

Measurements in microchannel by laser induced molecular tagging and micro-PIV

by

T. Yamamoto⁽¹⁾, S. Inaba⁽¹⁾, Y. Sato⁽²⁾, K. Hishida⁽¹⁾ and M. Maeda^{(1)†}

- (1) Department of System Design Engineering, Faculty of Science and Technology, Keio University
Hiyoshi 3-14-1, Kohoku-ku, Yokohama, 224-8522, JAPAN
- (2) Mechanical Engineering Systems, National Institute of Advanced Industrial Science and Technology
Namiki 1-2-1, Tsukuba Science City, Ibaraki, 305-8564, JAPAN

† E-Mail : maeda@sd.keio.ac.jp

ABSTRACT

Rapid technological progress has required reliable measurement techniques for velocity fields in microspace, particularly in the biochemical or electrochemical engineering. This paper focuses on how to accomplish quantitative measurement techniques applicable to investigating fluid dynamics in a microchannel that is utilized in lab-on-a-chip and *m*TAS. Micro-resolution particle image velocimetry (micro-PIV) is one of promising techniques to measure fluid velocities in a microchannel using a time-averaging method in order to eliminate the effect of Brownian motion of sub-micron tracer particles on velocity detection, however, this technique has no ability to detect an unsteady flow that is frequently observed in microfluidic devices. A spatial averaged time-resolved particle tracking velocimetry (SAT-PTV) method was proposed to measure velocity fields in a pulsating flow considering Brownian motion. A wide-use methodology for velocity measurements in microspace using sub-micron particles has been established by the authors' group, so that the next strategy is aimed at the practical application of lab-on-a-chip or *m*TAS.

The objectives of the present study are to develop quantitative measurement techniques for electrokinetically driven flow (EKDF) using sub-micron particles and to validate these measurement results by a novel technique, i.e., laser induced molecular tagging (LIMT), using a photochromic dye. Quantitative three-dimensional velocity measurements of electroosmotic flow (EOF) were first accomplished using micro-PIV, however, this technique requires a measurement result of particles' velocity induced by electrophoresis, because the surface of particles is charged electrically, when sub-micron particles are injected into EKDF. Direct measurements of EOF were performed using LIMT, in which a velocity profile was calculated using two successive images. Figure 1(a) exhibits an instantaneous image and a velocity profile of pressure-driven flow detected by LIMT, which shows a good agreement with a theoretical result. Measurement results of EOF were illustrated in Fig. 1(b), whose velocity profile shows a plug pattern, which was also observed in the measurement result by micro-PIV. Measurement techniques such as micro-PIV, SAT-PTV and LIMT will be a key feature for further development of microfluidic devices.

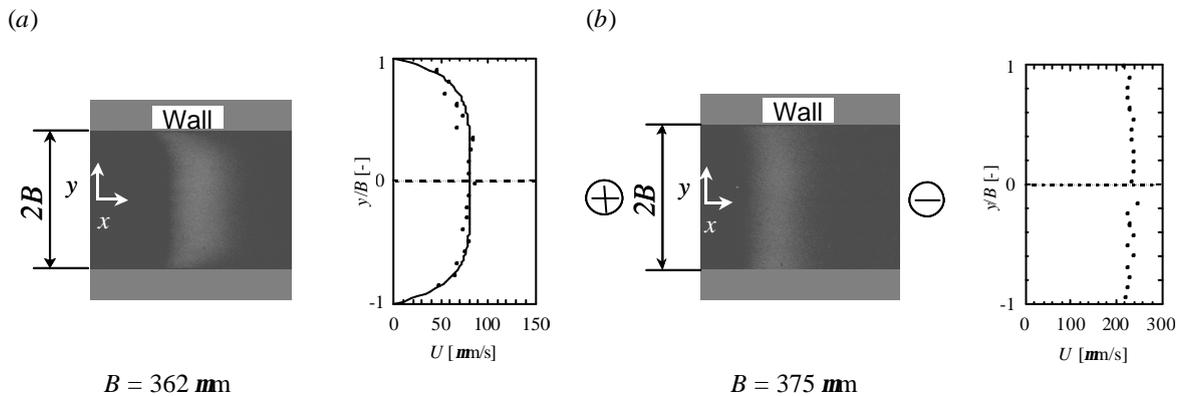


Fig. 1. Instantaneous image and velocity profile of (a) pressure-driven flow and (b) EOF with 185 V/cm measured by LIMT.

1. INTRODUCTION

Recent progress in micro- and nano-technologies has yielded microfluidic devices that comprise lots of microchannels in order to develop the assaying process using a very-small-amount-of fluid flow in the biological or electrochemical engineering. For further development of devices, e.g., lab-on-a-chip and μ TAS, quantitative measurements for a microchannel flow have been strongly required to understand the physics of transport processes in a microflow, to date, experimental efforts such as Santiago *et al.* (1998) and Meinhart *et al.* (1999) have contributed to investigate a velocity field in a microchannel. Micro-resolution particle image velocimetry (micro-PIV) is one of promising techniques to measure fluid velocities in a microchannel using a time-averaging method in order to eliminate the effect of Brownian motion of sub-micron tracer particles on velocity detection (Santiago *et al.* 1998, Meinhart *et al.* 1999). However, a dominant flow pattern in an electrophoresis DNA chip is an unsteady flow such as a pulsating flow, therefore a time-averaging method is no longer applicable to a flow of this kind. Inaba *et al.* (2001) proposed a spatial averaged time-resolved particle tracking velocimetry (SAT-PTV) method in order to measure an unsteady flow without losing time resolution. Moreover a fluid flow is usually driven by electrokinetic force in μ TAS, so that sub-micron particles are no longer tracer particles of the fluid, because the surface of particles is charged electrically. To overcome these difficulties novel measurement techniques have been strongly required for further development of microfluidic devices.

The objectives of the present study are;

- i) to develop measurement techniques for electrokinetically driven flow (EKDF) using micro-PIV (Sato *et al.* 2002) and
- ii) to propose a laser induced molecular tagging (LIMT) technique using a photochromic dye in order to measure electroosmotic flow (EOF) directly.

The above techniques established by the authors' group will bring about great changes in a methodology for velocity measurements in microspace. This paper focuses on how to accomplish quantitative measurement techniques and has been prepared in four sections.

2. VELOCITY MEASUREMENT TECHNIQUES USING SUB-MICRON PARTICLES

2.1 Measurement System

An optical measurement system used in the present study is illustrated in Fig. 2. A cooled CCD camera (Hamamatsu Photonics K.K., C4880-80) of 656×494 pixels was mounted on a microscope (Nikon Corp., E800). A continuous mercury lamp was used as a light source and the excitation wavelength was filtered by a band-pass filter. A dichroic mirror and a low-pass filter were used to detect fluorescence from sub-micron fluorescent particles as compiled in Table 1.

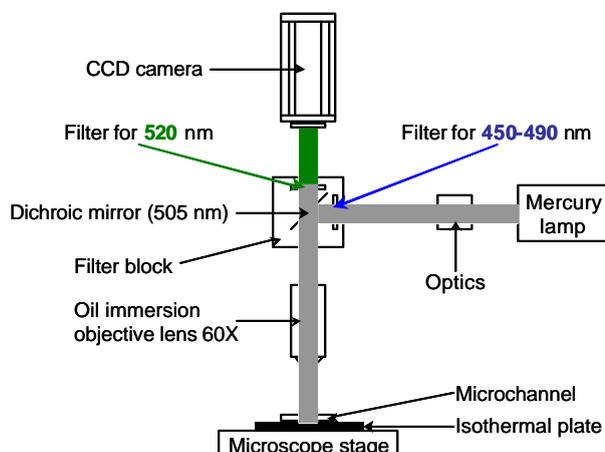


Fig. 2. Schematic of the measurement system.

Table 1. Properties of fluorescent particles

Number mean diameter	[nm]	400
Stan. dev. of diameter	[%]	<5
Density	[g/cm ³]	1.05
Absorption wavelength	[nm]	468
Emission wavelength	[nm]	508

2.2 Micro-Resolution Particle Image Velocimetry (Micro-PIV)

The pioneering study of micro-PIV by Santiago *et al.* (1998) and Meinhart *et al.* (1999) conducted to obtain velocity vectors in microspace using sub-micron fluorescent particles with diameters of a few hundred nm as a flow tracer, which induces a measurement error associated with Brownian motion. Santiago *et al.* (1998) reduced the effect of Brownian motion on velocity detection by ensemble averaging and Meinhart *et al.* (1999) by ensemble-averaged correlation maps in time series.

The most flow patterns in Lab-on-a-chip and $m\Gamma$ AS are an unsteady flow, so that the method above has no ability to detect the velocity field in time series. A novel technique is strongly required to measure velocity variations of a microflow.

2.3 Spatial Averaged Time-Resolved Particle Tracking Velocimetry (SAT-PTV)

A spatial averaged time-resolved particle tracking velocimetry (SAT-PTV, Inaba *et al.* 2002) method is proposed to be applicable to an unsteady flow such as a pulsating flow, which will be discussed in the next subsection. The SAT-PTV method can eliminate the effect of Brownian motion on velocity detection averaging PTV vectors in an interrogation window of the PIV method.

Figure 3 exhibits a schematic of the SAT-PTV method. A vector of a particle displacement obtained by PTV, v_p , is comprised of a vector of a fluid flow, v_f , and a vector due to Brownian motion, v_B , between two successive images. When N particles are contained in an interrogation window where spatial averaging is performed, the SAT-PTV vector is expressed as follows:

$$\frac{1}{N} \sum_{\text{window}} v_p = \frac{1}{N} \sum_{\text{window}} v_f + \frac{1}{N} \sum_{\text{window}} v_B \quad (1)$$

As Brownian motion is statistically random and unbiased, an increase in the number of particles, N , results in a zero value of the second term in the right-hand side of equation (1). It means that the spatial-averaged vector of a particle displacement becomes equal to that of a fluid flow, therefore it is possible to eliminate the effect of Brownian motion and obtain the accurate vector of a fluid flow in time series. A time interval of the SAT-PTV system depends on a frame interval of the CCD camera (37 ms for C4880-80).

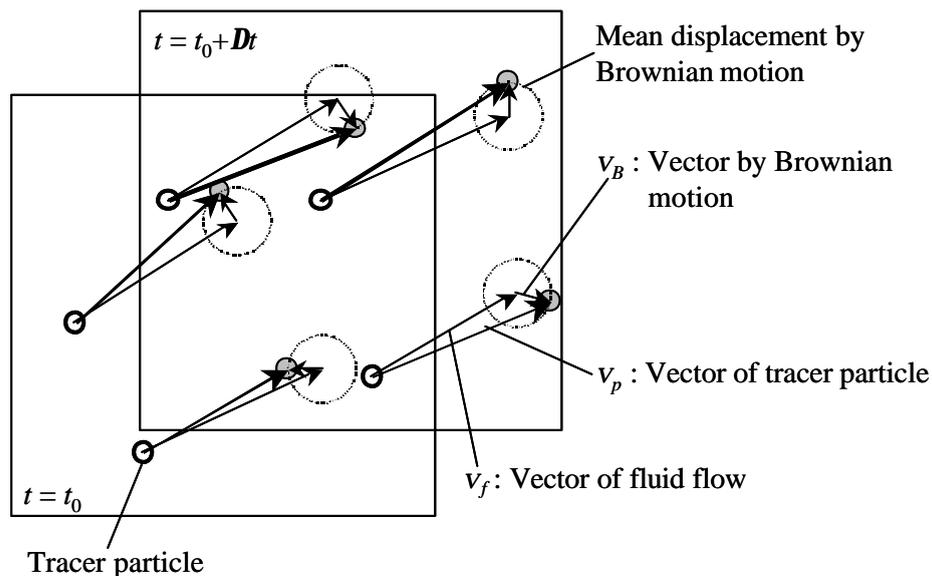


Fig. 3. Schematic of the SAT-PTV method considering the effect of Brownian motion on velocity detection.

Another important feature of the SAT-PTV method is the ability to detect velocity fields with velocity gradients. Synthetic particle images considering Brownian motion of tracer particles in a velocity field with a linear velocity gradient were generated in order to validate the SAT-PTV's ability. It is concluded that the SAT-PTV method can be applicable to velocity fields with velocity gradients, which was left unanswered in experimental works by Santiago *et al.* (1998) and Meinhart *et al.* (1999).

2.4 Measurement Results of a Pulsating Flow Detected by SAT-PTV

A pulsating flow is considered in this subsection, which was generated by an EK pump of 5 Hz attached to the microchannel. Figure 4 shows time evolution of velocity-vector field detected by the SAT-PTV method. The size of the first interrogation window was $6.7 \mu\text{m} \times 6.7 \mu\text{m}$ (40×40 pixels) and approximately six vectors were averaged. A spatial resolution was $6.7 \mu\text{m} \times 6.7 \mu\text{m} \times 2.5 \mu\text{m}$, based upon the size of the first interrogation window and the depth-of-field of the microscope. SAT-PTV can detect the pulsating flow considering the effect of Brownian motion, which is also confirmed from Fig. 5 that shows a power spectrum at a center point, as shown as a circle in Fig. 4, obtained from FFT. It is concluded that SAT-PTV is a promising technique in investigating unsteady fluid dynamics phenomena in microspace.

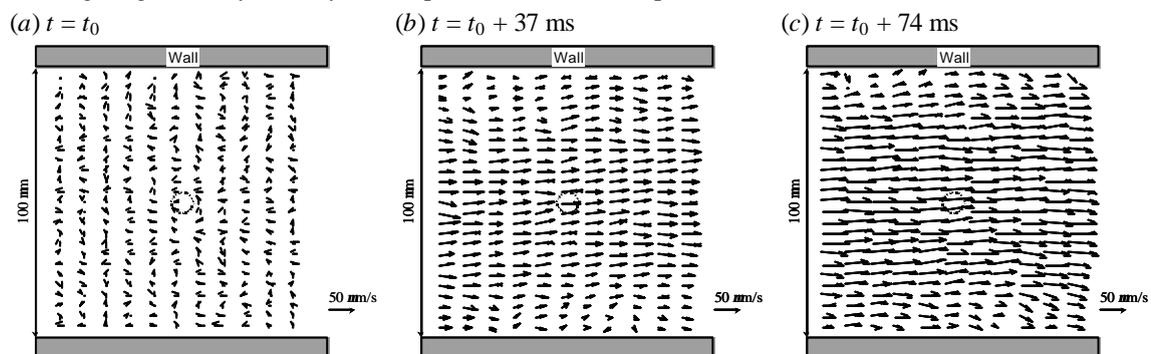


Fig. 4. Time evolution of velocity-vector field of a pulsating flow in the microchannel at 5 Hz generated by an EK pump of 60 V/cm detected by the SAT-PTV method. A time interval of each vector map was 37 ms.

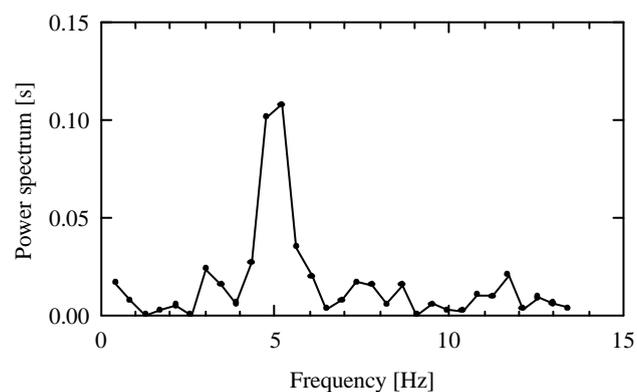


Fig. 5. Profile of power spectrum at a center point, as shown as a circle in Fig. 4, obtained from FFT.

3. VELOCITY MEASUREMENT TECHNIQUES OF ELECTROKINETICALLY DRIVEN FLOW BY MICRO-PIV

3.1 Fidelity of Sub-Micron Particles in Electrokinetically Driven Flow (EKDF)

A combination of micro-PIV and SAT-PTV may be a powerful investigating tool for a microflow, however, these techniques have never been examined in EKDF, so that none of quantitative information of the EKDF structure existed. When sub-micron particles are injected into EKDF, the surface of particles is charged electrically, so that the particles are no longer tracer particles of the fluid. Although experimental efforts using fluorescence imaging

(e.g., Tsuda *et al.* 1993, Paul *et al.* 1998 and Ross *et al.* 2001) have shown qualitative information and advanced our understanding, the EKDF structure is not fully resolved up to this day.

The objective of this section is to investigate the EKDF structure using micro-PIV considering the effect of electrophoresis (EP) on particle's motion. Measurement results for EKDF velocities, U_{ob} , obtained from the micro-PIV technique comprise a particle velocity by EP, U_{EP} , and velocities of EOF, U_{EO} (Mori and Okamoto 1980), as given by:

$$U_{ob} = U_{EP} + U_{EO}. \quad (2)$$

First particle velocities at each plane in the depth direction are measured in a closed cell, finally the three-dimensional velocity field of EOF is obtained subtracting the particle velocity by EP from measured velocities in a microchannel by micro-PIV (Sato *et al.* 2002).

3.2 Particle Velocity Induced by Electrophoresis (EP)

Measurements of the particle velocity induced by EP were performed in the closed cell with an electric field of 10 V/cm as shown in Fig. 6. Figure 7 illustrates schematic of a flow pattern in the closed cell, which shows that particle velocities are shifted toward the anode due to EP. Velocities of particles, as compiled in Table 1, in a steady state were measured at 11 planes in the depth, z , direction by micro-PIV and obtained from time averaging 100 instantaneous measurements in order to eliminate the effect of Brownian motion on velocity detection. The depth-of-field of the microscope using 400 nm diameter particles resulted in 2.5 μm (Meinhart *et al.* 2000).

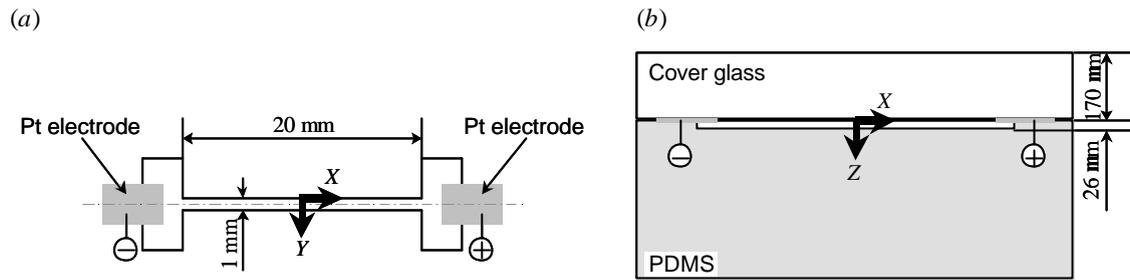


Fig. 6. (a) Top and (b) cross-sectional views of the closed cell.

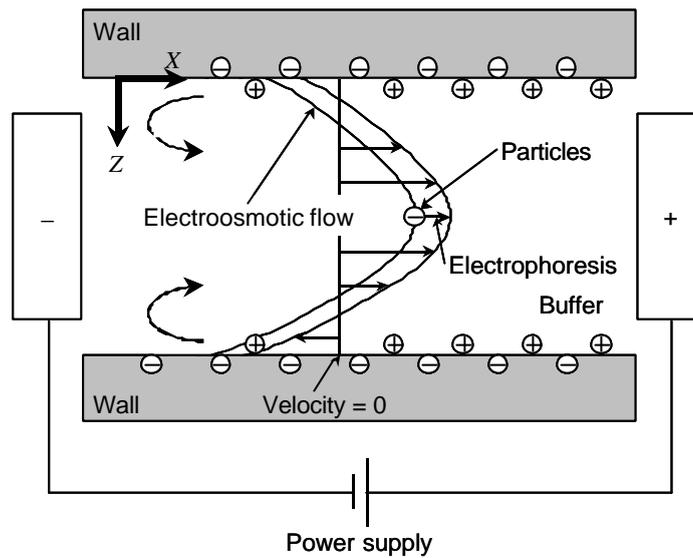


Fig. 7. Schematic of EKDF in the closed cell.

Figure 8 shows a velocity profile of particles induced by EP in the closed cell that comprises of a cover glass and PDMS. Due to the zeta potential at each wall, it is observed that particle velocities had a different value at each wall. According to equations proposed by Mori and Okamoto (1980), the particle velocity by EP shows 36.2 $\mu\text{m/s}$ toward the anode, as shown in Fig. 8.

3.3 Three-Dimensional Velocity Measurements of EOF

Velocity measurements of EOF were conducted using a 100 μm width microchannel with an electric field of 10 V/cm as illustrated in Fig. 9. The microchannel comprised of a cover glass and PDMS. Velocities in a steady state were detected at three planes in z direction.

Velocities of EOF were obtained subtracting U_{EP} from measured velocities detected by micro-PIV. Figure 10

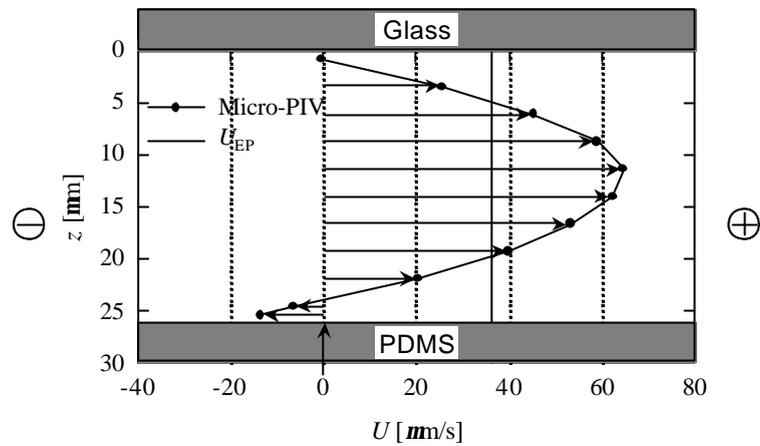


Fig. 8. Velocity profile of 400 nm diameter particles induced by EP in the closed cell with 10 V/cm. U_{EP} was calculated as 36.2 μm using equations by Mori and Okamoto (1980).

shows velocity profiles of EOF near the glass wall, in the center and near the PDMS wall obtained from time averaging 100 instantaneous measurements. Velocity profiles at each wall show a plug pattern, which is consistent with the experimental results using fluorescence imaging.

Using the available data by micro-PIV, a three-dimensional velocity field of EOF was first obtained as exhibited

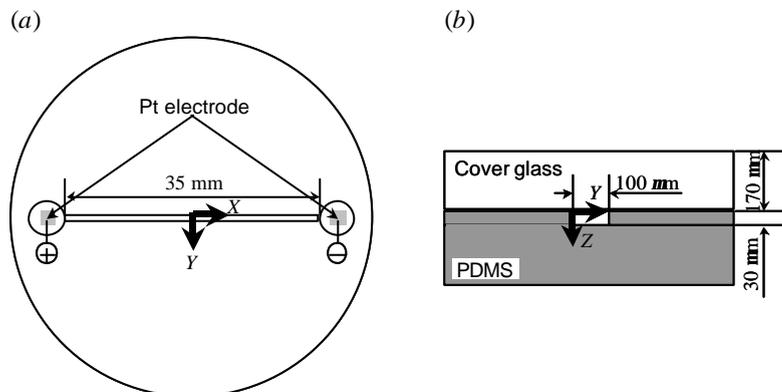


Fig. 9. (a) Top and (b) cross-sectional views of the microchannel.

in Fig. 11. Velocity measurements described in this section are the indirect technique, nevertheless quantitative information of EOF obtained in the present study is expected to contribute to further development of μTAS . The value of the depth-of-field, i.e., 2.5 μm , was so small that it is possible to detect the three-dimensional structure of EOF using sub-micron particles.

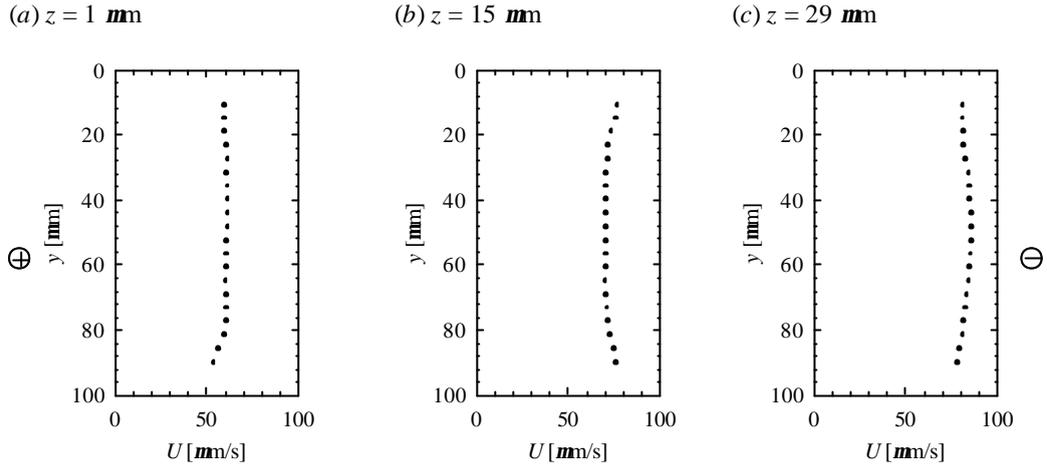


Fig. 10. Velocity profiles of EOF at each plane in the z -direction. (a) : near the glass wall ($z = 1 \text{ mm}$), (b) : in the center ($z = 15 \text{ mm}$), (c) : near the PDMS wall ($z = 29 \text{ mm}$).

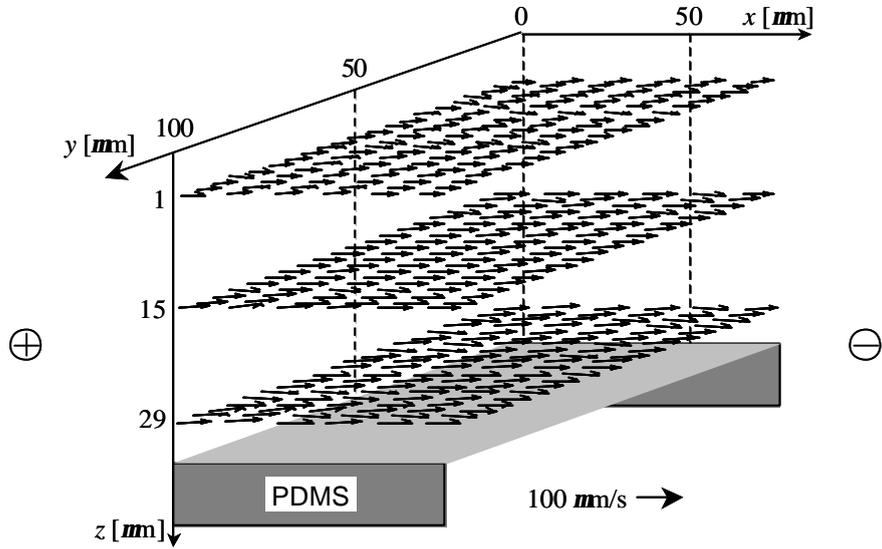


Fig. 11. Three-dimensional velocity-vector field of EOF in a 100 mm width microchannel with 10 V/cm measured by micro-PIV.

4. LASER INDUCED MOLECULAR TAGGING (LIMT)

4.1 Advantage of LIMT

Measurement techniques using sub-micron particles depend strictly on their fidelity, therefore precise measurements are no longer expected in EOF. Moreover measurements of molecule diffusion will be strongly required for the future microfluidic devices considering sensing and controlling selective diffusion and transport of molecules, so that the present study proposes a novel technique, i.e., a direct measurement technique for EOF. A laser induced molecular tagging (LIMT) technique using a photochromic dye was examined in pressure-driven flow and EOF.

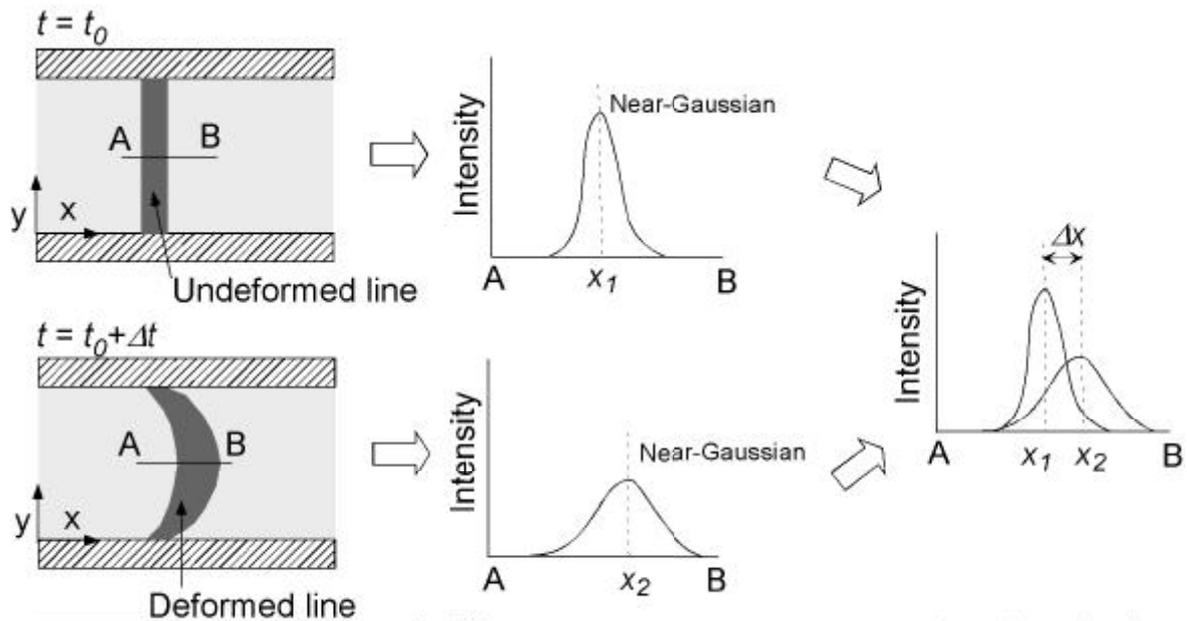


Fig. 12. Schematic of the LIMT technique.

Figure 12 exhibits the velocity detection process of the LIMT technique (Hill *et al.* 1996) which is based on determining a displacement between the line center of a deformed line and that of an undeformed line. The laser beam intensity is assumed to be Gaussian. First step is to calculate the x position associated with each y location along the undeformed laser line. At each y position for the undeformed line, smoothing is applied to the intensity versus position curve. Then the maximum intensity location is found from the smoothed curve. Gaussian fitting is applied to the region surrounding this peak location. The location of the maximum intensity considering the sub-pixels correction, x_1 , which corresponds to the line center is finally found. The next step is to find the line center of the deformed line, x_2 , using the same procedure.

Velocities at each y position along the line are calculated from a time interval, Δt , and the displacement, Δx , between both line center of the undeformed line and deformed line.

4.2 Properties of Photochromic Dye

In the present experiments, 1,3,3-tri-methylindolino-6-nitrobenzopyrlospiran dye (TMINBPS) illustrated in Fig. 13 was selected as a photochromic dye tracer. In a photochromic process a molecule M (the left hand side of Fig. 13) is activated to produce a high energy form of M , designated M' (the right hand of Fig. 13), which has a different absorption spectrum changing its color from clear to violet. The newly produced M' is the long lifetime dye tracer, which persists for several seconds. The tagging process from M to M' occurs rapidly within the duration of a UV laser pulse in a few nano seconds. M' thermally converts back, therefore the photochromic dye is reusable. Photochromic dyes are not generally in soluble in water, so that organic liquids such as kerosene, alcohol are used as a solvent. In the present study, an ethanol solution with 0.01 mol/l concentration of TMINBPS was used as a working fluid. Using the photochromic dye requires two photon sources: a UV laser to activate the photochromic dye, while a white light source to illuminate the entire flow fields.

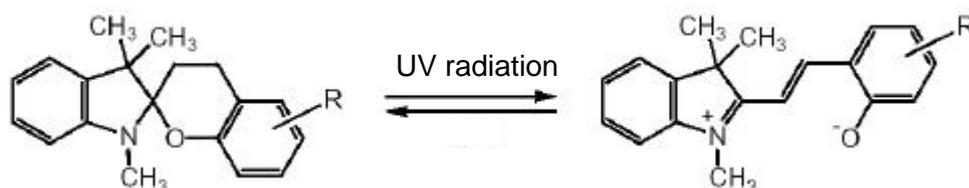


Fig. 13. Schematic of photochromic process.

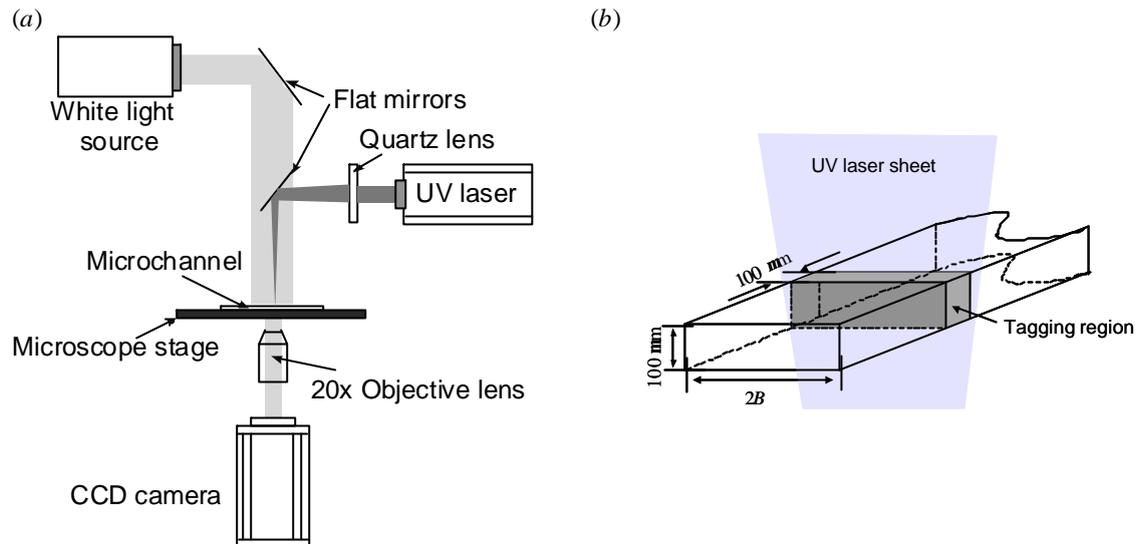


Fig. 14. Schematic of (a) the measurement system for LIMT and (b) the microchannel illuminated by the UV laser flash.

4.3 Measurement System

Figure 14(a) shows a measurement system using an inverted microscope (Nikon Corp., TE300). A pulse UV laser with a wavelength of $\lambda = 336 \text{ nm}$ was used to activate the photochromic dye. The UV laser was focused with a single 30 cm focal-length quartz lens and the laser thickness of approximately 100 μm was illuminated in the microchannel. A white light source illuminates the entire field of a microchannel to visualize a fluid flow and images were recorded by a cooled CCD camera of $378 \times 247 \text{ pixels} \times 12 \text{ bit}$ (Hamamatsu Photonics K.K., C4880-80). A frame interval was set at 50 ms. An objective lens (Nikon Corp., Plan Fluor) with a $20 \times$ magnification was attached to the microscope.

A microchannel used in this experiment as shown in Fig. 14(b) had a width of 370 μm and a depth of 100 μm . The ethanol solution with TMINBPS was moved by the height difference between both solution surfaces and an electrokinetically driven force.

4.4 Velocity Measurement of Pressure-Driven Flow

The LIMT technique was demonstrated in pressure driven flow. A Reynolds number based on a measured centerline velocity and a channel width was 1.2×10^{-5} . The ethanol solution with the photochromic dye in the microchannel was illuminated by the UV pulsed laser flash. These images were postprocessed subtracting a background noise. The focus field of the microscope was in the center of the microchannel. A spatial resolution was 8.3 μm (in y direction) \times 3.3 μm (the depth of field).

Figure 15(a) displays time evolution of instantaneous image of the photochromic dye tracer detected by the LIMT technique. Near the wall velocities were close to zero due to the non-slip condition, while the centerline velocity had a maximum value, which was correctly captured by LIMT. Distribution of the intensity in time series after Gaussian fitting is shown in Fig. 15(b), with which velocities were calculated using the peak position and a time interval, yielding a velocity profile as shown in Fig. 15(c). A theoretical result is also plotted as a solid line in Fig. 15(c). This result was calculated according to equations introduced by Brody *et al.* (1996). It is observed that the experimental result shows a good agreement with the theoretical solution, even though measurement errors were found near the wall, because of the diffusion of photochromic dye and noises of images due to penetration of the photochromic dye into PDMS during the UV laser flash.

4.5 Velocity Measurement of EOF

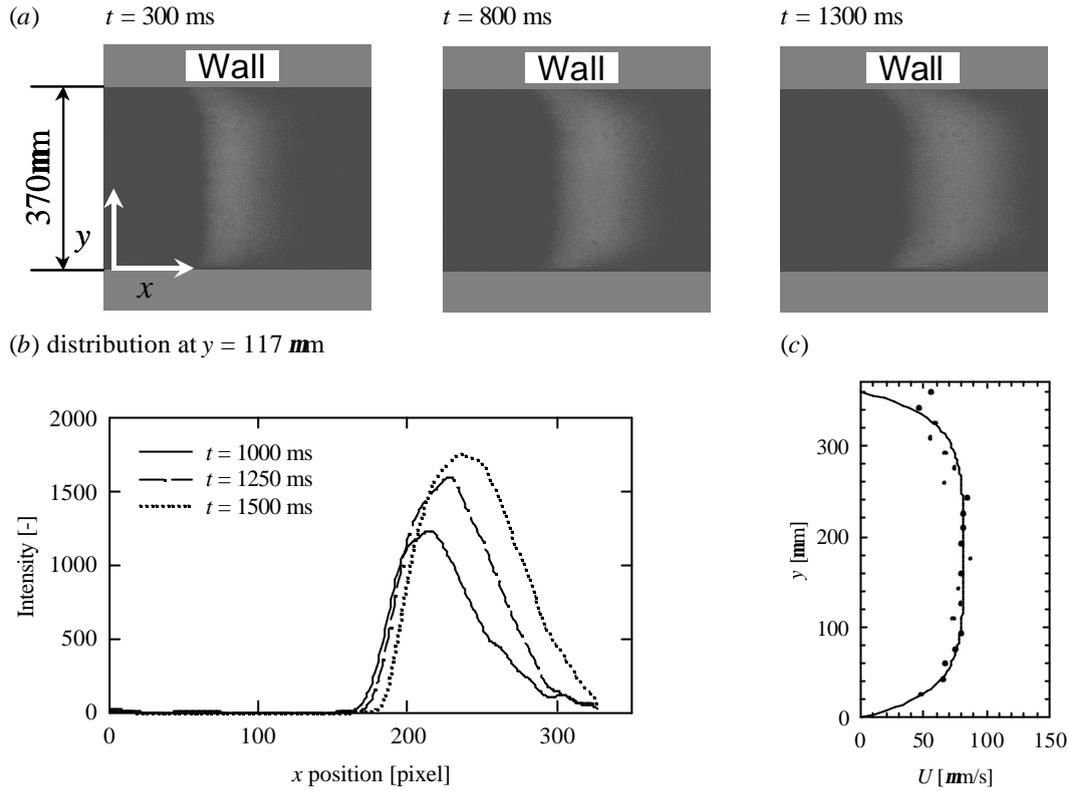


Fig. 15, (a) Time evolution of instantaneous image, (b) intensity distribution of the photochromic dye and (c) velocity profile of pressure driven flow.

The LIMT techniques was validated applying to EOF. EOF was generated by an electric field of 185 V/cm. Figure 16(a) shows time evolution of instantaneous image of EOF, in which it is found that the flow shows a plug pattern as reported by the previous experimental work. Postprocessing was performed calculating distribution of the intensity as shown in Fig. 16(b). A slope of distribution became wider in terms of time due to molecular diffusion of the photochromic dye. Using these distributions a velocity of EOF was calculated yielding a velocity profile as plotted in Fig. 16(c). A spatial resolution was 8.3 mm (in y direction) \times 3.3 mm (the depth of field).

In the present study the LIMT technique was developed using a photochromic dye and it can be concluded that LIMT has an ability to detect velocity fields in a microchannel. More elaborated measurement technique using a caged fluorescent dye is now currently underway.

5. CONCLUSIONS

Velocity measurement techniques for EKDF have been developed in order to obtain quantitative information for further development of microfluidic devices. The advantage of velocity measurement techniques using sub-micron particles is that it is possible to obtain precise information of velocity fields due to a small value of the depth-of-field of a microscope. One can easily measure various flow patterns using micro-PIV or SAT-PTV in microspace. The LIMT technique using the photochromic dye enables us to measure EOF directly and will be a promising technique for sensing and controlling molecules in microfluidic devices. It is important to recognize that each measurement technique has its own advantage, therefore a combination of micro-PIV, SAT-PTV and LIMT pioneers micro- and nanoscale technologies.

ACKNOWLEDGEMENTS

The authors would like to thank Mr. M. Ishizuka at National Institute of Advanced Industrial Science and

Technology, and New Energy and Industrial Technology Development Organization (NEDO) for his suggestions. This work was subsidized by the Grant-in-Aid for Scientific Research (No. 13555057).

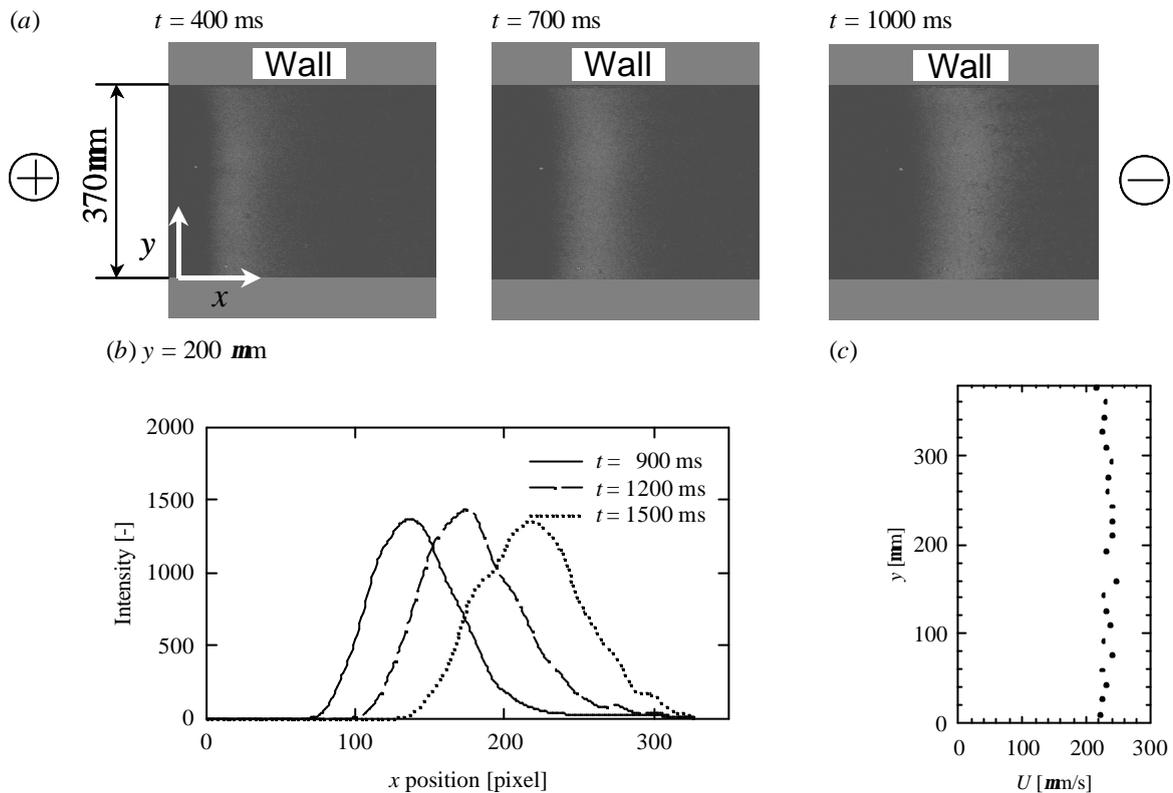


Fig. 16, (a) Time evolution of instantaneous image, (b) intensity distribution of the photochromic dye and (c) velocity profile of EOF with 185 V/cm.

REFERENCES

- Brody, J.P., Yager, P., Goldstein, R.E. and Austin, R.H. (1996). "Biotechnology at Low Reynolds Numbers", *Biophys. J.*, Vol. 71, pp. 3430-3441.
- Hill, R.B. and Klewicki, J.C. (1996). "Data Reduction Methods for Flow Tagging Velocity Measurements", *Exp. Fluids*, Vol. 20, pp. 142-152.
- Hosokawa, K. and Maeda, R. (2001). "In-line Pressure Monitoring for Microfluidic Devices Using a Deformable Diffraction Grating", *IEEE*, pp. 174-177.
- Inaba, S., Sato, Y., Hishida, K. and Maeda, M. (2001). "Flow Measurements in Microspace Using Sub-Micron Fluorescent Particles—An Effect of Brownian Motion on Velocity Detection—", *Proc. Fourth Int. Symp. Particle Image Velocimetry*, 1141.
- Meinhart, C.D., Wereley, S.T. and Santiago, J.G. (1989). "PIV Measurements of a Microchannel Flow", *Exp. Fluids*, Vol. 27, pp. 414-419.
- Mori, S. and Okamoto, H. (1980). "A Unified Theory of Determining the Electrophoretic Velocity of Mineral Particles in the Rectangular Micro-Electrophoresis Cell", *Fusen*, Vol. 27, pp. 117-126.
- Paul, P.H., Garguilo, M.G. and Rakestraw, D.J. (1998). "Imaging of Pressure- and Electrokinetically Driven

- Flows through Open Capillaries”, *Anal. Chem.*, Vol. 70, pp. 2459-2467.
- Ross, D., Johnson, T.J. and Locascio, L.E. (2001). “Imaging of Electroosmotic Flow in Plastic Microchannels”, *Anal. Chem.*, Vol. 73, pp. 2509-2515.
- Santiago, J.G., Wereley, S.T., Meinhart, C.D., Beebe, D.J. and Adrian, R.J. (1989). “A Particle Image Velocimetry System for Microfluidics”, *Exp. Fluids*, Vol. 25, pp. 316-319.
- Sato, Y., Hishida, K. and Maeda, M. (2002). “Quantitative Measurement and Control of Electrokinetically Driven Flow in Microspace”, *Sixth Int. Conf. mTAS* (to be submitted).
- Tsuda, T., Ikedo, M., Jones, G., Daddo, R. and Zare, R. (1993). “Observation of Flow Profiles in Electroosmosis in a Rectangular Capillar”, *J. Chromatography*, Vol. 632, pp. 201-207.
- Yurechko, V.N. and Ryazantsev, Yu.S. (1991). “Fluid Motion Investigation by the Photochromic Flow Visualization Technique”, *Exp. Therm. Fluid. Sci.*, Vol. 4, pp. 273-288.