

Measurements of Nanoparticle Velocity Distribution in a Square Micrometer-Channel by Defocusing Nanoparticle Tracking Velocimetry

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ABSTRACT

Understanding nanoparticle transport in confined micro/nanospaces is becoming increasingly important with recent advances in micro/nanofluidic devices for particle manipulation, chemical analysis, and biomedical applications. In particular, particle transport in square channels, where the width and depth are comparable, is expected to exhibit more complex behavior than in plate channels, where the width is larger than depth, because of confinement effects induced by surrounding walls. In the present study, we measured the three-dimensional velocity distribution of 200 nm fluorescent nanoparticles flowing through a square micrometer-channel with width and depth of $1.5\mu\text{m}$ using defocusing nanoparticle tracking velocimetry (defocusing nano-PTV). Defocusing nano-PTV determines the three-dimensional positions of nanoparticles from defocused particle images, thereby enabling super-resolution particle tracking beyond the optical diffraction limit, accompanied by spherical aberration. In highly confined square channels, however, even slight channel inclination and field curvature can introduce significant errors into particle velocity distribution measurement. Therefore, correction methods for particle positions in both spanwise y and depthwise z directions were developed by statistically analyzing experimentally obtained particle distributions. In addition, calibration experiments using particles attached to channel walls at different depths were conducted to determine the relationship between the particle image radius and depthwise particle position. Consequently, accurate three-dimensional velocity measurements were successfully achieved in the square micrometer-channel, and the velocity data of 5064 nanoparticles were obtained. The measured particle velocity distributions in both the spanwise and depthwise directions showed good agreement with the theoretical velocity profile for pressure-driven flow in a square channel. Furthermore, the results indicated that the influence of particle-wall hydrodynamic interactions was negligibly small under the present experimental conditions because the particle size was sufficiently smaller than the channel dimensions. The proposed method is expected to contribute to detailed investigations of nanoparticle transport phenomena in highly confined micro/nanospaces and to the future advancement of micro/nanofluidic technologies.

1. Introduction

The advent of micro and nanofluidics has led to the emergence of methodologies for the manipulation of particles within microspaces, which have found application in a myriad of disciplines. In 1990, Manz et al. proposed the micro-TAS (Total analysis system)^{1,2}, which uses MEMS (Micro Electro Mechanical System) technology³ to fabricate microscale channels on glass or silicon substrates. This development enabled chemical reactions and blood tests that

previously required large-scale equipment to be performed on a single microfluidic device.

Advances in MEMS fabrication technology have enabled the development of nanofluidic devices with nanoscale channels (depth: 10-1000 nm)⁴. This technology is expected to enable single-molecule analysis and transport^{5,6}. For the development of micro/nanofluidics devices, understanding the particle transport in micro/nanospaces is important.

In microscale laminar flows, it is well known that when the particle size is relatively small compared to the depth of the channel, the particle velocity becomes the same as the flow velocity. However, in case of particle size close to the channel depth, Staben et al. performed simulations⁷ of particle behavior in spaces where particle-wall interactions are dominant and demonstrated experimentally that particle velocity significantly slows relative to the flow near the channel wall in a region of several hundred micrometers.

On the other hand, in nanospaces, the augmented specific surface area in has been demonstrated to engender surface effects, giving rise to liquid properties that deviate from those exhibited by bulk fluids. This includes an increase in the viscosity of water and a decrease in its dielectric constant⁸. However, there have been few reports on measurement of particle behavior in spaces of a few micrometers or less, owing to lack of methods. Therefore, it is necessary to establish a methodology for measuring particle velocity distribution in micro- and nano-spaces.

Particle image velocimetry (PIV)⁹ is a measurement method that has been demonstrated to be effective in visualizing and observing fluid flow and particle movement. PIV utilizes suspended particles as tracers to capture particle images at multiple times. From the acquired particle images, the movement of the particles is calculated as flow velocity. This method's wide application measuring particle velocity in fluid flows, ranging from the macroscale to the nanoscale. A representative PIV with microscale spatial resolution is to micro-PIV, developed by Santiago et al. in 1999¹⁰. Micro-PIV is a method of measuring particle velocity that involves the observation of particles on the focal plane of a microscope. This technique utilizes the movement of the focal plane in the direction of the depthwise of the flow channel to obtain 3D position information of the particles. The depth of field, which is equivalent to the spatial resolution in the

measurement process, has been demonstrated to be effective in quantifying the flow velocity distribution in 10 to 100 μm with a spatial resolution that is on the order of microns. This measurement method has been employed in experimental studies to elucidate the characteristics of blood flow in circular microchannels¹¹. However, due to the fact that nanoscale spaces are extremely small compared to the depth of field, micro-PIV is incapable of tracking particles within these nanoscale spaces. In addition, previous studies have employed defocusing particle tracking velocimetry (PTV). Defocusing-PTV is a single-camera, 3D optical measurement technique used to determine the three-component velocity fields of a fluid flow. Defocusing-PTV employs the defocusing effect that occurs when a particle moves away from the camera's focal plane. The depth position is determined from the change in the radius of defocused particles. In earlier studies, this measurement method has been employed to visualize the three-dimensional flow of oil inside a clutch, a component of an automobile¹². However, it should be noted that these measurements were also performed on a microscale.

Recently, Kazoe et al. developed defocusing nano-PTV¹³ as a method for tracking particles in nanospaces. The defocusing nano-PTV can actively utilize spherical aberration to increase the spatial resolution. The velocity of the particles is determined by measuring the displacement of the particles in sequential images of the particles. The optimization of spherical aberration enables spatial resolution on the order of 10 nm. Kuwano et al. applied defocusing nano-PTV to measure particle velocity in a plate microchannel where the width is sufficiently greater than the depth¹⁴. The results indicated that hydrodynamic interactions were the predominant force. However, there was a problem where measurement errors occurred due to Brownian motion of particles and noise in the image. Thus, Tanaka et al. proposed guidelines for determining the requisite temporal and spatial resolutions to mitigate these error factors through simulations employing the Langevin equation¹⁵. Tsuda et al. developed image processing methods and depth position determination methods necessary to capture actual-phenomena¹⁶.

Although defocusing nano-PTV has been applied to plate microchannels, its application to square micrometer-channels which possesses approximately equal width and depth, has not yet

been sufficiently investigated. In square channels, the presence of surrounding walls in both spanwise and depthwise directions produces a more complex three-dimensional flow field and particle behavior than those observed in plate geometries. Therefore, direct velocity measurements in square channels are important for accurately understanding particle transport under strongly confined conditions. Furthermore, square micrometer-channels are fundamental geometries in microfluidic and nanofluidic devices. Establishing a reliable measurement method in such geometries is therefore important for future applications to highly confined nanoscale system, such as square nanochannels with dimensions of several hundred nanometers.

In the present study, the velocity distribution of 200 nm nanoparticles flowing in a square micrometer-channel with a width and depth of $1.5\ \mu\text{m}$ was measured. Because of the smallness of the channel, even minor variations in the slope of the channel or field curvature will lead to significant errors. Therefore, to accurately determine the particle position not only in the depthwise z direction but also in the spanwise y direction, we analyzed the experimentally obtained images and performed statistical correction by plotting many particle positions. Consequently, we were able to perform 3D particle velocity distribution measurement in a confined space measuring only a few micrometers. This work contributes to the advancement of knowledge in the field of particle transport in confined spaces and improves the design of micro and nanofluidic devices.

2. Method and principle

As illustrated in Figure 1, defocusing nanoparticle tracking velocimetry is a method for measuring particle velocity distribution in nanospace by utilizing spherical aberration in the out-of-focus region. Spherical aberration is a type of monochromatic aberration, defined as the dispersion of light rays rather than their convergence at a focal point. In the presence of spherical aberration, the capture of particle images in out-of-focus areas can result in the formation of interference fringes, which manifest as rings in the particle image. Typically, spherical aberration

necessitates correction to mitigate its impact. However, defocusing nano-PTV leverages the change in particle image shape due to spherical aberration and utilizes it to ascertain the particle's depth position. The depthwise z direction of the particle within the flow channel is determined by leveraging the linear relationship between the radius R of the interference ring and the particle's distance from the focal plane.

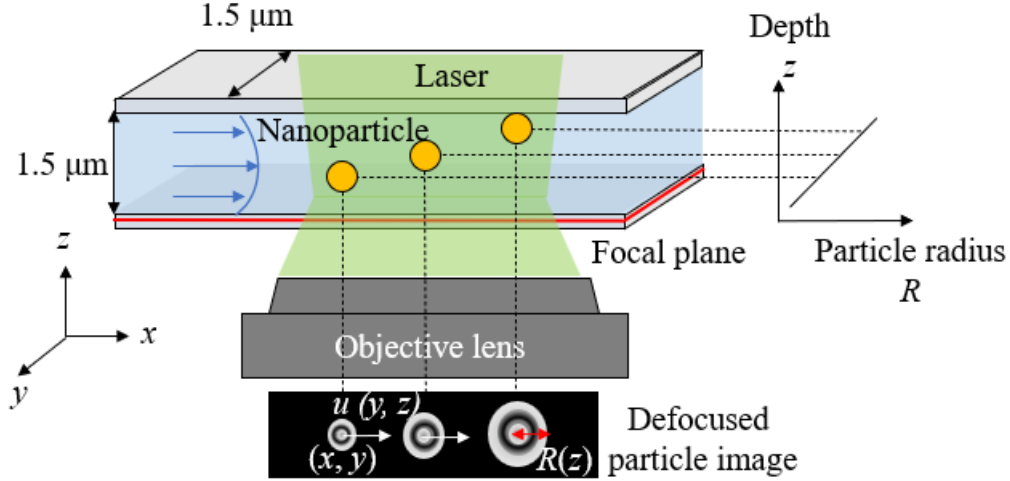


Figure. 1 Principle of defocusing nano-PTV.

In this study, we conducted measurements on pressure-driven flows. One of the sources of error in defocusing nano-PTV is Brownian motion, which is caused by irregular collisions due to thermal motion of particles. In the proximity of the wall surface of the flow channel, the particles are unable to move symmetrically, resulting in a flatted velocity profile (Tanaka et al. 2024). This, in turn, leads to a bias in the depthwise z direction of the particles. In order to effectively mitigate this error, it is imperative to employ a sufficiently high temporal resolution. In this case, the quantity of particle movement, designated as $\langle r^2 \rangle$, resulting from Brownian motion, is given by equation (1),

$$\langle r^2 \rangle = \sqrt{2Dt} \quad (1)$$

where t is specified time interval, D is the diffusion coefficient. The diffusion coefficient is given by the Stokes-Einstein equation,

$$D = \frac{k_B T}{6\pi\mu a} \quad (2)$$

where a is the particle radius, k_B is the Boltzmann constant, T is the temperature, μ is the viscosity.

In the context the 200 nm fluorescent particles utilized in this study, the particle movement amount $\langle r^2 \rangle$ due to Brownian motion is determined to be approximately 36 nm when the time resolution is set at 300 μ s, as indicated by equation (1). Given the comparatively reduced dimensions of the square channel utilized in this study, with a width and depth of 1.5 μ m, the impact of Brownian motion can be regarded as negligible.

In this study, measurements are performed in a square channel with width and depth of several μ m, so particle velocity is expressed as $u(y, z)$. Therefore, it is necessary to identify particle positions not only in the depthwise z direction but also in the spanwise y direction.

3. Experimental section

3.1 Materials and chemicals

In this study, carboxylate-modified fluorescent polystyrene nanoparticles with a diameter of $2a=200$ (199 ± 30) nm and a fluorescence spectrum with a peak at 560 nm (FluoSpheres, Thermo Fisher Scientific Inc., MA, USA) respectively were employed. In the experiment, 200 nm nanoparticles were suspended in a 10 mmol/L $\text{Na}_2\text{B}_4\text{O}_7$ solution at a volume fraction of $3.3 \times 10^{-3}\%$ for defocusing nano-PTV. For the preliminary calibration experiments, the particles were dissolved in phosphate-buffered saline at a volume fraction of ranging from 1/100 to 1/1000 of the particle concentration for defocused nano-PTV.

3.2 Experimental apparatus

A microfluidic device for measuring particle velocity distribution was prepared based on a top-down fabrication process. The device contains square micrometer-channels interfaced with microchannels (width: 1700 μ m, depth: 10 μ m) for sample injection. Micrometer-channels for measurement were patterned on a quartz glass substrate sputtered with 100 nm of chromium by electron beam lithography using a photoresist (ZEP-520A), followed by dry etching using a mixture of gaseous CHF_3 and SF_6 . The microchannels for introduction were also fabricated on this

substrate by photolithography using a photoresist (KMPR®1035, Kayaku Co., Ltd., Tokyo, Japan) and dry etching using a mixture of gaseous C_3F_8 , CHF_3 and Ar. Inlet holes were created using a diamond-coated drill, and the substrate was sealed with a 0.170 ± 0.005 mm thick borosilicate cover glass by a low-temperature bonding method. This method prevents glass breaking during the annealing process due to the different thermal expansion coefficients of quartz glass and borosilicate glass. The substrates were washed in a mixed solution of sulfuric acid and hydrogen peroxide (3:1), and following surface activation by irradiation with oxygen plasma for 40 s, were contacted and heated at 100°C for 5 hours. The temperature was subsequently increased to 100°C over the course of 1 hour in order to prevent cracking of the device due to the difference in thermal expansion coefficient between the glass substrate and the cover glass.

As illustrated in Figure 2, the fabricated microfluidic device and a micrometer-channel with dimensions of 1628 nm in width, 1700 nm in depth, and $200\ \mu\text{m}$ in length were examined using SEM and stylus profiler.

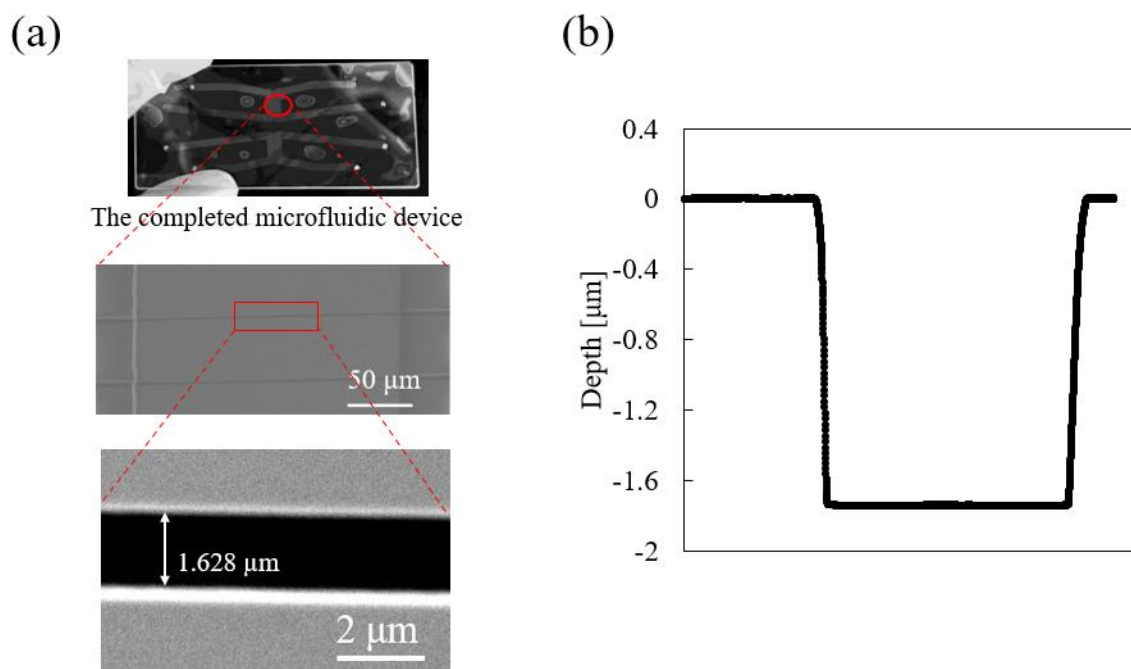
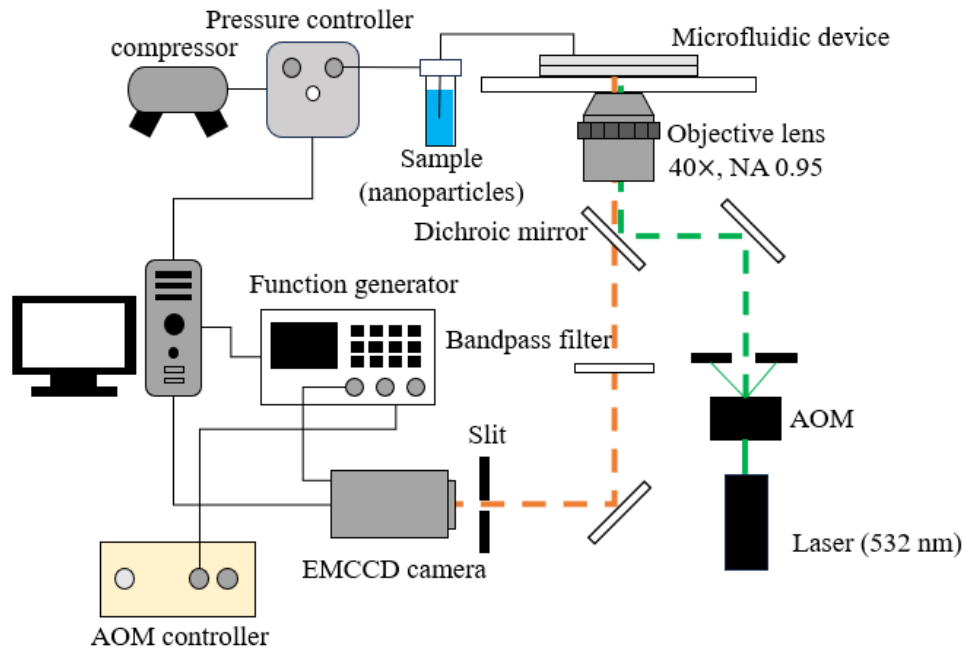


Figure. 2 Results of device fabrication. (a) Photograph of a microfluidic device and scanning microscopy image of micrometer-channels. (b) Depth of micrometer-channel obtained by stylus profiler for a reference channel.

Additionally, a microfluidic device and a nanofluidic device were fabricated for calibration experiment. The calibration devices have plate-channels of depths of 620, 1140, 1780, and 2490 nm, which were measured by stylus profiler.

Figure 3(a) shows a schematic representation of the defocusing nano-PTV system. In the context of an objective lens with a high numerical aperture (NA), the defocused image of a nanoparticle typically manifests as diffraction rings with spherical aberration. As previously reported, the radius R of the diffraction ring is proportional to the depthwise position, and nanoscale particle tracking was achieved with a spatial resolution that overcomes the optical diffraction limit by exploiting this proportional relationship (Kazoe et al. 2021). The measurement system was composed of an inverted microscope (IX 81, Olympus Corp., Tokyo, Japan) combined with an oil-immersion 40 $\times$ objective lens (NA 0.95, UALSAPO 40X2, Olympus Corp., Tokyo, Japan), a solid-state continuous wave laser (wavelength: 532 nm, GEM, Novanta Photonics, Manchester, UK) and an electron multiplying charge-coupled device (EMCCD) camera (ProEM: 512B, Teledyne Princeton Instruments, NJ, USA; 512 $\times$ 512 pixels, 16 μm /pixel, vertical shift rate of electron: 0.6 μs /row). The spherical aberration was adjusted to 0.1 λ for a reduced spatial resolution Δz , using the correction collar of the objective lens, as previously established (Kuwano et al. 2022). The fluorescence emitted from the nanoparticles was collected by the objective lens and subsequently detected by the EMCCD camera. The laser beam was converted to laser pulses by an acoustic optics modulator (AOM) to illuminate fluorescent nanoparticles at an exposure time of 60 μs . The temporal resolution Δt was calibrated to 300 μs by manipulating the frame interval using the previously developed measurement system (Kazoe et al. 2013, 2021). The generation of pressure-driven flow in the nanochannel was achieved by means of the application of air pressure, which was provided by a pressure controller (Institute of Microchemical Technology Co., Ltd., Kanagawa, Japan). The micrometer-channel demonstrated a substantially higher pressure resistance compared to microchannels. Consequently, the external pressure applied to the microfluidic device was approximated to that applied to the micrometer-channel. An external pressure of 10 kPa was applied to induce a pressure gradient of 50 MPa/m.

(a)



(b)

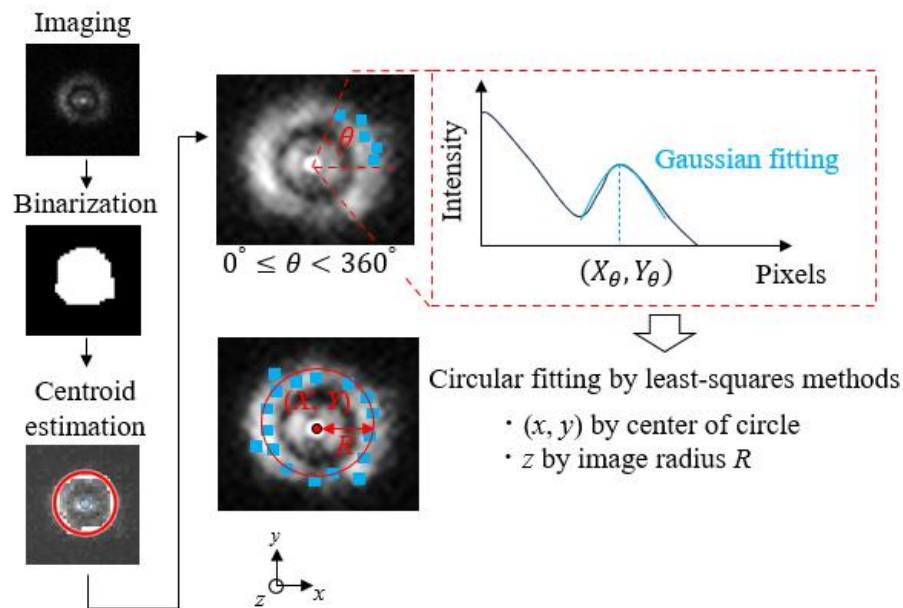


Figure. 3 Schematics of (a) measurement system and (b) image processing.

3.3 Image processing

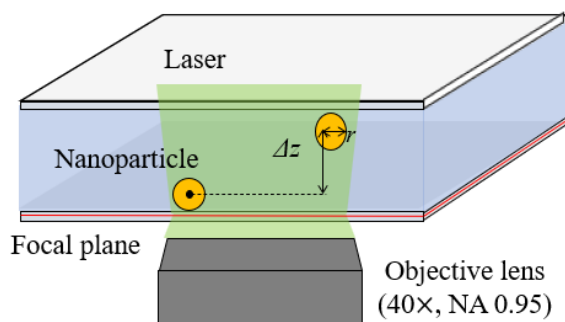
The radius R of the obtained nanoparticle images was determined using the image processing method proposed in a previous study (Tsuda and Kazoe. 2025). Initially, the obtained image noise was mitigated through the implementation of a bilateral filter. Subsequently, the particle image was detected by employing binarization process. A provisional center of the particle image was

determined by applying a circular approximation to the binarized particle image. Using the detected provisional particle image center as the origin, the radial intensity distribution is extracted by rotating the image through an angle θ , as shown in Figure 3(b). A search for a maximum value is performed on this intensity distribution. A Gaussian fit was applied to the intensity distribution over several pixels near each detected peak to determine the peak position. The ring peak coordinates on the image plane were then calculated from the distance between origin and the peak position and the rotation angle θ . Finally, a least-squares circle fitting was performed using the coordinates detected over the range $0^\circ \leq \theta < 360^\circ$, where the center coordinates and radius parameter of the fitted circle were defined as the particle image center and particle image radius, respectively.

3.4 Calibration experiments for z-direction

The depthwise z position of the particle was determined through a calibration experiment. Calibration was executed by flowing fluorescent particles through a microchannel of known depth and permitting their adhesion to and fixation to the upper and lower walls of the channel. Initially, nanofluidic devices and microfluidic devices of varying depths are prepared. The calibration experiments for 200 nm fluorescent particles utilized microfluidic devices with depths of 620, 1140, 1780 and 2490 nm. Subsequently, as illustrated in Figure 4(a), particles are actually flowed through the channel and absorbed onto the channel wall with varying depths thereby controlling the distance between two particles. Then, the change of image size of defocused fluorescent nanoparticles was measured. The depthwise z position of particle can be determined from the calibration line obtained in this way, which shows the relationship between particle radius and depth. As shown in Figure 4(b), calibration lines were successfully obtained with $\frac{dR}{dz} = 0.444$ for 200 nm fluorescent nanoparticles.

(a)



(b)

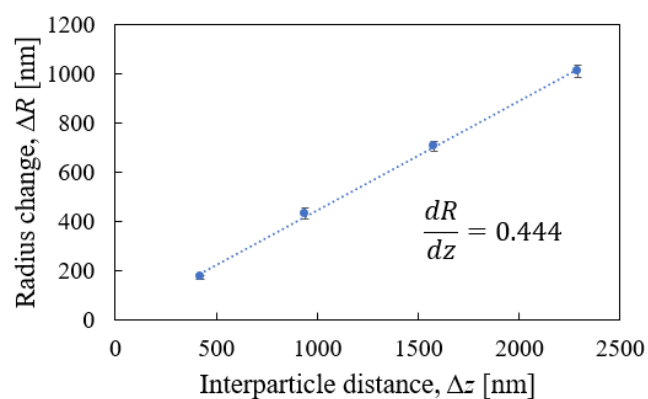
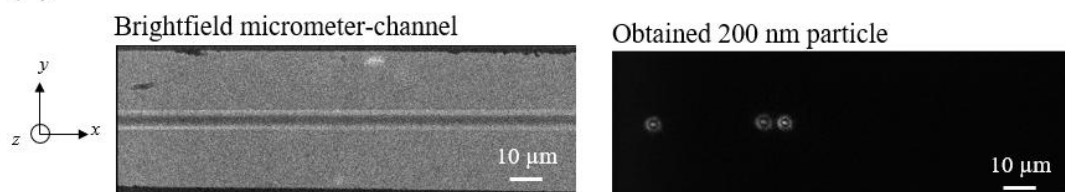


Figure. 4 (a) A schematic of calibration experiment. (b) Results of calibration.

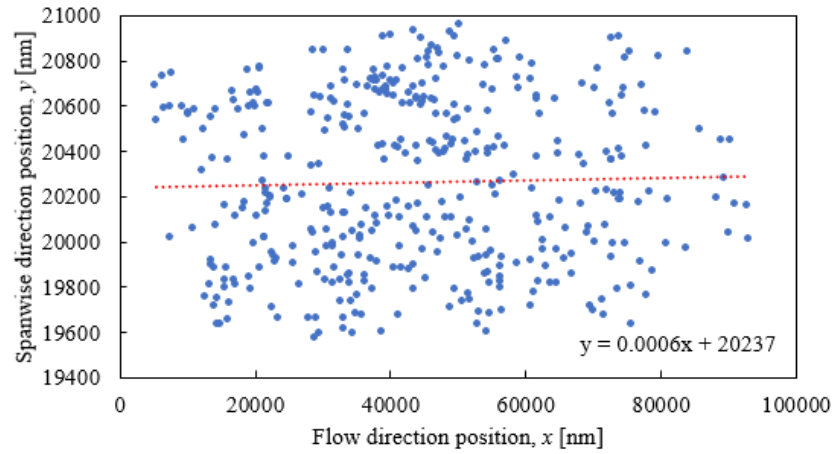
4. Results and Discussion

Figure 5(a) shows bright field image of the micrometer-channel and fluorescence image of 200 nm particles flowing in the channel. Consequently, we were able to obtain particle images in a square micrometer-channel. The acquisition of 5,064 data points pertaining to the velocity of the particles was successfully achieved. In the context of particle velocity distribution measurements, it is imperative to implement a correction to ensure the attainment of precise particle positions in both the spanwise y and depthwise z directions.

(a)



(b)



(c)

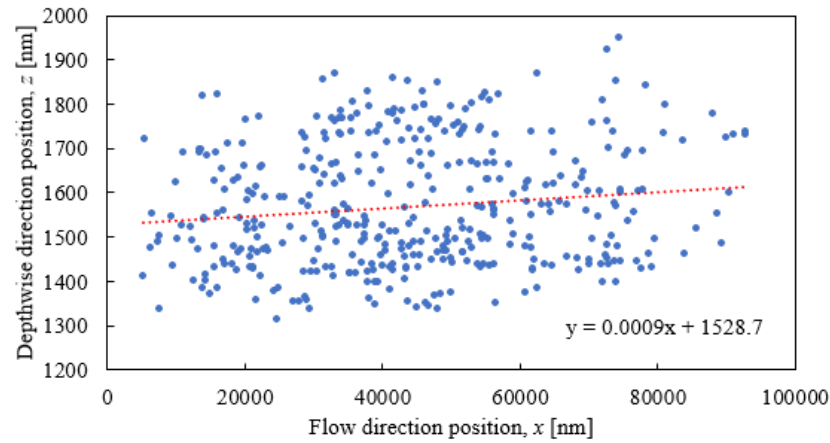


Figure. 5 (a) Bright field of channel and fluorescent particles. (b) Particle position in x, y plane. (c) Particle position in x, z plane.

The particle position in the image plane direction is determined from the center position of the defocused particle image. However, the inclination of micrometer-channel can lead to significant errors in determining the particle position in the channel. Therefore, it is necessary to correct the particle positions in the spanwise y direction by taking into account the inclination of the channel. To address this issue, the particle position data was obtained by means of analyzing the experimentally acquired particle images. These images were projected onto the flow x direction and the spanwise y direction plane. Thereafter, an approximate straight line was drawn as shown in Figure 5(b). The approximate straight line was used to determine the flow-channel slope, which was then used to calculate correct the gradient. In this section, the corrected particle velocity distribution is hypothesized to be a pressure-driven Hagen-Poiseuille flow, and is thus

approximated by a quadratic function utilizing the least squares method. The vertex is set to the center of the flow in the y-direction, thereby determining the particle position in the y-direction. Upon plotting the particle positions on the x, z plane, a slope was observed in the depth direction, as illustrated in Figure 5(c). We elected to implement the particle position correction method, which had been considered for the spanwise direction, in the depthwise direction as well. Subsequent to the rectification of the slope in the depth direction from the obtained approximation curve, the particle radius was converted to the particle depth position using the calibration curve obtained in the calibration experiment with particles in the flow channel described in 3.4. In a manner consistent with the spanwise direction, the corrected particle velocity distribution was approximated by a quadratic function, and the particle position was determined by establishing the vertex as the center in the depthwise direction.

Figure 6(a) illustrates streamwise velocities of individual nanoparticles. Consequently, the velocity distribution of the particles was successfully measured in a square micrometer-channel. Figures 6(b) and (c) illustrate the distribution of particle velocities in the spanwise y direction and the depthwise z direction, respectively. A comparison of the theoretical flow velocity with the particle velocity obtained experimentally shows agreement between the two in both the spanwise y and depthwise z directions. This finding indicates that, given the sufficiently small size of the particles relative to the channel dimensions, the influence of hydrodynamic interactions between the particles and the wall is negligible in micrometer scale.

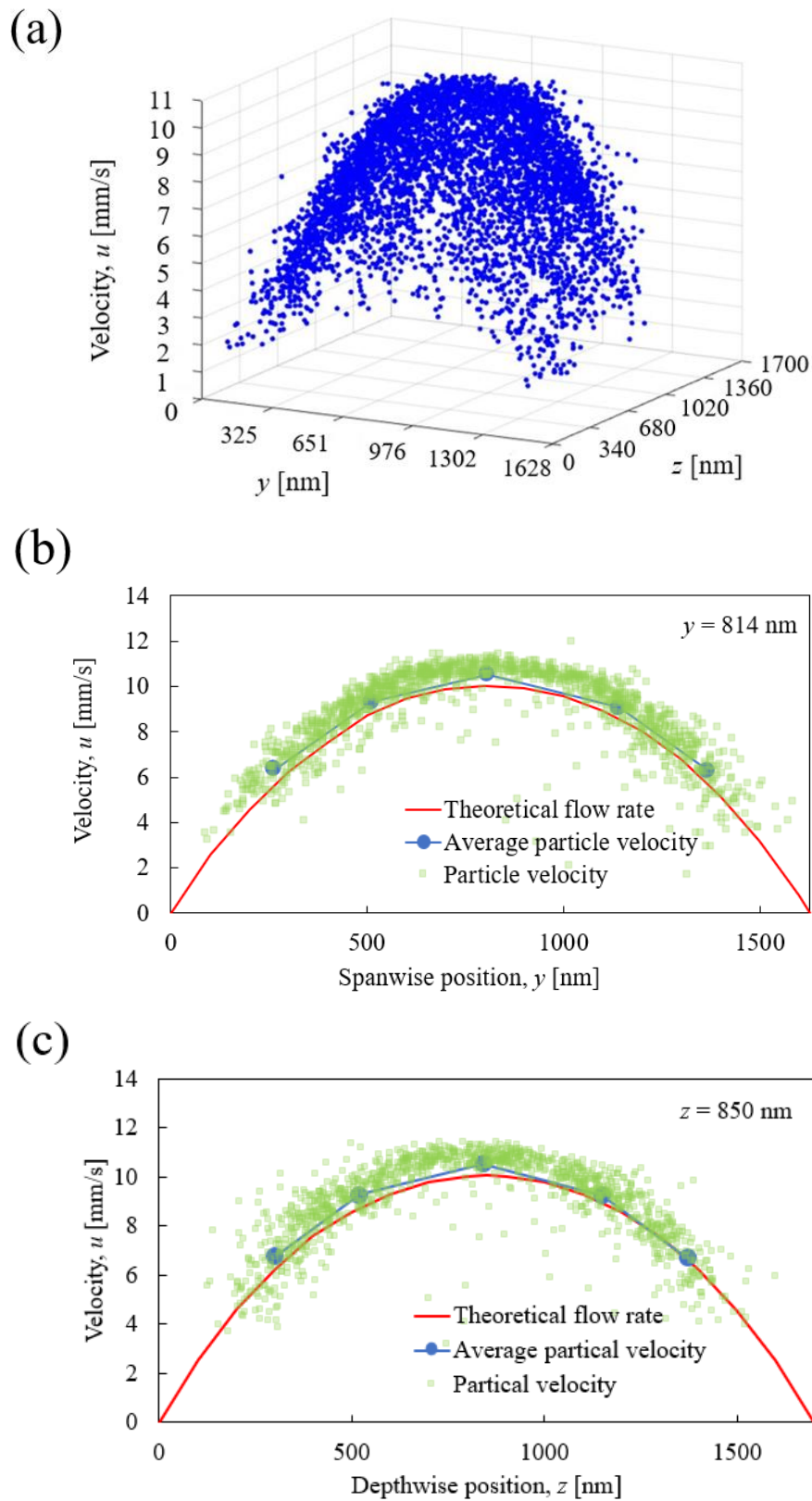


Figure. 6 (a) Stramwise velocities of individual nanoparticles. (b) Spanwise velocity distribution. (c) Depthwise velocity distribution.

5. Conclusions

In the present study, the particle velocity distribution in a square micrometer-channel with a width and depth of $1.5\ \mu\text{m}$ was measured using defocusing nano-PTV. A microfluidic device with a glass square micrometer-channel was fabricated. The position of particles in the depthwise z direction was determined by calibration experiments based on previously used methods. Since slight channel inclination and field curvature can introduce significant errors into particle velocity distribution measurements in channels with widths and depths as small as $1.5\ \mu\text{m}$, correction methods for particle positions in the width and depth directions were developed. The channel inclination and field curvature, which were used for the correction, were statistically evaluated using the positions of nanoparticles in x , y and x , z planes. Consequently, the 3D particle velocity distribution was obtained in a remarkably confined space, with a width and depth of a few μm . In this experiment, the velocity of 200 nm fluorescent particles flowing through the fabricated square channel was examined. It was confirmed that the particle velocity was nearly equivalent to the theoretical flow velocity, and the particle velocity distribution in a square micrometer-channel was successfully measured. This study makes a significant contribution to the advancement of knowledge regarding the mechanism of particle transport in confined spaces. This phenomenon is of general relevance across diverse disciplines, including micro/nanofluidics, medicine, and biofluids.

Acknowledgments

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