscope, the *x*-*y* translation movement of the front lenses drives the laser beam laterally relative to the microscope objective lens, yet maintains the beam close to the optical center. This permits a wide translation capability (effectively the field of view) of a trapped particle within a channel. The positions of the stages are controlled through either manual micrometers (BM17.25, Newport) or motorized actuators (LTA-HL, Newport) linked to a controller (XPS-C6, Newport) via drivers (XPS-DRV01, Newport). The translation stage resolution coupled with the 1:1 telescope and optical magnification of the microscope objective allows sub-micron position movement and object resolution. Before entering the fixed back lens, the two independently controlled beams are recombined into a single path by a second polarizing cube beamsplitter. Once in the microscope, the infrared beams reflect off a low-pass filter (cut-off wavelength of 1000 nm), pass through a high-pass filter (cut-on wavelength of 550 nm) and are focused through one of two available high numerical aperture (NA=1.45, 60 $\times$ or NA=1.49, 100 $\times$) oil immersion objective lenses (Plan Apo TIRF Nikon) into the sample.

Once a particle is trapped, its position and shape deformation may be imaged with either a moderate frame rate, low-resolution (1 pixel = 125 nm) CCD camera (Gevicam GP-3360 running a visualization imaging software Coyote V2.3.0, Gevicam Inc.), or the high-resolution μ PIV camera (1 pixel = 109 nm) described in the following section. Captured images are further analyzed with object tracking software (Spotlight-8, NASA) for characterizing position and size/deformation of trapped particles with a resolution of ± 0.5 pixels.

3.2. The μ PIV system

The μ PIV system consists of 532 nm wavelength Nd:YAG lasers (Solo III, 15 Hz, New Wave Research, Fremont, CA) connected with a fiber optic cable to the upper optical port of the inverted microscope. The very short laser pulses (3 ns to 5 ns duration, with a maximum energy of 50 mJ per pulse) are reflected by the high-pass filter cube noted previously and directed into the described high numerical aperture objective lens illuminating the flow field which is seeded with 275 nm diameter fluorescent polystyrene spheres (nile red fluorescent 535/575, carboxylate-modified, Molecular Probes, Carlsbad, CA). The emitted fluorescent light ($\lambda = 575$ nm) is then captured by the objective lens, passes through both the low- and high-pass filter cubes, and is focused onto a CCD camera (PIVCAM 14–10 Model 630055, TSI, Shoreview, MN). The image pairs are viewed and saved using a data acquisition, analysis and display software (Insight 3G, TSI). The data processing includes the use of a viewer (IrfanView freeware, Wiener Neustadt, Austria) to convert images to process compatible formats, a customized program for μ PIV interrogation (written in Matlab, The Mathworks, Natick, MA), a PIV vector validation software (CleanVec V1.13.41, freeware), and finally software for viewing flow images and displaying the calculated vector fields and flow streamlines (Tecplot 10, Tecplot Inc., Bellevue, WA).

4. Validation method: experimental protocol

4.1. Static environment

In order to test the different laser techniques and their influence on each other, a static condition was first set up. A stagnant solution containing a typical concentration of μ PIV nanoparticles (concentration of 50 μ L nanoparticle, 2% solid solution for 2 mL of distilled water), and/or a small concentration of particles to be trapped, was placed between two coverslips separated by double face tape. The OT laser was then fired and the sample region was imaged in real time by one of the two cameras. The laser power was measured at the entrance of the microscope, using a power meter (1916-C, Newport) linked to a detector (818P-010–12, Newport), with either both of the OT lasers on, or one shuttered for single trap experiments. The trap power has been observed to fluctuate during the first 45 min of firing time. For the typical trap power of 0.49 W, the measured variation was less than 1%. The average trap power between the beginning and end of each experiment was reported.

4.2. Dynamic environment: flow around a freely suspended sphere

The objective of the experimental validation was to create a controlled and measured constant flow around a stationary particle not attached to any surface or mechanical restraint. Two types of flow were examined: (1) a uniform flow field generated by moving the fluid sample with an automated translation stage and (2) a gravity-driven flow through a straight microchannel.

4.2.1. Stationary sphere in a uniform flow—Figure 2(a) provides a schematic of a spherical particle trapped by OT in between two coverslips. A very low concentration of polystyrene microspheres solution (polystyrene crosslinked DVB copolymer, Duke Scientific, Fremont, CA), with diameters ranging from 10 to 35 μ m (sizes of biological cells), was diluted in a solution of typical concentration fluorescent nanoparticles (for μ PIV measurements around the suspended microsphere) and placed between two coverslips. A sphere was trapped at a depth of 1.5 times the sphere diameter (between 20 and 55 μ m) from the bottom coverslip to maintain a constant dimensionless distance (depth/sphere diameter) from the bottom wall. The depth was set with the objective lens of the microscope (vertical resolution of 250 nm). The upper coverslip is greater than 150 μ m from the suspended sphere, and thus minimally affects the trapped sphere hydrodynamically (Ho and Leal 1974). The uniform flow field was generated by moving the coverslips on an automated stage (H117 ProScan™ II stage, Prior

Scientific, Rockland, MA) at constant velocity. The stage was controlled to execute displacement rates of $50 \mu\text{m s}^{-1}$ to $500 \mu\text{m s}^{-1}$ in the x -direction (linear resolution of 20 nm).

The captured μPIV images were examined with an initial interrogation window of 64×64 pixels and 75% image overlap, then further refined to 32×32 pixels (a common μPIV interrogation domain) with a 50% overlap (yielding a $1.7 \mu\text{m}$ velocity spacing). This resolution is sufficient for the particle sizes examined. The time difference between images (Δt) was set from 2.5 ms (for a stage velocity of $500 \mu\text{m s}^{-1}$) up to 20 ms (for a stage velocity of $50 \mu\text{m s}^{-1}$) so that the typical movement of the nanoparticles was approximately 10 pixels between image frames.

4.2.2. Stationary sphere in a gravity-driven microchannel flow—Figure 2(b) provides a schematic of a spherical particle trapped by OT in a $50 \mu\text{m} \times 500 \mu\text{m}$ microchannel. This straight channel was fabricated using a standard soft lithography approach with polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning, Midland, MI) (figure 3) as the soft peeled material (Xia and Whitesides 1998). The chip was then chemically bonded to a coverslip using the PDMS replica molding and microfluidic device finishing protocol (Pang 2006). A gravity-driven microchannel flow was generated by simply attaching input and output open syringes (filled barrel with no plunger) placed at different heights. The output syringe height was held constant while the input syringe height was controlled in order to vary the flow rate. The syringes were connected to the microfluidic chip via Tygon tubing (Fisher Scientific, Pittsburgh, PA) attached to metal pins ($0.025''$ OD $\times$ $0.017''$ ID, Type 304, New England Small Tubes Corp., Litchfield, NH) that were directly inserted at the entry/exit ports of the microchannel. This setup allowed for a controllable, constant, and steady flow.

The microfluidic chip was initially primed with particle free de-ionized water. A low concentration of polystyrene microspheres solution with an average diameter of $26 \mu\text{m}$, diluted in distilled water at a volume ratio of 1:1000, was then inserted through the upstream tubing. A single microsphere was trapped far from the entry/exit ports in order to be in a fully developed flow region, and the remaining microspheres were flushed out of the microchannel. All the experiments were performed at $z = 0$ (center of the channel), calculated by locating the top and bottom of the channel then fine tuning the microsphere position by observing its rotation. No rotation corresponds to symmetry in the velocity field experienced by the sphere. This occurs only at the mid-plane between the two walls. The gravity-driven microchannel flow was then turned on. This fluid was seeded with fluorescent nanoparticles for μPIV measurements around the microsphere. The captured μPIV images were examined as described in the above section. Δt ranged from $500 \mu\text{s}$ to 2 ms for a 6 to 10 pixels movement between image frames.

4.3. Computational model

4.3.1. Stationary sphere in a uniform flow—The flow around a sphere suspended in a uniform velocity field was modeled with computational fluid dynamics software (STAR-Design 4.14.003, CD-adapco, Melville, NY, for model and meshing, and STAR-CCM+ 2.10.017, for the computation). For the experimental conditions (microscale, low velocities), the flow is laminar ($Re < 10^{-2}$) and steady. The fluid was modeled as an incompressible Newtonian fluid with a density (998 kg m^{-3}) and viscosity ($0.992 \times 10^{-3} \text{ N s m}^{-2}$) equal to that of water at room temperature (20.5°C). Following the described experimental setup, a sphere was set to be $20 \mu\text{m}$ in diameter, and placed $30 \mu\text{m}$ from the bottom coverslip. The bottom coverslip is modeled as a no-slip boundary moving at a constant velocity of $200 \mu\text{m s}^{-1}$ while a symmetry plane replaces the remaining computational boundaries including the top coverslip (there is no hydrodynamic interaction with the sphere) to reduce computational effort. The computational domain, shown in figure 4(a), was meshed with 134 375 polyhedrals

to provide a numerical resolution greater than experimental measurements. We extracted velocities along the inflow/outflow axis (x -direction).

The solver uses the numerical ‘SIMPLE’ algorithm that iteratively solves the coupled Navier–Stokes equation (2) and the mass continuity equation (3):

$$\rho \left(\frac{\partial \mathbf{v}}{\partial t} + \mathbf{v} * \nabla \mathbf{v} \right) = - \nabla p + \mu \nabla^2 \mathbf{v} + \mathbf{f} \quad (2)$$

$$\nabla * \mathbf{v} = 0 \quad (3)$$

where ρ is the fluid density, $\mathbf{v}$ is the fluid velocity vector, t is time, ∇p is the pressure gradient, μ is the fluid viscosity, and $\mathbf{f}$ is an added vector representing other possible forces applied to the fluid ($\mathbf{f} = 0$ in the cases studied).

4.3.2. Stationary sphere in a gravity-driven microchannel flow—In the second experimental setup, the width of the microchannel ($500 \mu\text{m}$) was much larger than the height of the microchannel ($50 \mu\text{m}$). Under these conditions, the flow at the center of the microchannel resembles Poiseuille flow between two infinite stationary parallel plates which was confirmed experimentally. However, the theoretical solution for Poiseuille flow around a suspended sphere is relatively complex with solutions limited to spheres with diameters sufficiently smaller than the plate separation (Ho and Leal 1974, Ganatos *et al* 1980). These solutions are not applicable to the single sphere experiments described here. As a result, the flow around a sphere suspended in a microchannel was modeled with computational fluid dynamics software (OpenFOAM, with the SimpleFOAM solver, OpenCFD, Berkshire, UK).

The solver uses the same numerical ‘SIMPLE’ algorithm as described in the previous section and the fluid was also modeled as previously described. The boundary conditions were the no-slip condition at the channel walls and at the sphere surface. In addition, the simulation required an inlet velocity. The computational domain is sufficiently large that effects of the sphere at the inlet are negligible, and the velocity field is consistent with flow through a microchannel without a sphere. The velocity for flow in the microchannel without a sphere (at the same conditions of the sphere experiment) was measured experimentally with μPIV and the average input as the inlet velocity. To reduce computational effort, the flow domain was subdivided into quadrants (dictated by flow symmetry) and solved with 5000+ tetrahedron elements for a single quadrant. Figure 4(b) provides the quadrant computation domain and shows representative flow characteristics of this scenario. From this 3D model, we examine the 2D velocity profile at the mid-plane of the channel, coincident with the center of the microsphere (the x – y plane in figure 2(b)) which corresponds to the experimentally analyzed region. We also extract velocities along the inflow/outflow axis (the x -direction).

5. Device validation

The μPIV and OT techniques have successfully been integrated as the μPIVOT with the individual capabilities of each technique maintained and simultaneous application of both techniques enabled. A major concern in integrating the techniques was the influence the integration may have on each technique and the compatibility of the simultaneous application of both.

5.1. OT alone

With dual optical tweezers, we have trapped and manipulated small glass microspheres (soda lime glass, model 07666-1, Polysciences Inc., Warrington, PA), polystyrene microspheres, as well as skin, blood, bone and cartilage cells (diameter range: $2 \mu\text{m}$ to $40 \mu\text{m}$) in a static environment.

Applying the drag force method, the trap stiffness was calculated for suspended microspheres (diameters between 20 and 23 μm) with laser power settings varying between 0.105 W and 1.481 W. The fluid velocities were set such that the drag force was insufficient to displace the trapped particle outside the linear trap regime. Figure 5(a) shows the trap stiffness varying linearly between 4 and 70 $\text{pN } \mu\text{m}^{-1}$ with laser power, while figure 5(b) shows the trap stiffness decreasing toward a plateau with increasing particle diameter. This behavior is consistent with previously reported stiffness values (Williams 2002). This indicates that the integration of the OT and the μPIV laser techniques, which involved the placement of an additional filter cube in the OT path, did not interfere with the capabilities of the OT system.

5.2. μPIV alone

With μPIV , measurement of fluid velocities in custom microfluidic devices was achieved. Figure 6 shows a 2D velocity field adjacent to the side wall at the mid-plane of a microchannel with a velocity vector vertical spacing of 436 nm. The velocity approaches its free-stream value at approximately 20 μm (y-direction) and smoothly decreases to zero at the wall. This profile is consistent with the analytical solution for flow through a rectangular duct assuming the no-slip boundary condition (White 1974), and concurs with previous studies performed with μPIV (Tretheway and Meinhart 2002). This indicates that the addition of optical components, specifically the low-pass filter necessary for the setup of the integrated device, does not decrease the capabilities of μPIV .

5.3. Interaction between OT and μPIV without a trapped sphere

The μPIV lasers volume illuminate the entire field of view during two 5 ns pulses. As expected, due to the short and diffuse nature of this lighting, no apparent effect of the μPIV lasers on a particle trapped with OT was observed. The particle remained stationary and in focus during each pulse even at the maximum pulse energy of 50 mJ. During μPIV measurements, the laser power is reduced to the minimum power (less than 25 mJ per pulse) necessary to excite the fluorescent nanoparticles.

A greater concern was the effect of the OT on the μPIV nanoparticles. The fluorescent nanoparticles are much smaller than the IR wavelength of the OT laser, however, the influence of the OT on the nanoparticles is not known. In order to successfully apply both techniques simultaneously, the influence of the OT on the flow trajectory of the μPIV nanoparticles (275 nm diameter) must be determined.

Figure 7(a) shows the effect of the OT laser power on a stagnant solution of μPIV nanoparticles while figure 7(b) shows the μPIV solution moving at a constant velocity set with the automated translation stage (streaklines indicate the direction of flow). In each case, the laser was off then turned on at a given power. For all laser powers, a small region of trapped nanoparticles is observed. These trapped nanoparticles fluoresce and appear as a bright spot (note, due to the resolution of figure 7, the fluorescent spot at 0.11 W may be difficult to discern). This trapped clump of particles exhibits a dynamic growth (accumulation of particles) and collapse (release of particles) behavior. Even with this dynamic clumping, no attraction of nanoparticles to the OT in the field of view is observed for either the static solution or moving fluid. The streaklines in figure 7(b) are straight throughout the field of view even near the fluorescent spot, which indicates a localized region of influence (ROI). This is further verified by calculating the fluid velocity with μPIV . The measured velocities are within 1% of the translation stage velocity except in the ROI where velocities cannot be calculated due to pixel saturation in the μPIV images. This suggests that the dynamic behavior occurs along the optical axis. The maximum ROI (as shown in figure 7) increases from a measured diameter of 0.7 μm for 0.11 W to a diameter of 4.0 μm for 1.45 W. While the actual size of the nanoparticle clump cannot be

definitively determined, the nanoparticle clump is within the ROI. Thus, the ROI represents the upper limit of nanoparticle clump size.

It is interesting to note that figure 7 shows the μ PIV nanoparticles fluorescing within the ROI in each image without applying the required excitation wavelength of 532 nm. This is most likely due to two-photon excitation from the trapping laser light (Florin *et al* 1996,1997). While this phenomenon interferes with μ PIV by saturating pixels as stated above, the phenomenon is limited to the localized ROI.

5.4. Interaction between OT and μ PIV with a trapped sphere

Figure 8 shows a large microsphere (diameter of $26\ \mu\text{m}$) trapped in a stagnant solution of nanoparticles. Since the trapped sphere is sufficiently larger than the ROI of the OT, the dynamic collection of nanoparticles at the trap location noted in figure 7 is not observed. Brownian motion with a time average zero velocity is measured with μ PIV for the surrounding nanoparticles. Therefore, for particle/cell studies, if the trapped particle/cell covers the OT ROI, the effects of the OT on μ PIV measurements should be negligible. Future applications of the μ PIVOT will require quantifying the microfluidic environment of a trapped particle/cell under dynamic (non-stationary) flow conditions.

5.4.1. A sphere in a uniform flow—As described in section 3, we validate the μ PIVOT under dynamic conditions by trapping a single sphere in either a uniform flow or in the middle of a straight microchannel with an imposed, gravity-driven laminar flow. Figure 9 quantitatively compares the measured and predicted velocities along the inflow and outflow axes of a stationary sphere in a uniform flow generated by translation of the automated microscope stage. With the velocity normalized by the free stream velocity (in this case the stage velocity) and the distance from the sphere normalized by the sphere radius, low Re hydrodynamics predicts the velocities collapse to a single curve for a sphere positioned at the same dimensionless distance from the wall. Figure 9(a) shows the measured velocities collapse to a single curve for sphere diameters of $15\ \mu\text{m}$ to $35\ \mu\text{m}$, while in figure 9(b) the velocities collapse for free stream velocities of $50\ \mu\text{m s}^{-1}$ to $500\ \mu\text{m s}^{-1}$. Figure 9 shows strong quantitative agreement between the predicted and observed velocity as a function of dimensionless distance (x/a) from the sphere for diameters of $15\ \mu\text{m}$ to $35\ \mu\text{m}$ ($R^2 = 0.9131$, $\text{RMS} = 0.0776$ along the inflow axis and $R^2 = 0.9311$, $\text{RMS} = 0.0652$ along the outflow axis) and for free stream velocities of $50\ \mu\text{m s}^{-1}$ to $500\ \mu\text{m s}^{-1}$ ($R^2 = 0.9310$, $\text{RMS} = 0.0635$ along the inflow axis and $R^2 = 0.9389$, $\text{RMS} = 0.0605$ along the outflow axis).

Note the discrepancy between the experimental velocities and computational predictions with the theoretical predictions of a sphere in a uniform flow without wall effects. The significant wall effects of the bottom coverslip force the actual velocity profile to reach the free stream velocity sooner. Thus, the μ PIVOT is capable of resolving the hydrodynamic interaction between the suspended sphere and the bottom wall.

5.4.2. A trapped sphere in microchannel flow—To further validate the technique, figure 10(a) shows the measured velocity field in the vicinity of a trapped $28\ \mu\text{m}$ sphere (every third vector is shown for clarity) in an imposed, gravity-driven microchannel flow. This flow is more complicated than uniform flow as a result of a non-uniform free stream velocity and significant wall effects. The measured velocity field is qualitatively and quantitatively consistent with the computationally predicted velocity field (figure 10(b)). For quantitative comparison, figure 11 shows the predicted and measured velocities along the inflow and outflow axes (the x -axis at $y = z = 0$ in figure 4). As expected, the x -direction velocity at the sphere surface (when x/a is equal to 1 and -1) is zero (no-penetration condition), and the velocity increases towards its free stream velocity away from the sphere. Figure 11 shows strong quantitative agreement

between the predicted and observed velocity as a function of dimensionless distance (x/a) from the sphere ($R^2 = 0.988$, RMS error = $13.04 \mu\text{m s}^{-1}$ along the inflow axis and $R^2 = 0.973$, RMS error = $18.89 \mu\text{m s}^{-1}$ along the outflow axis). While not shown for brevity, the measured and predicted velocities along the cross-flow axis (the y -axis at $x = z = 0$ in figure 4) agree quantitatively as well.

6. Discussion

The validation measurements were made in the equatorial plane of a suspended sphere. Future work may require the complete 3D velocity field in the vicinity of the sphere be determined. As noted in section 5.3, without a trapped sphere a localized dynamic nanoparticle accumulation and release in the vicinity of the OT is observed. Outside this ROI, the clumping process has no measurable effect on velocity measurements in the image plane. This indicates the dynamic process occurs along the optical axis (out of the image plane). As the out of plane extent is unknown, it is not clear, at this time, how significant this effect would be on 3D velocity measurements. For measurements with a suspended sphere in the image plane, the clumping process is generally negligible. However, two circumstances exist where the influence is significant and OT interferes with μPIV . Both of these occur when the nanoparticles are not excluded from the optical trap ROI. First, when the trapped particle is smaller than the region of influence, nanoparticles are attracted toward the center of the trap. Their concentration at that spot becomes problematic due to the fact that they are excited by the OT laser which induces image saturation. Second, when the trapping power is relatively low and a large particle (microsphere or cell) is trapped in a sufficiently fast flow, the ROI may extend beyond the sphere. During flow, the imposed drag force displaces the particle relative to the trap center (trap stiffness behavior). If the flow is sufficient, the particle displacement may approach the sphere radius which means the trap center is located near the sphere edge. If the trap ROI extends beyond the sphere edge, nanoparticles may be attracted and conglomerate. This results in a saturated image on the inflow axis. Regardless, these two cases of significant interference can be mitigated with the proper selection of trap power and particle size. Overall, the quantitative agreement between the measured and predicted velocities, shown in figures 9 and 11, indicates limited influence of OT on μPIV during flow conditions and validates the integration of OT and μPIV for trapped particle diameters of $15 \mu\text{m}$ to $35 \mu\text{m}$ and velocities from $50 \mu\text{m s}^{-1}$ to $500 \mu\text{m s}^{-1}$.

With the μPIVOT , theoretical models characterizing microscale fluid and particle phenomena may be verified experimentally, and previously unattainable measurements of direct particle–particle interaction and the stress fields around deformed elastic and viscoelastic bodies, such as biological cells may be explored. With its dual trap capability, the μPIVOT enables the suspension of two particles in an imposed fluid flow and the simultaneous measurement of the velocity field surrounding the interacting particles. From these measurements the hydrodynamic interaction and the detailed flow characteristics required for validating non-Newtonian constitutive equations may be determined. Coupled with a microfluidic testbed, the μPIVOT provides multimodal cellular manipulation with the ability to characterize the mechanical microenvironment (Nève *et al* 2007). This combination provides a unique platform toward characterizing cellular biomechanics (stress–strain; the precursor toward deciphering mechanotransduction) by allowing a sequence or combination of mechanical stresses to be applied to the same individual cell while monitoring the strain response for each stress state. This technique eliminates the effects of mechanical restraints associated with many single-cell approaches (Leipzig and Athanasiou 2005). In addition, the proposed technique is a single-cell approach that extends the range of applicable fluid induced stresses to include global and extensional stresses, which are limited in cell cultured monolayer shear stress studies.

7. Summary

A novel instrument has been developed by integrating two laser-based techniques: micron-resolution particle image velocimetry (μ PIV) and optical tweezers (OT) within a single microscope. The μ PIVOT maintains the individual capabilities of μ PIV and OT. The integrated techniques were validated by comparing computational predictions to the measured velocity profile around a trapped particle in either an imposed microchannel flow or a uniform flow. Good quantitative agreement between measured and predicted velocities is observed for 15 μm to 35 μm diameter trapped particles subjected to fluid velocities from 50 $\mu\text{m s}^{-1}$ to 500 $\mu\text{m s}^{-1}$ even at the highest laser power (1.45 W). This validation demonstrates the first reported full field velocity measurements around a freely suspended particle in either an imposed microchannel flow or a uniform flow. Finally, the μ PIVOT enables a new experimental method to explore fluid–particle interactions at the microscale. Moreover, previously unattainable full field measurements of direct particle–particle interaction and the stress fields around deformed elastic and viscoelastic bodies such as biological cells may now be explored.

Acknowledgments

The authors gratefully acknowledge a National Science Foundation Major Research Instrumentation grant (CBET-0521637), an Academic Research Enhancement Award from the National Institutes of Health (EB007077), and the Engineering Technology and Industry Council for funding. We would also like to thank Joerg Klatte, ZARM, Bremen University, Germany, for providing simulation expertise; Dr Steve Wereley, Purdue University who has written the customized Matlab program for μ PIV interrogation; Dr Shelley Winn, OHSU, for access to biological cells; and Dr Charles Asbury, University of Washington, for his expertise in optical tweezing. Finally, we would like to express our gratitude towards the Stanford Microfluidics Foundry for fabrication of the microfluidic chips.

References

- Asbury CL, Fehr AN, Block SM. Kinesin moves by an asymmetric hand-over-hand mechanism. *Science* 2003;302:2130–4. [PubMed: 14657506]
- Ashkin A. Trapping of atoms by resonance radiation pressure. *Phys. Rev. Lett* 1978;40:729–32.
- Ashkin A. Forces of a single-beam gradient laser trap on a dielectric sphere in ray optics regime. *Biophys. J* 1992;61:569–82.
- Ashkin A, Dziedzic JM. Optical trapping and manipulation of viruses and bacteria. *Science* 1987;235:1517–20. [PubMed: 3547653]
- Florin EL, Horber JK, Stelzer EH. High-resolution axial and lateral position sensing using two-photon excitation of fluorophores by a continuous-wave Nd/YAG laser. *Appl. Phys. Lett* 1996;69:446–8.
- Florin EL, Pralle A, Horber JK, Stelzer EH. Photonic force microscope based on optical tweezers and two-photon excitation for biological applications. *J. Struct. Biol* 1997;119:202–11. [PubMed: 9245760]
- Ganatos P, Pfeffer R, Weinbaum S. A strong interaction theory for creeping motion of a sphere between plane parallel boundaries: part2. Parallel motion. *J. Fluid Mech* 1980;99:755–83.
- Happel, J.; Brenner, H. *Low Reynolds Number Hydrodynamics*. Springer; Berlin: 1983. Sphere moving relative to plane walls; p. 322–30.
- Ho BP, Leal LG. Inertial migration of rigid spheres in two-dimensional unidirectional flows. *J. Fluid Mech* 1974;65:365–400.
- Kamm RD, Kaazempur-Mofrad MR. On the molecular basis for mechanotransduction. *Mech. Chem. Biosystems* 1 2004;3:201–9.
- Knöner G, Parkin S, Heckenberg NR, Rubinsztein-Dunlop H. Characterization of optically driven fluid stress fields with optical tweezers. *Phys. Rev. E* 2005;72
- Kohles SS, Wilson CG, Bonassar LJ. A mechanical composite spheres analysis of engineered cartilage dynamics. *ASME J. Biomech. Eng* 2007;129:473–80.
- Lang M, Asbury C, Shaevitz J, Block SM. An automated two-dimensional optical force clamp for single molecule studies. *Biophys. J* 2002;83:491–501. [PubMed: 12080136]

- Leipzig ND, Athanasiou KA. Unconfined creep compression of chondrocytes. *J. Biomech* 2005;38:77–85. [PubMed: 15519342]
- Leonardo RD, Leach J, Mushfique H, Cooper JM, Ruocco G, Padgett MJ. Multipoint holographic optical velocimetry in microfluidic systems. *Phys. Rev. Lett* 2006;96:134502. [PubMed: 16711992]
- Meinhart CD, Wereley S, Santiago J. A PIV algorithm for estimating time-averaged velocity fields. *J. Fluid Eng* 2000;122:285.
- Neuman KC, Block SM. Review article: optical trapping. *Rev. Sci. Instrum* 2004;75:2787–809. [PubMed: 16878180]
- Nève N, Lingwood JK, Winn SR, Tretheway DC, Kohles SS. Microfluidics supporting an optical instrument for multimodal single cell biomechanics. *ASME Int. Congr. Exposition* (Seattle, WA). 2007paper 42004
- Pang, WL. PDMS Replica Molding and Microfluidic Device Finishing Revision 1.3. Systems Biodynamics Laboratories; University of California, San Diego: 2006. http://ori.ucsd.edu/download/docs/pdmsprocessing_v1p3.pdf
- Polin N, Ladavac K, Lee S-H, Roichman Y, Grier D. Optimized holographic optical traps. *Opt. Exp* 2005;13:5831–45.
- Santiago JG, Wereley ST, Meinhart CD, Beebe DJ, Adrian RJ. A particle image velocimetry system for microfluidics. *Exp. Fluids* 1998;25:316–9.
- Simmons RM, Finer JT, Chu S, Spudich JA. Quantitative measurements of force and displacement using an optical trap. *Biophys. J* 1996;70:1813–22. [PubMed: 8785341]
- Svoboda K, Block SM. Biological applications of optical forces. *Annu. Rev. Biophys. Biomol. Struct* 1994;23:247–85. [PubMed: 7919782]
- Tretheway DC, Meinhart CD. Apparent fluid slip at hydrophobic microchannel walls. *Phys. Fluids* 2002;14:L9–12.
- Visscher K, Gross S, Block SM. Construction of multiple beam optical traps with nanometer-resolution position sensing. *Sel. Top. Quantum Electron.* 2 1996;4:1066–76.
- White, F. *Viscous Fluid Flow*. McGraw-Hill; New York: 1974. p. 123
- Williams, MC. Optical tweezers: measuring picoNewton forces. In: Schwille, P., editor. *Single Molecule Techniques*. Biophysical Society; Bethesda, MD: 2002. a volume of the *Biophysics Textbook Online*, editor-in-chief L DeFelice
- Xia Y, Whitesides GM. Soft lithography. *Ann. Rev. Mater. Sci* 1998;28:153–84.
- Zubarev AY, Kats ES. Rheological properties of finely dispersed suspensions. *J. Eng. Phys. Thermophys* 1989;57:1437–42.

Figure 1.
Schematic of the μ PIVOT device and the distinct optical arrangement.

Figure 2.

A 3D simplified sketch of the dynamic single sphere experiment. (a) A sphere held stationary in between two coverslips moving at a constant velocity. The sphere is more than 1 mm below the top coverslip. (b) A particle trapped in the middle of a straight channel with laminar flow ($Re < 10^{-2}$).

Figure 3. A PDMS chip bonded to a glass coverslip with four $50\ \mu\text{m} \times 500\ \mu\text{m}$ straight channels. The μ PIVOT validation experiments were conducted in a single channel.

Figure 4. A 3D representation of the computational flow domain. (a) Uniform flow half-space. (b) Gravity-driven flow quadrant. The color scales represent the magnitude of the velocity in the general flow direction, in $\mu\text{m s}^{-1}$ (x -axis).

Figure 5.

(a) Trap stiffness as a function of the applied laser power for trapped polystyrene microspheres.
 (b) Trap stiffness as a function of sphere diameter for a constant laser power of 0.48 W. Stiffness values were determined from linear curve fits of the drag force versus microsphere displacement experimental data. Drag force calculations accounted for channel wall proximity.

Figure 6.

Laminar flow velocity profiles acquired with μ PIV. The near wall velocity resolution is 436 nm. The no-slip condition can be observed as the velocity decreases (vector length = magnitude) to zero at the wall.

Figure 7.

Response of μ PIV nanoparticles to the applied trap laser power (measured at the microscope entrance). The number of trapped nanoparticles (bright spot) increases with trap power within (a) a static fluid and (b) a fluid moving at constant velocity (shown by the streaklines).

Figure 8.

A trapped polystyrene microsphere among a static solution of nanoparticles at a laser power of 0.49 W. The μ PIV laser is on and therefore the nanoparticles fluoresce. No trapping of the nanoparticles is observed due to the shield created by the size of the microsphere.

Figure 9.

Quantitative comparison between measured velocities (points), computationally predicted velocities (line) and theoretical velocities without wall effects (dashed line). The inflow and outflow axes ($-$ and $+$ x -axes, respectively) is normalized with the sphere radius, a , and the velocity is normalized with the automated stage velocity. (a) A $21.8 \mu\text{m}$ sphere in a uniform flow at different velocities. (b) Different diameter microspheres with a constant uniform flow of $200 \mu\text{m/s}$.

Figure 10.

Comparison between the experimental velocity field and the computational prediction for a stationary sphere held in a gravity-driven flow. (a) μPIV 2D velocity field around a trapped $28 \mu\text{m}$ diameter polystyrene microsphere held in a straight flow. (b) Model simulation of the experimental flow conditions.

Figure 11.

Comparison between measured (points) and predicted (line) velocities for a $28 \mu\text{m}$ sphere in a gravity-driven flow with a maximum plane velocity of $460 \mu\text{m s}^{-1}$.