

Optically trapped particle tracking velocimetry

Tetsuro Tsuji¹,* Shoma Hashimoto, and Satoshi Taguchi²
Graduate School of Informatics, [Kyoto University](#), Kyoto 606-8501, Japan



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In this paper, we propose a microflow velocimetry based on particle tracking with the aid of optical trapping of tracers, namely, *optically trapped particle tracking velocimetry* (ot-PTV). The ot-PTV has two phases: a trap phase, in which individual tracers are trapped by an optical force and held at a measurement position; and a release phase, in which the tracer is released and advected by the fluid flow, without interference from the optical force. The released tracer is subsequently trapped again by the optical force. By repeating the set of trap and release phases, we can accumulate the sequential images of the tracer that have the same initial position. Although the data acquisition rate of ot-PTV is lower than that of standard micro-resolution particle image velocimetry (μ PIV) due to the nature of pointwise measurement, an advantage of ot-PTV is that the measurement positions can be chosen by the experimenter. That is, even when tracers are scarce in a test section because of some external effects and/or the small size of the test section—ill-suited cases for μ PIV since the experimenter has to wait for tracers to diffuse into the test section (i.e., diffusion-limited situation)—ot-PTV remains efficient. The concept of ot-PTV is validated using a benchmark experiment, i.e., a pressure-driven flow in a straight microchannel with a square cross section. An application to thermally induced microflows is also demonstrated, where tracers can be scarce in a test section due to thermophoresis.

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I. INTRODUCTION

Flow velocimetry is a fundamental tool to investigate the characteristics of fluid flow fields. In microflows, where the spatial dimension is microscale or even nanoscale, conventional yet effective methods are micro-resolution particle image velocimetry (μ PIV) [1,2] and micro-particle tracking velocimetry (μ PTV) [3]. These methods are successful in measuring microflows and have been further developed in various manners, e.g., by extending μ PTV to a three-component measurement [4].

In particle tracking velocimetry (PTV), we acquire the sequential images of individual particles. Then, the displacements between the images, divided by a frame interval, are computed as the tracer velocities. Although the spatial resolution of PTV can be as fine as the tracer diameter, the noise inherent in the Brownian motion of tracers may be problematic, for instance, in the flow measurement of inhomogeneous creeping flows near boundaries [5] or highly localized flows around point-like heat sources [6,7]. To eliminate the effect of noise, ensemble averaging over many samples (i.e., the displacement data) is a standard approach. However, measurement positions in PTV are determined by tracer positions, which are not controllable. Therefore, to suppress the effect of noise sufficiently, the accumulation of the displacements at the same measurement position is

*Contact author: tsuji.tetsuro.7x@kyoto-u.ac.jp

necessary, which becomes increasingly difficult as the regions of interest become smaller and/or the flow becomes more localized.

A typical problematic case is found in a pioneering experimental study [8], where the thermally induced microflows around heated Janus microparticles (diameter 1 μm or 8 μm) were investigated. Because of the small size of the test section, i.e., the region near the microparticle, the authors needed 7 hours for a single trial, waiting for Brownian tracers to diffuse into the test section. Such a case can be classified as “diffusion-limited;” that is, experimenters have to wait for tracers until they diffuse into the region of interest. Another problematic case arises when external forces act on the tracers, pushing them out of the test section. For instance, in thermally induced microflows around heated Janus microparticles, the tracers are subject to thermophoretic forces that repel them from the heated part, thereby forming a tracer-depleted region. Such low-tracer-density regions (i.e., tracer-sparse regions), including diffusion-limited cases, are ill-suited for μPIV or μPTV . Thus, controlling tracer positions at desired locations is helpful when the regions of interest are small and a large number of samples is required. In this paper, we propose the optical trapping of tracers as a solution.

Optical trapping was proposed by Ashkin [9] to manipulate the motion of tiny particles using a focused laser. Practical guides for in-house experimental setups for optical trapping are available [10,11], and the technique has been widely applied to life sciences, nanosciences, and analytical chemistry. In flow measurement, optical trapping has been introduced in various forms [12–27]. In Ref. [12], the optical trapping of a target microparticle was used to effectively investigate the flow around the target by μPIV . In Refs. [13,14], two methods using optical trapping were proposed. The first method (hereafter referred to as method 1) uses the balance between drag and optical forces; that is, the displacement of the optically trapped tracers due to the flow is measured by camera or quadrant photodetectors. This method is called optical tweezer-based velocimetry (OTV). The OTV (or its variants) has been applied to measuring oscillatory microflows [15,16], rotlets [17], *in vivo* blood flows [18], the flow field around the cilia of microorganisms [19,20], the measurement of three-dimensional flows [22], and the Marangoni flows around thermoplasmonic bubbles [23,24]. In Ref. [21], many-body hydrodynamic interactions between colloidal particles exhibiting translation and rotation have been quantified using the optical trapping of partially fluorescent particles. The second method (say, method 2) is basically a standard PTV, but the measurement position is controlled by positioning the optically-trapped tracers as desired [13,14,25–27]. Although methods 1 and 2 have their own advantages, some challenges remain. Method 1 (i.e., OTV) requires the calibration of the optical force to quantify the flow velocity. However, the optical force is sensitive to the fluid temperature and/or the diameter d of the particle, since its magnitude scales as $\propto d^n$ ($n \geq 3$) [28]. Moreover, because any misalignment of optical components or temporal changes in the entire apparatus can affect the magnitude of the optical forces, careful and persistent maintenance is necessary for reproducible experiments. Method 2 requires a large number of samples to average out the effects of noise. To address this difficulty, Refs. [25,26] introduced the repeated cycle of the trap and release of tracers.

In this paper, we propose optically trapped particle tracking velocimetry (ot-PTV) as a solution to the above-mentioned tracer-sparse cases. The concept is simple, consisting of two stages, as illustrated in Fig. 1(a). First, an optically trapped tracer is held at a measurement position (a trap phase). Second, the tracer is released and advected in a flow (a release phase), during which sequential images are acquired. Then, after image acquisition, the same tracer is pulled back to the measurement position by the optical force, returning to the trap phase. By repeating the cycles of trap and release phases, we can achieve rapid image acquisition for the same measurement position. Note that the ot-PTV does not require quantification of the optical force, which was necessary in OTV. Although the concept of the trap-and-release strategy was demonstrated in Ref. [25], the detailed statistical analysis and the connection to the tracer-sparse cases have not been discussed. As a proof of concept, we present a validation of ot-PTV using pressure-driven microflows in a straight microchannel, with a particular interest in the analysis of slow flows with speeds on the order of $O(1)$ $\mu\text{m/s}$. The measurement of these slow flows is important, e.g., in thermally induced

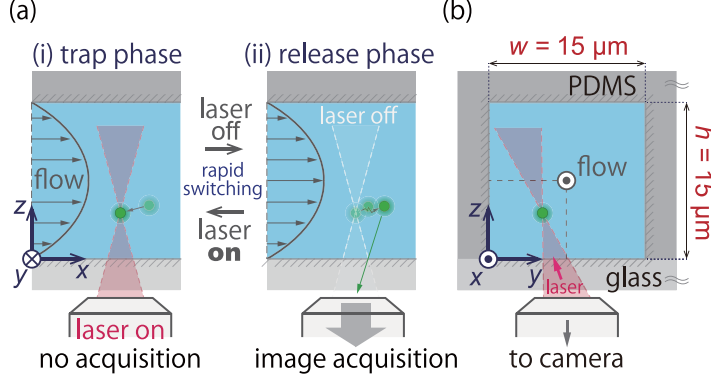


FIG. 1. Schematic description of the present paper. (a) Concept of optically trapped particle tracking velocimetry (ot-PTV). In the trap phase, a tracer is captured at a measurement position using the optical force. In the release phase, the tracer follows the fluid flow in the absence of the optical force. These two phases are instantaneously switched and repeated. (b) Cross-sectional view of the microchannel. The laser focus, i.e., the trap position of a tracer in the y direction plane, can be controlled by AOD.

microflows [5]. As an application example of the proposed ot-PTV, preliminary results of the thermally induced microflows experiment are also given at the end of the paper in Sec. IV.

II. EXPERIMENTS

Experimental details are given in Supplemental Material S1 [29], and we provide an overview below. Note that only steady flows are considered here.

A. Setup

The test section is a straight microchannel with a square cross section. The width w and height h of the cross section are both $15 \mu\text{m}$, as shown in Fig. 1(b). An aqueous solution containing tracers fills the microchannel. Then, the fluid flow is driven by a pressure gradient $|\nabla_x p|$ with a resolution of approximately 0.01 Pa . The x component of the flow is denoted by $V = V(y, z)$. Using the incompressible Navier–Stokes equation and no-slip boundary condition on the channel walls, an analytical solution $V(y, z)$ is given as [30]

$$V(y, z) = \frac{4h^2}{\pi^3 \mu} |\nabla_x p| \sum_{n, \text{ odd}} \frac{1}{n^3} \left[1 - \frac{\cosh\left(\frac{n\pi w}{2h} - \frac{n\pi y}{h}\right)}{\cosh\left(\frac{n\pi w}{2h}\right)} \right] \sin\left(\frac{n\pi z}{h}\right), \quad (1)$$

where μ is the fluid viscosity and $|\nabla_x p|$ is assumed constant.

The tracers are fluorescent, made of polystyrene (PS), and the tracer diameter is $d = 1 \mu\text{m}$. The Stokes number of the tracer is much smaller than unity for a typical microfluidic setup (say, reference length $\approx 10 \mu\text{m}$ and reference speed $< 1 \text{ m/s}$; see Supplemental Material S1 A [29]), and thus tracers follow the fluid flows. Moreover, the Reynolds number of the flow around the tracer is estimated as $Ud/\nu = 10^{-5} \ll 1$ with the reference flow speed $U = 10 \mu\text{m/s}$ and the kinematic viscosity $\nu = 1 \times 10^{-6} \text{ m}^2/\text{s}$, allowing us to consider the tracer in the Stokes-flow regime.

The tracers are optically trapped at a measurement position, as shown in Fig. 1(b). The y positions of the trapped tracer are controlled by electrical input signals to the acousto-optic deflector (AOD). The z position of the trapped tracer is controlled by the z position of the focal plane. The switching between the trap and release phases [Fig. 1(a)] should be as instantaneous as possible; at least, the transition time must be much shorter than the frame intervals of image acquisition. Therefore, we use the AOD, which has a response time on the order of microseconds. To be more specific, we move

the focal spot away during the release phase, rather than shutting off the laser using mechanical shutters.

The power of the trapping laser is estimated to be ≈ 24 mW at the measurement position. According to Refs. [31,32], the temperature increase caused by the laser absorption is less than 1 K with this power, due to the low absorption coefficient of water 14.2 m^{-1} and that of polystyrene 6 m^{-1} at the wavelength of 1064 nm. The effects of thermo-osmosis and thermophoresis caused by this temperature increase are estimated as $< 0.5 \text{ }\mu\text{m/s}$ [5,33–35] (see Supplemental Material S2 [29] for details). Therefore, we neglect the effect of the laser-induced temperature increase.

The tracer diameter determines the spatial resolution of the flow measurement. Therefore, in principle, the smaller the tracer diameter, the higher the resolution. However, as the tracer diameter decreases, the optical gradient force that traps the tracer particles is significantly weakened [28], and, in addition, Brownian motion becomes more intense. These factors are the main bottlenecks of using smaller tracers. In our preliminary experiments with several tracer diameters, we find that a tracer diameter of $1 \text{ }\mu\text{m}$ is practically suitable in our setup. For this tracer diameter, the radial range r_c of the region in which we can pull back the tracers to the initial position is obtained as $r_c \approx 1 \text{ }\mu\text{m}$, using the estimate on the optical force for the Gaussian beam [36] (see Supplemental Material S3 [29] for details). The critical range r_c and the magnitude of the optical force both decrease as the diameter d decreases, thus leading to more frequent failure of optical trapping.

B. Procedure

The experimental procedure is summarized in Fig. 2. First, the measurement position is determined using an optically trapped tracer. To find the bottom wall (i.e., $z = 0 \text{ }\mu\text{m}$), we move the trapped tracer in the z direction by adjusting the objective slowly, and look for a specific z position at which the tracer starts to become defocused. We define this position as $z = 0 \text{ }\mu\text{m}$. Then, we move the tracer to the measurement position with the desired (y, z) coordinates. In ot-PTV, we repeat the sets of the trap and release phases sequentially. As shown in Fig. 2(a), AOD alternates between the trap and release phases, which have the time durations of $T_{\text{trap}} = 40 \text{ ms}$ and $T_{\text{release}} = 60 \text{ ms}$, respectively. The release time duration of 60 ms is determined in preliminary experiments in accordance with the target flow speed, which is less than $10 \text{ }\mu\text{m/s}$ in this paper. A longer duration would result in more frequent failure of trapping of tracers; a shorter release duration would result in smaller displacements and thus a lower signal-to-noise ratio. In the case of the failure of trapping, we discard the associated data in the present paper, to make the number of samples the same for all measurement positions. To reduce the amount of unnecessary data, images are acquired only during the release phases.

Figure 2(b) shows the snapshots of the tracer during a release phase ([i]–[iii] in Fig. 2) and the subsequent release phase ([iv] in Fig. 2) (see also Supplemental Material S4 [29] for movie). It can be seen from the snapshots [i] and [iv] that tracer positions in the first frames of the release phases are almost identical due to the return to the measurement position in the trap phase. This enables us to acquire the tracer displacements originating from the same measurement position. The error in the initial positions (see a slight difference between the positions of the tracer in snapshots [i] and [iv]) is due to the timing jitter T_{jitter} in Fig. 2(a). We define Δx_0 as the displacement of the tracer’s initial position from its ensemble average. Then, we confirm that Δx_0 obeys the Gaussian distribution with the standard deviation of 123 nm (see Supplemental Material S5 [29]), which represents the uncertainty of the measurement position. The timing jitter can be reduced further to decrease the uncertainty, as long as experimenters are sure that the acquisition duration does not include the trap phase. The acquired image sequence is then analyzed using the software ImageJ and its plug-in PTA (Particle Track and Analysis, version 1.2).

Finally, some technical remarks are given as follows. (i) The fluorescence intensity decreases over time. Therefore, the trapped tracer is replaced by a fresh one occasionally in the sequential data acquisition. (ii) The optical trapping of single tracers is less feasible when the measurement position is located at a large z value. More specifically, in the upper half region of the microchannel

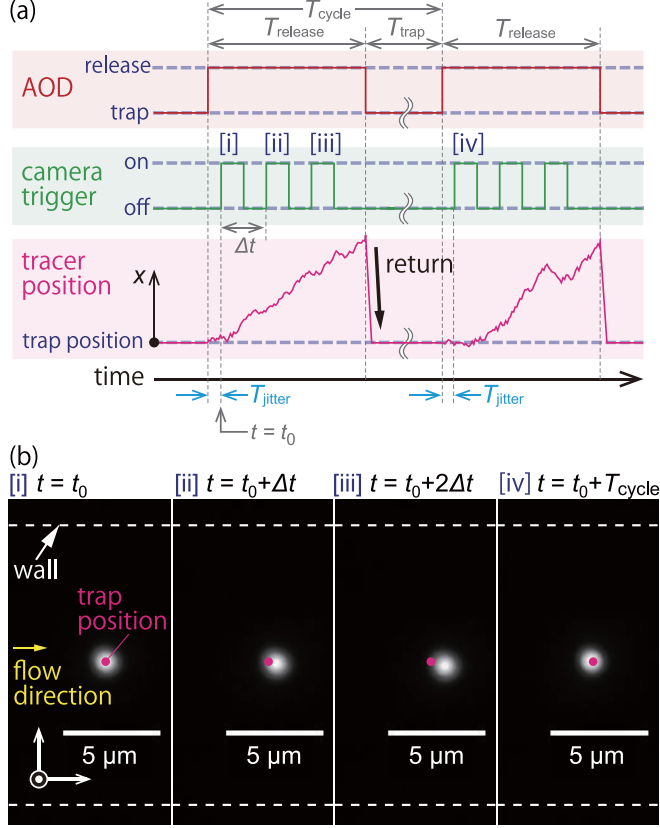


FIG. 2. Schematic illustration of the experimental protocol. (a) AOD alternates between the trap and release phases. The time duration for the trap and release phases is $T_{\text{trap}} = 40$ ms and $T_{\text{release}} = 60$ ms, respectively. Trigger signals are sent to the camera to acquire images during the release phase. A time jitter between the start of the release phase and the first acquisition is $T_{\text{jitter}} \approx 6$ ms. The released tracer is advected in the flow direction on average. Note that the tracer position curve is hand drawn for schematic description. (b) Snapshots during a release phase ([i]–[iii]); a snapshot in the subsequent release phase [iv].

$z > 7.5 \mu\text{m}$, the possibility of trapping two or more tracers increases, because the laser basically pushes upward (i.e., in the positive z direction) the tracers passing through the region lower than the measurement position. Therefore, the test section is limited to the lower half region $z < 7.5 \mu\text{m}$.

III. RESULTS AND DISCUSSION

The set of measured positions is listed as follows: $y = 3.4, 4.5, 5.5, 6.6, 7.6 \mu\text{m}$ and $z = 1, 2, 3, 5, 6 \mu\text{m}$. That is, $25 (= 5 \times 5)$ measurement positions are evaluated. It should be noted that y positions cannot be set closer to the wall because the optical trapping of the tracers becomes unstable near the wall. We consider that this instability is possibly due to the interference of the laser with the wall.

A. Probability density of tracer velocities

1. Convergence of probability density to Gaussian

First, let us focus on the probability density of tracer velocities. Figure 3(a) shows a typical result for the probability density as a function of the tracer velocity v_x at the measurement position $y = 5.5 \mu\text{m}$ and $z = 5 \mu\text{m}$. Here, the average tracer velocity $\bar{v}_x$ is considered equivalent to the flow

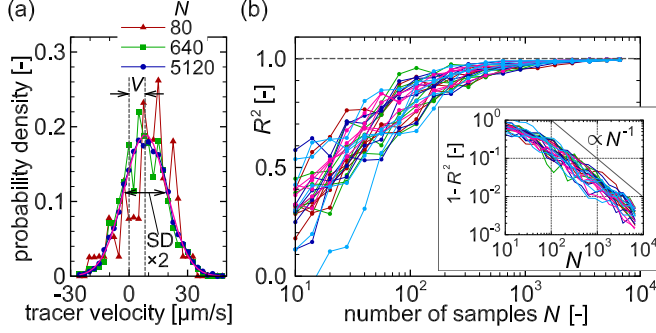


FIG. 3. (a) Probability density of tracer velocity v_x at $y = 5.5 \mu\text{m}$ and $z = 5 \mu\text{m}$ for sample sizes $N = 80$ (red triangle), 640 (green square), and 5120 (blue circle), where the bold-solid curve (magenta) is the Gaussian fit to the case of $N = 5120$. In the panel, “V” and “SD” indicate the mean and the standard deviation, respectively. (b) Coefficient of determination R^2 for $y = 3.4 \mu\text{m}$ (red), $4.5 \mu\text{m}$ (green), $5.5 \mu\text{m}$ (blue), $6.6 \mu\text{m}$ (magenta), and $7.6 \mu\text{m}$ (cyan), with $z = 1, 2, 3, 5$, and $6 \mu\text{m}$. The inset shows $1 - R^2$ in a double-logarithmic scale.

velocity V . To construct the probability density, sample sizes of $N = 80, 640$, or 5120 are used. The solid magenta curve represents a Gaussian fit. Due to the central limit theorem, the probability density converges to the Gaussian distribution as the sample size increases. The average tracer velocity $\bar{v}_x$, i.e., the mean of the Gaussian distribution, is approximately $10 \mu\text{m/s}$ in Fig. 3(a), but this is not very clearly visible when the sample size N is small (i.e., $N = 80$). The standard deviation (SD) of the probability density [Fig. 3(a)] is given by $\text{SD} = \sqrt{2D\Delta t/\Delta t}$, where $2D\Delta t$ indicates the mean squared displacement (MSD), $\Delta t = 12.5 \text{ ms}$ is the frame interval, and D is the diffusion coefficient. The data of SD at all measurement positions result in $D = 0.63 \pm 0.03 \mu\text{m}^2/\text{s}$. This value is well comparable to the diffusion coefficient computed as $D = 0.55 \mu\text{m}^2/\text{s}$ using the Stokes-Einstein relation, where the diameter $d = 1 \mu\text{m}$, the viscosity $0.8 \times 10^{-3} \text{ Pa}\cdot\text{s}$, and $T = 300 \text{ K}$ are used as a representative case. Therefore, the randomness of the data stems from the diffusion of tracers.

Note that, if the flow speed is sufficiently high (e.g., 1 mm/s), the smallness of N is not harmful since the mean is sufficiently away from zero, that is, the signal-to-noise ratio (or equivalently, mean-to-standard deviation ratio) is favorable. Therefore, the difficulty of small N is specific to the measurement of slow microflows on the order of $O(1) \mu\text{m}$ using small Brownian tracers. As Fig. 3 demonstrates, the ot-PTV can offer reliable results even under a poor signal-to-noise ratio (i.e., low V/SD). Therefore, the lower bound of the measurement speed is not determined by the Brownian characteristics, but rather the image acquisition and image analysis processes (e.g., pixel size, the accuracy of particle position identification).

Figure 3(b) shows the coefficient of determination R^2 (i.e., the goodness of fit) for various values of y and z , where the inset displays the deviation from unity, i.e., $1 - R^2$, in a double-logarithmic scale. The values of R^2 for all the cases converge to unity as the sample size N increases, with the rate of convergence proportional to N^{-1} . Therefore, the probability densities at all the measured positions can be well approximated by the Gaussian distributions, indicating the adequacy of the obtained samples.

Let us make a small remark on the advantage of ot-PTV. In ordinary PTV, one has to wait until tracers reach the region of interest (i.e., diffusion-limited). If the supply of tracers to the region of interest is not easy, obtaining a large number of samples using ordinary PTV would be problematic. Such a case can happen, e.g., (i) when the length scale of the region of interest is small due to the steep spatial variation of the flow, or (ii) tracers are scarce in the region of interest due to, e.g., external forces. The latter case will be demonstrated in Sec. IV using the case of flows around a heated particle, where tracers tend to deplete from the region of interest due to thermophoretic forces.

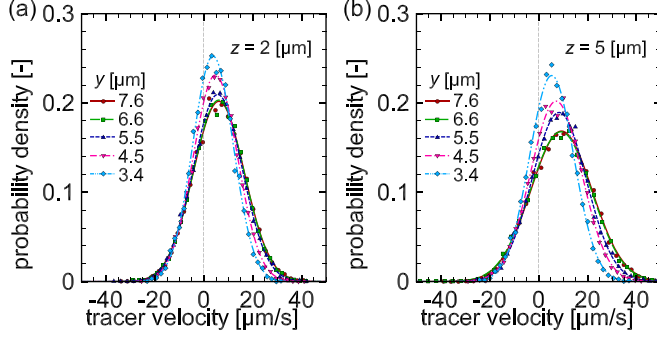


FIG. 4. Probability density of tracer velocity v_x at (a) $z = 2 \mu\text{m}$ and (b) $5 \mu\text{m}$ for $y = 7.6 \mu\text{m}$ (red circle), $6.6 \mu\text{m}$ (green square), $5.5 \mu\text{m}$ (blue triangle), $4.5 \mu\text{m}$ (magenta lower triangle), and $3.4 \mu\text{m}$ (cyan diamond). The curves show the corresponding Gaussian fits. The sample size is $N > 6600$ for all cases. Results for other values of z are presented in Supplemental Material S6 [29].

2. Position dependency of probability density

Next, let us focus on the position dependence of the probability densities. Figures 4(a) and 4(b) show the probability densities at $z = 2 \mu\text{m}$ and $z = 5 \mu\text{m}$, respectively, for various values of $y = 3.4, \dots, 7.6 \mu\text{m}$. All the experimental results (symbols) are well fitted by the Gaussian distributions (curves). As y varies from $y = 3.4 \mu\text{m}$ to $7.5 \mu\text{m}$, the probability density varies as well, shifting the mean from smaller to larger magnitudes. The differences among the means are quite subtle. In other words, to distinguish flow velocity differences at different y positions in the present flow conditions, the accurate reconstructions of the probability densities are required. This is the reason we need a large number of samples for slow microflow measurements.

It should be noted that the SD of the probability densities in Fig. 4 increases as y . A similar tendency has been reported in other microflow experiments, such as in Refs. [37,38], where the finite-thickness imaging range was considered to be the cause. In our experiments, since ot-PTV analyzes the tracers at the same position, the cause is considered different. The SD contains the information about temperature, viscosity, and particle diameter, and thus the quantification of SD can lead to the measurement of these quantities. However, since only the mean values are relevant in velocimetry, we leave the origin of the varying SD as future work.

B. Flow velocity analysis

1. Spatial profiles

Figure 5 summarizes the measurement results of $\bar{v}_x (= V)$ at 25 positions. Figures 5(a) and 5(b) show the same data plotted as a function of y and z , respectively. The experimental results (symbols) are fitted by the analytical solution (curves), Eq. (1).

First, let us explain the fitting method. In Eq. (1), we assume that the viscosity $\mu = 1 \times 10^{-3} \text{ Pa}\cdot\text{s}$ and the channel dimensions $h = w = 15 \mu\text{m}$ are given. Therefore, $|\nabla_x p|$, y , and z are treated as fitting variables.

The pressure gradient is recast as $|\nabla_x p| = \Delta p / L_x$ where Δp is the pressure difference across the microchannel of the length $L_x = 500 \mu\text{m}$ (see Supplemental Material S1 C and S1 D [29]). The coordinates at measurement positions are specified as $y = 3.4, \dots, 7.6 \mu\text{m}$ and $z = 1, \dots, 6 \mu\text{m}$ in the experiments, using the method described in Sec. II B. However, it is possible that these coordinates contain systematic submicron scale errors due to the difficulty of microscopic observation. Therefore, we replace (y, z) of Eq. (1) by $(y + \delta y, z + \delta z)$ in the fitting process, and search for a set of $(\Delta p, \delta y, \delta z)$ that minimizes the mean squared error with respect to the experimental data. The values $(\Delta p, \delta y, \delta z)$ for the best fit are found to be $\Delta p = 0.302 \text{ Pa}$, $\delta y = -1.01 \mu\text{m}$,

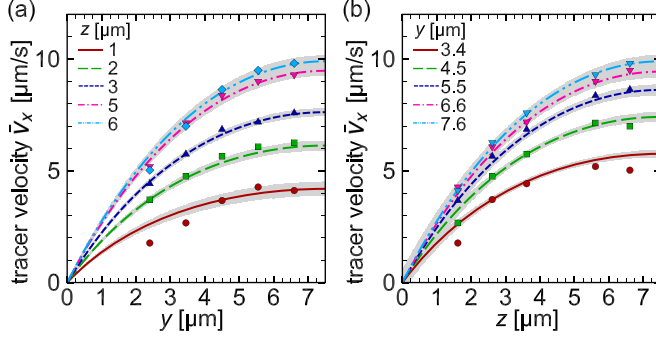


FIG. 5. Average tracer velocity $\bar{v}_x$ as a function of (a) y and (b) z , where symbols show the experimental data, curves indicate the results of fitting using Eq. (1), and shaded regions indicate the 95 % confidence interval. Note that experimental data (symbols) are shifted by (a) $\delta y = -1.01 \mu\text{m}$ in the y direction and (b) $\delta z = 0.61 \mu\text{m}$ in the z direction to consider submicron scale errors due to the difficulty of microscopic observation. (a) $z = 1 \mu\text{m}$ (red circle), $2 \mu\text{m}$ (green square), $3 \mu\text{m}$ (blue triangle), $5 \mu\text{m}$ (magenta lower triangle), and $6 \mu\text{m}$ (cyan diamond), and (b) $y = 3.4 \mu\text{m}$ (red circle), $4.4 \mu\text{m}$ (green square), $5.5 \mu\text{m}$ (blue triangle), $6.5 \mu\text{m}$ (magenta lower triangle), and $7.6 \mu\text{m}$ (cyan diamond).

and $\delta z = 0.61 \mu\text{m}$. We consider that the pressure difference $\Delta p = 0.302 \text{ Pa}$ estimated from the fitting to be reasonable, since the pressure difference estimated from the experimental condition is $\Delta p = 0.294 \pm 0.039 \text{ Pa}$, as described in Supplemental Material S1 D [29]. The values of δy and δz are also reasonable considering the observational and fabrication accuracies of the present experimental system.

Next, let us focus on the results of the fitting in Fig. 5. It is evident that the experimental values are well fitted by the analytical profile in Eq. (1). We remark that the measured flow speeds ($< 10 \mu\text{m/s}$) are slow compared with the maximum instantaneous tracer speed ($> 30\text{--}40 \mu\text{m/s}$; see the probability density in Fig. 4), and thus averaging over many samples is essential to obtain the accurate position dependence of the flow speed with a resolution of $O(1) \mu\text{m/s}$. It should be mentioned that the data at $z = 4$ and $7 \mu\text{m}$, which are not presented in Fig. 5, are discarded from the figure by the observation of the probability distribution, as described in Supplemental Material S6 [29].

Finally, we make a comment on the comparison with a standard μPIV . For the present simple flow with x uniformity, only a 10 s-measurement is enough to reproduce the theoretical expectation Eq. (1) (see Supplemental Material S7 [29] for the details of PIV analysis). However, there are some cases where the μPIV will not be effective, e.g., (i) the spatial averaging is not available; and (ii) the tracers are scarce in the region of interest due to some external forces. As for (i), if we do not take the spatial average in the x direction for this PIV analysis, a longer operation with several minutes will be necessary to reduce the statistical noise. The latter case (ii) will be discussed in Sec. IV.

2. Effect of the number of samples

To reduce the experimental duration, it is useful to know the optimal number of samples N which is required to realize the desired accuracy. Figures 6(a) and 6(b) show the average tracer velocity $\bar{v}_x$ as a function of N at $z = 2 \mu\text{m}$ and $z = 5 \mu\text{m}$ for various y , respectively (see also Fig. 4 for the corresponding probability densities). In the figure, the horizontal dashed lines represent the results of full samples with $N > 6000$. It is vaguely seen that $\bar{v}_x$ approaches a certain value as N increases, as expected, but the manner of approach is not clear due to statistical noise. Qualitatively, the number of samples in the range $100 < N < 1000$ seems to yield reasonable results under the present experimental condition with $\bar{v}_x < 10 \mu\text{m/s}$.

For more quantitative evaluation, Fig. 6(c) shows the relative and absolute errors of $\bar{v}_x$, which are calculated in the following manner. The relative error, shown by the red circle and the left axis,

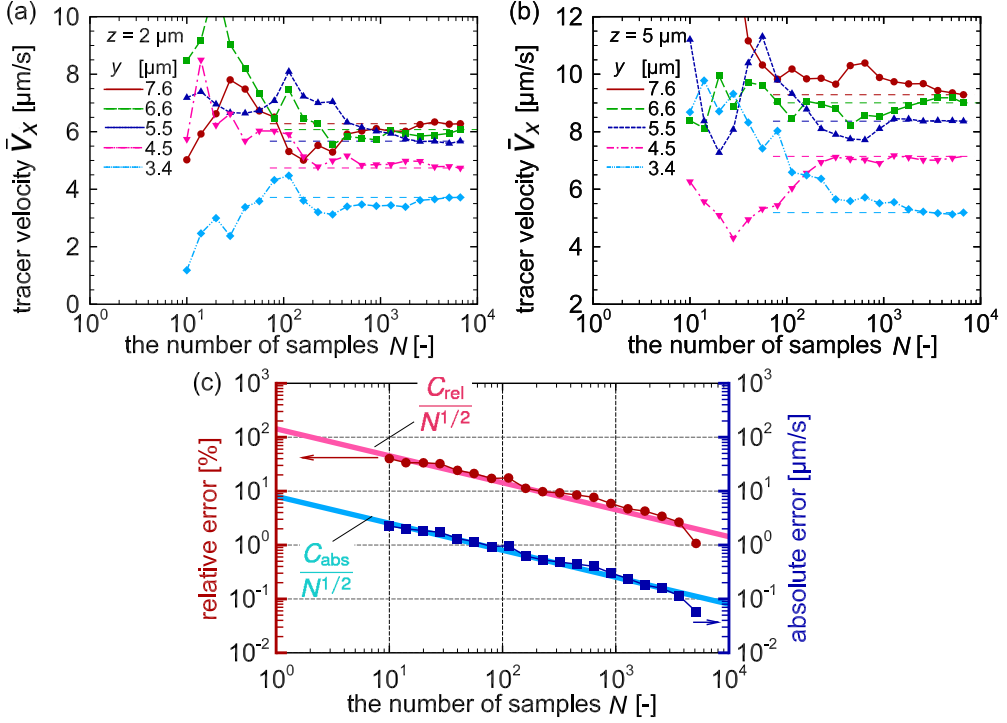


FIG. 6. Average tracer velocity $\bar{v}_x$ at (a) $z = 2 \mu\text{m}$ and (b) $z = 5 \mu\text{m}$ as a function of the number of samples N for various y . See also Fig. 4 for the corresponding probability density distributions at the same position (y, z). (c) Relative error (left axis, red circle) and absolute error (right axis, blue square) as a function of N averaged over whole measurement positions. The dashed lines for relative and absolute errors represent $\propto N^{-1/2}$ with proportionality constants $C_{\text{rel}} = 160\%$ and $C_{\text{abs}} = 8 \mu\text{m/s}$, respectively.

is the average over all 25 measurement positions of $|\bar{v}_x^{(\text{ref})} - \bar{v}_x^{(N)}|/\bar{v}_x^{(\text{ref})}$, where $\bar{v}_x^{(\text{ref})}$ is a reference value obtained with whole samples ($N > 6000$) and $\bar{v}_x^{(N)}$ is the value obtained with first N samples. Therefore, to obtain the results with a relative error less than 10 % for the present flow speed condition ($< 10 \mu\text{m/s}$), the figure indicates that N should be $N > 200$. The absolute error, shown by the blue square and right axis, is the average of $|\bar{v}_x^{(\text{ref})} - \bar{v}_x^{(N)}|$ over the whole measurement positions. To obtain the results with an absolute error less than $1 \mu\text{m/s}$, N is required to be $N > 200$.

The bold straight lines in Fig. 6(c) represent the best fit using power-law functions $\propto N^{-1/2}$. For the relative error (red circle), the power-law function is $C_{\text{rel}}/N^{1/2}$ with $C_{\text{rel}} = 143\%$. Note that C_{rel} indicates the relative error when only a single sample ($N = 1$) is used for the analysis. For much higher flow speed, C_{rel} is expected to decrease since only the denominator increases while the numerator is determined by the randomness of the data characterized by the Brownian motion. In the same manner, for the absolute error (blue square), the power law-function is $C_{\text{abs}}/N^{1/2}$ with $C_{\text{abs}} = 8.0 \mu\text{m/s}$. This value of C_{abs} is well consistent with the SD of the probability density, shown in Figs. 3 and 4. In fact, using the data of the whole measurement position, the SD of the probability density is computed as $10.0 \pm 0.2 \mu\text{m/s}$. In this way, we can evaluate the expected error in the ot-PTV.

The drawback of the ot-PTV is that it is a pointwise method, and thus a long experimental duration with area scanning is required when two-dimensional profiles are under concern. To address this difficulty, reducing experimental duration is possible by optimizing the number of samples N , as demonstrated above. In our case, the number of samples may be reduced to $N \approx 200$, which results in a 30-fold speed up, sacrificing the accuracy of $1 \mu\text{m/s}$.

C. Features of ot-PTV

We discuss some advantages and disadvantages of ot-PTV in this section by comparing it with other methods.

1. Comparison with μ PIV and TIRV

As mentioned in Sec. I, flow measurements near boundaries are one of the important issues in the microfluidics community. This is mainly due to velocity slips, which have been investigated experimentally using μ PIV [39,40]. The effects of the hydrophilic surface properties on slip lengths were pointed out in these references. To further improve spatial resolution near boundaries, the use of evanescent waves as an excitation light source was introduced [37,41]. This method is called total internal reflection velocimetry (TIRV) and has been applied to slip-length measurements [38,42]. The use of evanescent waves was further applied to flow measurements in nanochannels [43]. With the aid of an optical image analysis technique using defocusing, the measurement of nanoscale flow profiles was further refined [44].

The main differences between the present ot-PTV and the above-mentioned preceding studies are found in the range of the flow speed under investigation. The measured flow speed ranges were, approximately, 2 to 40 $\mu\text{m/s}$ in Ref. [41], 30 to 300 $\mu\text{m/s}$ in Ref. [37], 50 to 500 $\mu\text{m/s}$ in Ref. [45], and $> 1 \text{ mm/s}$ in Refs. [43,44]. Although Ref. [41] measured the flows with relatively slow speeds, the measured velocities were the spatial average over a certain distance near the boundaries. In contrast, the present ot-PTV focuses on the flow speed $< 10 \mu\text{m/s}$ and resolves the spatial profiles as functions of the distances from the boundaries. It should also be noted that TIRV applies to flows over a planar boundary, but ot-PTV can be used, in principle, for flows over curved surfaces such as those around spheres [5].

2. Comparison with MTV and LIFPA

Another class of velocimetry techniques is molecular tagging velocimetry (MTV) [45,46] and laser-induced fluorescence photobleaching anemometer (LIFPA) [47–50]. In these methods, molecules are added to a working fluid and are tagged by lasers via photochemical reactions such as phosphorescence and photobleaching. The subsequent motion of tagged molecules is tracked by imaging and analyzed to obtain flow velocities. While MTV has been applied to the measurement of flow spatial profiles, LIFPA is specialized for pointwise measurements with a high temporal resolution. For microscope analysis in microfluidics and nanofluidics, LIFPA is considered more convenient, since a single objective lens can be used for both laser irradiation and observation [48–50].

The main difference between the ot-PTV and the LIFPA is the range of the flow speed under investigation. The LIFPA utilizes molecular motion, which consists of the combination of both translational and diffusive components. Due to the strong diffusion of the molecules, the flow speed that can be measured by LIFPA is relatively high: for instance, Refs. [48,49] mainly considered the range $> 1 \text{ mm/s}$. In contrast to LIFPA, the ot-PTV can treat much slower flows with the resolution of 1 $\mu\text{m/s}$, as already discussed above in this section. To obtain flow profiles, both LIFPA and ot-PTV require scanning across a test section, which is a drawback compared to μ PIV, μ PTV, TIRV, and MTV.

3. Comparison with OTV

As introduced in Sec. I, OTV uses optical trapping of tracers, although the measurement principle is totally different from that of ot-PTV. In Refs. [18,20], photo detectors are used to acquire tracer positions (supplemented by camera observation). Therefore, the sampling rate is much higher than the present ot-PTV analysis (say, several kHz [18]). This high data acquisition rate enables the analysis of time-dependent periodic motion with $O(10) \text{ Hz}$ with amplitude 1.5 to 70 $\mu\text{m/s}$ [20]. Furthermore, the OTV is less affected by the Brownian motion, since the tracer is optically trapped

during data acquisition. However, the experimental setup of OTV is much more involved than that of PTV here, requiring the optical-force calibration, the simultaneous use of both photo detector and camera, and data analysis using a Kalman filter. The ot-PTV setup is easier to introduce because calibration is not necessary and the standard PTV protocol can be used.

One possibility to improve ot-PTV for faster flow speed is to use a photo detector as in Refs. [18,20] instead of using a camera. To realize this, we need to develop an algorithm to deduce particle velocity from a time-series data of intensity. This may be feasible, especially using fluorescent tracers, since they have a well-characterized intensity distribution.

4. Some technical issues of ot-PTV

Finally, the limitations and some technical issues of ot-PTV are explained. The durations of trap and release (T_{trap} and T_{release} in Fig. 2) are closely related to the expected flow speed. For instance, if T_{release} is too long, the optical trap cannot pull back the tracer to the measurement position; if T_{release} is too short, the obtained samples are more affected by the precision of particle-tracking image analysis. Since the optical force depends on available optical tools and the tracer diameter, trial and error will be necessary to find a suitable protocol of trap and release in practice. Another issue is the total time duration of experiments. In this paper, a single run to measure a point takes 330 s for full sample numbers $N = 6600$ (i.e., two samples per cycle and ten cycles per second). Therefore, to obtain a profile (e.g., 30 measurement points in a test section), it will take at least 3 hours. Such a long duration may cause undesired drifts in experimental parameters (e.g., flow speeds, observation position, adsorption of tracers, temperature, etc.), and should be avoided. To address this issue, the use of multiple optical traps using liquid-crystal-on-silicon devices will be useful; in Ref. [25], a holographic optical tweezer using a spatial light modulator was applied for multiple optical traps. By choosing an appropriate size of N in accordance with the requirement of the flow analysis (e.g., expected flow speed, allowable level of error; see Sec. III B 2), together with the multiple optical trap strategy, we can reduce the experimental time significantly.

IV. APPLICATION

A more compelling test case, in which the advantage of ot-PTV is significant, is demonstrated here. The data presented here are preliminary, and more detailed research is ongoing and will be reported in future work.

A. Experimental setup

The experimental system is so-called optothermal fluidics, as shown in Fig. 7(a). In this system, in addition to the laser with wavelength 1064 nm for optical trapping, a visible focused laser with wavelength 488 nm (20 mW) is irradiated to a gap of $h = 10 \mu\text{m}$ between two glass substrates. In particular, this visible laser is focused onto a light-absorbing particle (diameter = $3.9 \mu\text{m}$; PS-MAG-S2180, microParticle GmbH), which heats up upon laser irradiation (see Ref. [51] for details; similar particles with a smaller diameter were used there). Therefore, the visible laser is referred to as the heating laser hereafter.

Due to the laser heating, we expect two dominant thermal effects on the fluid: (i) thermophoresis of tracers [Fig. 7(b)] and (ii) thermo-osmosis along the substrate [Fig. 7(c)]. In panel (b), F_{TP} is a thermophoretic force that is proportional to the temperature gradient of the fluid (see, e.g., Refs. [35,52]). The force is directed toward the colder region in our test here, where the tris-HCl buffer of 1 mM is used as a solvent, as verified in Ref. [53]. In panel (c), v_{TO} is the thermo-osmotic flow velocity that is proportional to the temperature gradient of the substrate; thermo-osmosis is considered localized near the solid surface and directed toward the hotter region [5–7]. Note that thermal convection is negligibly small because of the small gap size [51,54], and the laser power is kept low enough so that the creation of bubbles never happens. The goal here is to investigate the flow characteristics of thermo-osmosis.

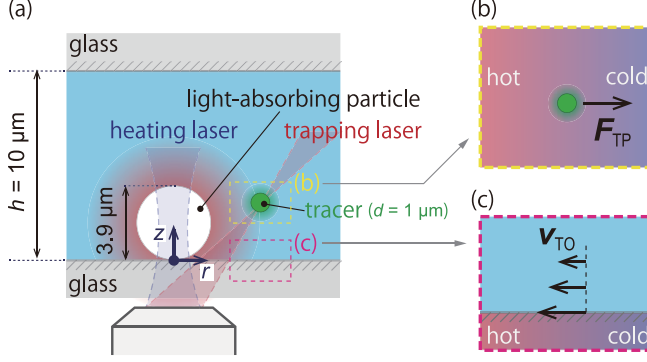


FIG. 7. Schematic of the experimental system demonstrated as an application of ot-PTV. (a) Configuration of the system. Heating laser (488 nm wavelength) is focused on a light-absorbing particle ($d = 3.9 \mu\text{m}$); trapping laser (1064 nm) is focused on a fluorescent tracer ($d = 1 \mu\text{m}$). (b) Thermophoretic force F_{TP} acts on the tracer in the opposite direction of the temperature gradient. (c) Thermo-osmosis v_{TO} occurs along the surface in the direction of the temperature gradient. Thermophoresis and thermo-osmosis coincide upon laser heating of a light-absorbing particle [panel (a)].

B. Why ot-PTV?

The advantage of ot-PTV over a standard μPIV or μPTV is described as follows. In this experiment, tracers are subject to a thermophoretic force directed toward the colder region. Therefore, even though the flow field (i.e., thermo-osmosis) is steady, the tracer distribution is unsteady and nonuniform, being scarce near the light-absorbing particle. These features make a standard μPIV inefficient. After a single experimental run, one must wait for the tracers to diffuse over the region of interest before carrying out the next run; in other words, the system is diffusion-limited. Since the expected flow speed is slow $< 10 \mu\text{m/s}$, many repeated runs are necessary to reduce statistical noise, but these diffusion-limited characteristics hinder efficient data acquisition.

To evaluate thermo-osmosis, we need to subtract the effect of thermophoresis of tracers. This needs two more steps: (i) an estimate of the thermophoretic force and (ii) a temperature profile measurement (or analysis using computational models). However, because these are not directly related to the scope of the present paper, we leave these steps for future work and evaluate only the tracer motion in the following.

C. Results

First, let us see the snapshots of the observed phenomena. Figures 8(a) and 8(b) show the cases of the focal plane set at $z = 2 \mu\text{m}$ and $4 \mu\text{m}$, respectively. (See also movies in Supplemental Material S4 [29].) Time $t = 0 \text{ s}$ indicates the start of the heating laser irradiation. Panels (c) and (d) are rough sketches to describe the motion of tracers in the snapshots (a) and (b), respectively.

In panel (a), the tracers near the focal plane look like white circles, whereas defocused tracers look dark. The snapshots indicate that only tracers near the focal plane come close to the light-absorbing particle, keeping certain distances at $t = 10 \text{ s}$. However, the defocused tracers are repelled from the center, indicating the thermophoretic depletion. Conversely, in panel (b), the tracers near the focal plane are depleted from the heat. These situations suggest that (i) the tracers near the bottom surface are brought to the heat source by thermo-osmosis (toward hot) near the boundary, finding the r position at which the effects of thermophoresis and thermo-osmosis are balanced [panel (c)]; (ii) the tracers away from the bottom surface are subject to only thermophoresis and are repelled from the heat [panel (d)].

As described in Sec. IV B, a standard PIV approach is not efficient due to the tracer depletion. Therefore, to investigate the flow characteristics, ot-PTV may be helpful. Figure 8(e) shows the

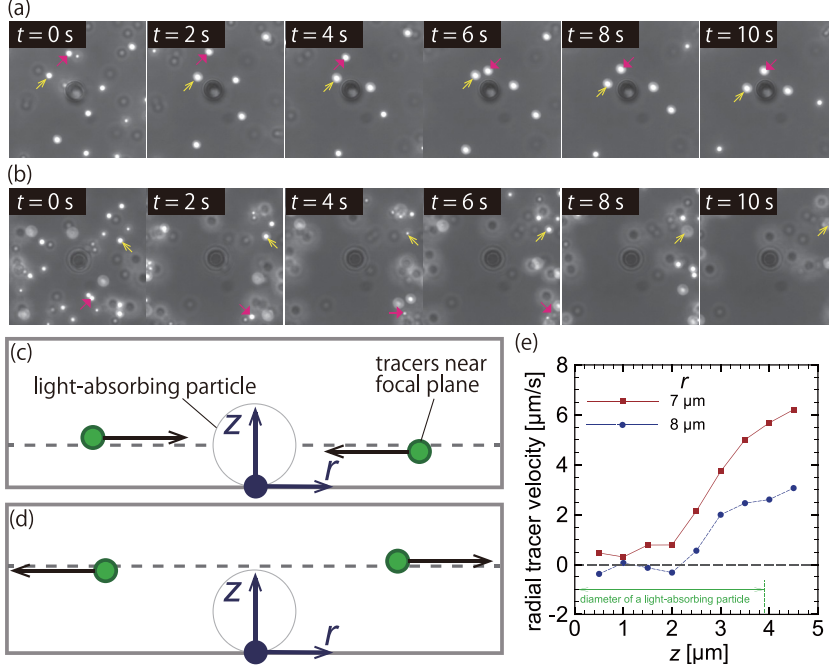


FIG. 8. Snapshots of optothermal fluidics experiment with focal plane at (a) $z = 2 \mu\text{m}$ and (b) $z = 4 \mu\text{m}$. Arrows indicate the same particles. See also movies in Supplemental Material S4 [29]. Panels (c) and (d) are rough sketches to describe the situations in panels (a) and (b), respectively. (e) Results of ot-PTV measurement. Radial component of tracer velocity is shown as a function of z for $r = 7 \mu\text{m}$ (red square) and $r = 8 \mu\text{m}$ (blue circle).

results of ot-PTV at $r = 7 \mu\text{m}$ and $r = 8 \mu\text{m}$ as a function of z . The number of samples is larger than 3000 for all the data points. For $z \gtrsim 3 \mu\text{m}$, radial tracer velocity v_r is positive and away from zero, indicating that the tracers are repelled by heat due to thermophoresis. The plots for $r = 8 \mu\text{m}$ show a smaller magnitude because the temperature gradient decreases as r becomes larger. Conversely, for $z \lesssim 2 \mu\text{m}$, v_r stays near zero, and v_r at $r = 8 \mu\text{m}$ becomes negative (i.e., tracers move toward hot). However, the magnitude of v_r for $z \lesssim 2 \mu\text{m}$ is so small and within the statistical noise. We consider that the tracers near the bottom boundary only show “Brownian motion,” possibly under the counterbalance between thermophoresis and thermo-osmosis. These data are consistent with the observation of snapshots. Therefore, ot-PTV is expected to serve as a velocimetry to quantify the flow characteristics of optothermal fluidics.

V. CONCLUSION

In this paper, we proposed optically trapped particle tracking velocimetry (ot-PTV) and demonstrated the measurement of a pressure-driven flow in a microchannel with a square cross section. By rapidly repeating the cycles of the trap and release phases of an individual tracer, several thousand samples (i.e., tracer displacements) were accumulated within minutes. Thanks to the large sample size, the probability densities of the tracer velocities sufficiently converged to Gaussian distributions, and the spatial profile of slow microflows $< 10 \mu\text{m/s}$ was successfully measured in good agreement with a theoretical prediction. Despite the drawback that ot-PTV is a pointwise method having a lower data acquisition rate as compared to μPIV , ot-PTV enables a well-controlled tracer seeding, which is advantageous in a situation where tracers are scarce in the test section. The proposed ot-PTV was applied to the measurement of thermally induced microflows, where the tracers are

depleted from the test section due to thermophoresis. Although the results are preliminary, it was shown that the ot-PTV can be an efficient measurement technique even when tracers are scarce, a situation that is inefficient for μ PIV.

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DATA AVAILABILITY

The data that support the findings of this article are openly available [55].

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