Optical path-length modulation for three-dimensional particle measurement in mirror-embedded microchannels

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Simple and low-cost implementation of three-dimensional (3D) particle measurement is vital for designing and characterizing microfluidic devices that show spatiotemporally varying characteristics in three dimensions. However, the conventional 3D particle image velocimetry or particle streak velocimetry has proven difficult to address the needs, requiring complex and expensive equipment, precise alignment between optical components, and specialized image-processing algorithms. Here, we report mirror-embedded microchannels and a method of optical path-length (OPL) modulation for 3D particle measurement in the channels. The mirror, ideally at 45°, reflects the side view of the channels and enables 3D positional information to be obtained easily from two different orthogonal-axis images with different optical paths. To offset the optical path difference between two image views, we utilized a cover glass as a medium of high refractive index and placed it in the light path through which the side-view image propagates, thereby prolonging the OPL of the side view and simultaneously shifting its depth of field (DOF) range. This modulation ensures imaging of in-focus side view as well as top view. This 3D imaging principle was verified by observing 3D positions of 6 μm-sized beads in the linear and grooved microchannels. The mirror-embedded scheme can be readily fabricated with existing microfluidic designs, and offer easy and simple implementation of 3D particle measurement.

Introduction

Advances in microfluidic technology often accompany an increase of complexity in channel geometry, fabrication, and flow phenomena. Chaotic mixers passively generate transverse flows from three-dimensional (3D) topological features such as grooves and rilles, thereby stirring the boundary layer in three dimensions and enhancing mixing in microchannels.1–3 Such stirring effect has also been used to continuously separate particles of different sizes by using a hydrodynamic principle known as “hydrophoresis” that means the movement of suspended particles under the influence of a microstructure-induced pressure field.4 Dean mixers exploit a secondary flow field where two vortices (called Dean vortices) exist rotating in opposite directions to each other and increase the interfacial surface area between different fluid streams.5,6 The counter-rotating vortices have also been employed for 3D hydrodynamic focusing by fully sheathing a sample flow with the convective, rotational flows.7 The spatially varying characteristics of these microfluidic technologies have mainly been explored by using confocal fluorescence scanning microscopes that reconstruct a 3D image with layer-by-layer images.1,5 However, the low temporal resolution of such systems makes it difficult to measure complex particle dynamics and flow fields varying in three dimensions.

To address the above need, a mirror-embedded microchannel has been proposed for real-time, 3D measurement of particle position.8 This imaging technique employs a mirror ideally at 45° to reflect the side view of a microchannel, thereby enabling simultaneous imaging of the top and side views of the channel with a single lens. This simplicity ensures easy implementation of 3D characterization of flowing objects in microchannels without complex, expensive optical equipments and specialized image-processing algorithms that are typically required for 3D particle image velocimetry and particle streak velocimetry.8–11 With this method, we observed that hydrophoresis is governed by both 3D convective vortices and steric hindrance, and enables 3D particle focusing without sheath flows. A silicon mirror is placed in close proximity to the area of interest in a microchannel. The mirror coated with an aluminium film yields a reflectivity of around 80–90% over the visible spectrum.12 In a mirror-embedded microchannel, an object has two different optical paths through a microscope for the side and top view. The side view reflected by a mirror has a longer optical path-length (OPL) than the top view. Therefore, the distance between the mirror and channel area should be smaller than the depth of field (DOF) to obtain an in-focus image of the side view as well as the top view. In optics, the DOF is determined by the distance from the nearest object plane to the farthest plane in focus, and varies with numerical aperture and magnification of the objective lens. The DOF values for 4× and 10× objective lenses are ≈130 and 25 μm, respectively.8 For mirror-embedded microchannels, a large DOF is more effective to simultaneously obtain two images with a difference in OPL.

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However, the limited DOF values make it difficult to image an object far from the mirror. This disadvantage prevents the use of the mirror-embedded scheme to reveal 3D characteristics of many microfluidic applications.

Herein, we report on OPL modulation for 3D particle measurement in mirror-embedded microchannels. In a fixed geometric condition, OPL can be modulated by replacing the medium through which light propagates. To offset the optical path difference between two image views in a mirror-embedded microchannel, we utilized a cover glass as a medium of high refractive index and placed it in the light path through which the side-view image propagates. The use of the medium with different refractive index makes it possible to prolong the OPL of the side view and to obtain an in-focus image of the side view as well as the top view. The OPL modulation capability was demonstrated by imaging a linear channel filled with fluorescein isothiocyanate (FITC) and 6 μm-sized fluorescence beads in the linear and grooved channels. This study yields a fundamental understanding of the OPL modulation in a mirror-embedded microchannel, which is crucial for designing a mirror-embedded microchannel for many other microfluidic applications.

Experimental

Design and fabrication of mirror-embedded microchannels

We fabricated the mold structures with multi-step photolithography in SU-8 photoresist (SU8-2010 and -2025; Microchem Corp., MA): The first layer of photolithography defined the groove structure (20 μm in depth) to place a mirror a certain distance away from the microchannel; the second layer was aligned to lie on top of the groove structure in the first layer and defined the pattern of linear channels (35 or 22 μm in depth). To fabricate a grooved microchannel, the third layer was aligned to lie on top of the channel structure (22 μm in depth) in the second layer and defined the pattern of grooved surfaces (30 μm in depth) on the linear channel. A polydimethylsiloxane (PDMS) block was then placed the same distance as the thickness of the PDMS block away from the groove (Fig. 1). The mirrors were made by dicing a silicon wafer onto which an aluminium film (1000 A in thickness) was deposited (12 mm in width and 10 mm in length). The mirror was placed in the groove of the mold. A mixture of PDMS and its curing agent (ratio 10 : 1) was then poured on the mold and cured for 3 h in a convection oven at 65 °C for complete cross-linking. The PDMS block functions to help position the mirror at a certain distance from the microchannel and remain fixed during curing of liquid PDMS. The mirror was embedded in the PDMS device at an angle (θ) of ≈43°. To seal the microchannel, two PDMS layers (one for the mirror-embedded channel and the other for a stepped PDMS substrate) were manually aligned and bonded after exposure to oxygen plasma for 30 s (Fig. 1). The gap between the two PDMS layers was filled with liquid PDMS to prevent diffraction around the edges of the layers. For complete curing, the bonded PDMS layers were stored in a convection oven for 1 h. For OPL modulation, we bonded two cover glasses separated by a certain distance to an acryl substrate with an opening. The mirror-embedded microchannel was then placed on the cover glasses.

Material preparation

Red fluorescent polystyrene beads with a diameter of 6 μm were purchased from Molecular Probes (Eugene, OR). The beads were prepared in 2% Pluronic F68 solution (Sigma–Aldrich, St. Louis, MO) supplemented with FITC dye (20 mg mL⁻¹) to visualize fluorescent streams simultaneously in both the horizontal and vertical directions of the microchannel.

Data acquisition

The microchannels and beads were imaged through an inverted optical microscope (TS100; Nikon Co., Japan) with an objective lens (CFI Plan UW2×; Nikon Co.) of magnification 2× and a numerical aperture of 0.06, and an objective lens (CFI Plan4×; Nikon Co.) of magnification 4× and a numerical aperture of
A syringe pump (Pump 11 Pico Plus; Harvard Apparatus, MA) was used to produce 0.2–0.4 μL min⁻¹ flows through the microchannels. A commercial image analyzing program, i-Solution (IMT i-solution Inc., Korea), was used to measure the positions of particles inside the microchannel. The program measures the lines drawn by users and converts their pixel information into metric information. Each pixel from the acquired images represents 2.4 μm.

Results and discussion
Characterization of mirror-embedded microchannel with optical path-length (OPL) modulation

Fig. 2 shows the schematic of a mirror-embedded microchannel and the principle of OPL modulation. The channel is composed of an embedded silicon mirror, functioning as a reflector of the side view of the channel. This imaging technique enables simultaneous imaging of the top and side view of the channel with a single lens. In optics, the DOF is the range of distance from the nearest object plane to the farthest plane in focus. OPL is defined as the geometric length of the light path multiplied by the refractive index of the medium. The mirror-embedded channel has two different optical paths, one of which is a kind of folded optics that light is bent at the mirror, thereby making the optical path longer than the direct path of the object to the lens. Since the side view reflected by the mirror has a longer OPL than the top view, it is important to obtain a wide resolving range (or DOF) that can cover both the side and top view. However, due to the limited DOF, an object can be out of the DOF and defocused, thereby decreasing its intensity and sharpness. For example, when the nearest object plane of the lens in focus is set to the bottom of the channel, the DOF is formed in the distance of the DOF value from the bottom along the z-axis (see the left panel of Fig. 2). For easy comparison, the virtual image of the side view positioned behind the mirror is plotted, while the light rays do not travel like the virtual image. In the configuration of the left panel in Fig. 2, the DOF does not cover the side view of the object farther from the mirror, thereby producing a blur, defocused spot. Therefore, to obtain the side- and top-view images both precisely in focus, one of the OPLs should be modulated (see the right panel of Fig. 2). As a solution, adding a medium with high refractive index only in the light path of the side view can prolong its OPL and simultaneously make its DOF range shift upward in the virtual image, while the DOF range of the side view shifts along the y-axis in the real image. The OPL is given by the product of the local refractive index and distance:

$$\text{OPL} = n \cdot l$$  \hspace{1cm} (1)

where $n$ is the refractive index of the medium through which light propagates, $l$ is the geometric length of the medium. OPL can be modulated simply by replacing the medium. To offset the optical path difference between two image views ($d$ in Fig. 1), a medium of high refractive index such as a glass can be placed in the light path through which the side-view image propagates, thereby prolonging the OPL of the side view and simultaneously shifting its DOF range (the right panel of Fig. 2). The prolonged OPL of the side view can be expressed by $(n_{\text{glass}} - n_{\text{air}})d$ from eqn (1). This expression should be equated with the optical path through the PDMS, $n_{\text{PDMS}}d$, to offset the optical path difference between two image views. The resulting thickness ($t$) of the glass to offset the optical path difference is given by eqn (2):

$$t = \frac{n_{\text{PDMS}}}{n_{\text{glass}} - n_{\text{air}}} d$$ \hspace{1cm} (2)

where $n_{\text{PDMS}}$, $n_{\text{glass}}$ and $n_{\text{air}}$, are the refractive indexes of the PDMS, glass, and air respectively; $d$ is the mirror-to-channel distance.

To demonstrate the OPL modulation capability, we imaged the linear channel of 400 μm in width and 35 μm in height filled with FITC (Fig. 3). The side-view width of a 34 μm well corresponds to the mold thickness of the channel. The mirror-to-channel distance ($d$) is ≈ 250 μm and corresponds to the optical path difference between two image views. The DOF is given by Inoue and Spring by eqn (3):

$$\text{DOF} = \frac{n\lambda}{\text{NA}^2} + \frac{ne}{\text{NA} \cdot M}$$ \hspace{1cm} (3)

where $n$ is the refractive index of the medium, $\lambda$ is the wavelength of illuminating light, NA is the numerical aperture of the objective lens, $M$ is the magnification of the system, and $e$ is the pixel spacing of a charge-coupled device (CCD) sensor. For a given wavelength and pixel spacing, increasing the magnification and numerical aperture decreases the DOF. The calculated

Fig. 2 Schematic of optical path-length (OPL) modulation for 3D particle measurement. The virtual image shows the symmetry of the real image along the mirror surface for easy comparison of the DOF of the side view with the DOF of the top view. In a mirror-embedded microchannel, there exists a difference in OPL between the side view reflected from the mirror and top view. An object appears sharp on the CCD sensor only within the DOF. The optical path difference makes a bead image (most far from the mirror) out of the DOF and defocuses the bead, thereby decreasing its sharpness and intensity (left panel). The OPL modulation prolongs the OPL of the side view and ensures in focus imaging of an object even far from the mirror.
DOF value for the \(4 \times\) objective lens is 130 \(\mu m\) for \(n = 1\), \(\lambda = 700\) nm, and \(e = 24\) \(\mu m\). Without OPL modulation and assuming that the nearest object plane in focus is formed right under the channel bottom (Fig. 2), the DOF of the side view is formed from \(-250\) to \(-120\) \(\mu m\) along the lateral position (Fig. 3B and D). In that case, the side-view image is out of the DOF and the decrease in sharpness is observed in its intensity profile. The OPL of the side view was modulated with a cover glass of 400 \(\mu m\) thickness. The optical path difference between the glass and air medium is 200 \(\mu m\). This value is converted into \(=143\) \(\mu m\) through the PDMS medium (\(n_{PDMS} = 1.4\)). Therefore, the DOF of the side view shifts into the lateral range from \(-107\) to \(+23\) \(\mu m\) and the range from \(-36\) to \(+94\) \(\mu m\) for the OPL modulation with a glass of 400 and 600 \(\mu m\) thickness, respectively (Fig. 4B, C and 5). Without OPL modulation, the DOF of the side view is formed from \(-250\) to \(-120\) \(\mu m\) along the lateral position (Fig. 4A and 5). As mentioned above, an object can be precisely focused only within the DOF, the decrease in its sharpness and fluorescence intensity is gradual on either side of the DOF. Without OPL modulation, the side views of 6-\(\mu m\)-sized beads are out of the DOF and the decrease in their fluorescence intensity and sharpness is observed in the whole channel width (Fig. 5). With
the OPL modulations, the fluorescence intensity and sharpness of 6-μm-sized beads are constantly maintained within each DOF range, while the fluorescence intensity gradually decreases going away from the DOF range (Fig. 4B, 4C, and 5). As mentioned before, the degree of the shift of the DOF range by the modulation depends on the thickness of the medium as well as its refractive index. These results provide a clear indication of the OPL modulation capability to shift the DOF range of the side-view.

Particle measurement in a grooved microchannel with OPL modulation

We demonstrated the proper functioning of the mirror-embedded microchannel and OPL modulation by employing it to image flowing 6-μm-sized beads through the grooved microchannel (Fig. 6). Fig. 6B and C show the trajectories of flowing 6-μm-sized beads in the grooved microchannel, imaged by fluorescence microscopy with a long-time exposure. The grooved channel is 140 μm wide and 52 μm deep (h_g + h_t) with h_g = 22 μm, p_g = 39 μm, d_g = 36 μm and θ = 140° (Fig. 6A). The mirror-to-channel distance (d) is ≈ 165 μm. As solving eqn (2) with n_{PDMS}, n_{glass} and n_{air} of 1.4, 1.5 and 1.0, respectively, we can obtain the thickness (t) of 462 μm for a cover glass to offset the optical path difference between the side and top view. For the OPL modulation, we used a cover glass of 400 μm in thickness. With the modulation, the DOF of the side view shifts into the lateral range from −22 μm to +109 μm, from the original lateral range of −165 μm to −35 μm without the OPL modulation. In the presence of V-shaped grooves, the microchannel generates counter-rotating flows by using steady y- and z-axial pressure gradients in the right and left plane of symmetry of the channel (Fig. 6A). As shown in Fig. 6B, the 6-μm-sized bead moves back and forth between the sidewall 1 and the channel-center, following the rotating flows. As reached at the channel-center, the 6-μm-sized bead goes upward and approaches near the groove- or channel-surfaces, following the upward flows. Before crossing from the channel center to the side-wall 1, the 6-μm-sized bead strongly collides with the groove (see the arrows in Fig. 6B). After this, the bead goes downward following the downward flows and crosses the channel in the direction from the channel-center to the sidewall 1. The two circulation flows are symmetry related, rotating in counter-clockwise and clockwise directions, respectively. We did not observe any transfer of the 6-μm-sized beads between two rotating flows (Fig. 6C). That is, the bead (1) entering from the sidewall 1 follows the counter-clockwise rotational track, and moves back and forth between the sidewall 1 and the channel-center (Fig. 6C). The bead (2) entering from the sidewall 2 follows the clockwise rotational track, and moves back and forth between the sidewall 2 and the channel-center (Fig. 6C). In the current experimental setup, it might be possible to draw the 3D trajectories of the beads with the obtained data.
containing 3D positional information. However, since the data are not temporally resolved, it is difficult to compute three components of the particle velocity. Incorporation with a high-speed camera and high-power laser will enable measurement of the particle velocity in three dimensions.

Conclusions

We have described a method of OPL modulation for 3D particle measurement in mirror-embedded microchannels by replacing the medium through which light propagates. This method makes it possible to offset the optical path difference between two images of the side and top view in the mirror-embedded microchannels by utilizing a cover glass as a medium of high refractive index and prolonging the OPL of the side view. With this method, we demonstrated the OPL modulation capability to image in-focus side and top views of a linear channel filled with FITC and 6 μm-sized fluorescence beads in the linear and grooved channels. The mirror-embedded microchannel is particularly attractive because it can be easily fabricated with a single cast of PDMS, and allows 3D positional information to be readily obtained from two different orthogonal-axis images without relatively complex and expensive equipments and the modification of a microscope and its optical components. This study yields a fundamental understanding for designing a mirror-embedded microchannel for many other microfluidic applications.

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