Development of three-dimensional integrated microchannel-electrode system to understand the particles' movement with electrokinetics

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An optical transparent 3-D Integrated Microchannel-Electrode System (3-DIMES) has been developed to understand the particles' movement with electrokinetics in the microchannel. In this system, 40 multilayered electrodes are embedded at the 2 opposite sides along the 5 square cross-sections of the microchannel by using Micro Electro-Mechanical Systems technology in order to achieve the optical transparency at the other 2 opposite sides. The concept of the 3-DIMES is that the particles are driven by electrokinetic forces which are dielectrophoretic force, thermal buoyancy, electrothermal force, and electroosmotic force in a three-dimensional scope by selecting the excitation multilayered electrodes. As a first step to understand the particles' movement driven by electrokinetic forces in high conductive fluid (phosphate buffer saline (PBS)) with the 3-DIMES, the velocities of particles' movement with one pair of the electrodes are measured three dimensionally by Particle Image Velocimetry technique in PBS; meanwhile, low conductive fluid (deionized water) is used as a reference. Then, the particles' movement driven by the electrokinetic forces is discussed theoretically to estimate dominant forces exerting on the particles. Finally, from the theoretical estimation, the particles' movement mainly results from the dominant forces which are thermal buoyancy and electrothermal force, while the velocity vortex formed at the 2 edges of the electrodes is because of the electroosmotic force. The conclusions suggest that the 3-DIMES with PBS as high conductive fluid helps to understand the three-dimensional advantageous flow structures for cell manipulation in biomedical applications. © 2016 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4943859>]

I. INTRODUCTION

A manipulation of target cells is a critical technique in the regenerative medicine applications.¹ Most of the conventional methods such as fluorescence-activated cell sorting (FACS)² and magnetic-activated cell sorting (MACS)³ are economically expensive and demand extraordinary precision to prevent cell deterioration.⁴ An effective cell manipulation means that the cells should retain the biological function without any deterioration; however, most of these conventional methods use special biochemical markers (labels) on the cell surface which jeopardize the cell viability. Hence, the label free manipulation technique of the target cells which does not rely on biochemical marker is highly desirable for the effective cell manipulation. In the quest to develop a label free manipulation technique, various methods based on ultrasonic,⁵ optical,⁶ and electrical^{7,8} principles have been widely reported.

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The manipulation technique with electrical method uses electrokinetic forces to drive particles such as cells and fluid to realize the particles' movement. After voltage and electrical current are applied to electrodes, the particles are driven by the electrokinetic forces which are dielectrophoretic (DEP) force, thermal buoyancy, electrothermal force, and electroosmotic force. DEP force is one of the major forces in the electrical method. The DEP force exerting on the dielectric particles in non-uniform electric fields is widely used to manipulate the particles.^{9–13} Conventionally, in the manipulation technique with electrical method using DEP force, the particles are immersed in low conductivity fluid. However, the low conductivity fluid is not consistent with the natural environment of cells which is highly conductive. In high conductive fluid, the particles only experience negative DEP force. Hence, it is difficult to manipulate the particles on the basis of switching frequency like in the case of low conductive fluid. Moreover, the particles' movement is also influenced by thermal buoyancy, electrothermal force, and electroosmotic force. Thermal buoyancy is generated by a phenomenon of physics where warm liquid is less dense than cool liquid, and thus, causes warm liquid to rise. The amount or "power" of thermal buoyancy depends upon the size of the temperature difference between the cold and warm fluid, and the height differential between where the cold fluid enters a space and where the warm fluid leaves. Increasing the temperature or increase the electric field strength and height differential increases the fluid movement due to thermal buoyancy.¹⁴ Electrothermal force arises in fluid with non-uniform permittivity and conductivity. In this fluid, a local free charge distribution presents when Gauss's Law and charge conservation are satisfied simultaneously. The density of local charge under the applied electric field results in a non-zero body force on the fluid.¹⁵ Electroosmotic force is generated from a fluid movement via a slip velocity around the electrode surface with electrical double layers (EDLs) in an AC electric field.¹⁶ In microfluidic systems, a pressure-driven flow by electroosmotic force is often applied to deliver the sample or cells into the target region.¹⁷

Exploiting the resultant effect of the combination of the above electrokinetic forces was used as field-flow fractionation (FFF)¹⁸ by Kuczenski *et al.*,¹⁹ Gielen *et al.*,²⁰ Wang *et al.*,²¹ and Yasukawa *et al.*²² to manipulate the particles in two dimensional scope, while the electrokinetic forces generated from a simple two dimensional planar electrodes. The overall mechanism of these forces in 2D is straightforward. Alternatively, fabrication of 3-D microfluidic devices has been realized with 3-D printing technologies and direct internal 3-D laser writing methods, which was critically reviewed by O'Neill *et al.*,²³ however, it is difficult to employ these 3-D fabrication technologies to embed electrodes in the microfluidic devices, that means, the electrokinetic forces are difficult to be employed to manipulate the particles in 3 dimensions.

Currently, a multilayered electrodes microchannel with diamond cross-sections was fabricated by our laboratory²⁴ to measure the concentration distribution of cells in the microchannel,²⁵ however, the flow characteristics of particles in the multilayered electrodes microchannel were not fully understood because the diamond cross-sections block optic to measure the particles' movement. Therefore, a new type of cross-sections to transparent optics is totally necessary. Hence, to make a breakthrough of the limitation of 3-D particle manipulation by electrokinetic forces, the proper understanding of the particles' movement in 3-D scope with these electrokinetic forces under different fluid conditions needs to be thoroughly studied with an optical transparent multilayered electrodes microchannel. Furthermore, in the conventional cell manipulation technique, the cells are immersed in the low conductive fluid, which is not consistent with the natural environment of the cells which is highly conductive. To overcome this issue, the alternative would be to put the cells in the high conductive fluid so that the natural cell environment remains unchanged.

Therefore, this study is to develop an optical transparent 3-D Integrated Microchannel-Electrode System (3-DIMES) to understand the 3-D particles' movement in the microchannel with electrokinetic forces. To understand the particles' movement driven by the electrokinetic forces in high conductive fluid (phosphate buffer saline (PBS)) with the 3-DIMES, the velocities of particles' movement with one pair of the electrodes are measured three dimensionally by Particle Image Velocimetry (PIV) technique in PBS; meanwhile, low conductive fluid (deionized water) is used as a reference; Furthermore, to determine the dominant forces exerting on the particles, the velocities of particles due to the electrokinetic forces which are DEP force, thermal buoyancy,

electrothermal force, and electroosmotic force are analysed theoretically. Finally, to fully understand the 3-D flow characteristics of the particles in the 3-DIMES observed in the experiments, the 4 types of the electrokinetic forces are discussed according to the experimental results.

II. CONCEPT AND FABRICATION OF THE 3-DIMES

We fabricated a system that is named as 3-DIMES for particle manipulation under optical transparency. In this system, multilayered electrodes are integrated in a microchannel with square cross-sections by using Micro Electro-Mechanical Systems (MEMS) technology. The concept is that the particles are manipulated by 3D electrokinetic forces generated from selecting the excitation electrodes among multilayered electrodes.

The 3-DIMES shown in Figure 1(a) is composed of a microchannel integrated with platinum multilayered electrodes, a printed circuit board (PCB) pin connectors, and a holder. As shown in Figure 1(b), the microchannel has three inlets, three outlets, and a main flow channel. The schematic diagram of the main flow channel is shown in Figure 1(c). The main flow channel has a channel length of $L = 20$ mm, a square cross-section of $h \times w = 550 \mu\text{m} \times 550 \mu\text{m}$, and five electrode-integrated positions with separation distance $l = 4.5$ mm. In each position, the main flow channel has altogether 8 electrodes with 4 electrodes in each side of the cross-sections. Each electrode has the thickness of $t = 10 \mu\text{m}$, length along the channel of $L_x = 200 \mu\text{m}$, and inter-electrode distance of $d_e = 100 \mu\text{m}$. The electrode tails are connected to PCB through copper terminal connectors. The PCB is equipped with pin connectors to connect a control and measurement systems of electrical excitation source.

The micro computed tomography (μCT) images (TDM1300-IS; Yamato Scientific, Japan) of the 3-DIMES along with integrated electrodes are shown in Figure 2. In the μCT images, as shown in Figures 2(a) and 2(b), the inlets and the outlets as well as the main-flow channel are smoothly grooved. Also, as shown in Figures 2(c) and 2(d), the platinum electrodes are perfectly aligned to the wall of the main flow channel. Similarly, the gap between the electrodes is uniform as expected.

The electrodes are integrated in the 3-DIMES using MEMS technology based fabrication, as shown in Fig. 3. The fabrication is carried out in the following steps. Step-1, a quartz substrate of $L_{\text{SL}} \times L_{\text{SW}} \times L_{\text{SH}} = 40 \text{ mm} \times 20 \text{ mm} \times 0.11 \text{ mm}$ with high transparency is prepared. Actually, glass is also proper to be substrate for the microchannel. However, to realize extensive applications (even in severe conditions) of the microchannel in the future, the quartz substrate is used. Though the quartz substrate is expensive, some advantages of the quartz substrate are also valuable to use, such as better transparency, high temperature resistance, wear resistance, especially chemical stability like good acid resistance, alkali resistance in the presence of chemical solvents. Step-2, 5 grooves ($18 \text{ mm} \times 0.2 \text{ mm} \times 0.01 \text{ mm}$) with separation distance of 4.5 mm for platinum vapor deposition of electrodes are drilled with very hard solid tungsten carbide micro twist drill bits (special size with diameter of 1 mm) at super-high speed. A shadow-mask is prepared with rectangular cuttings of $L_e \times L_x = 18 \text{ mm} \times 0.20 \text{ mm}$ at the five positions. The width of a rectangular cutting matches the length L_x of an electrode along the microchannel, and the distance between rectangular cuttings matches the distance l between

FIG. 1. 3-D Integrated Microchannel-Electrode System (3-DIMES), (a) the 3-DIMES is composed of a microchannel integrated with platinum multilayered electrodes, a printed circuit board (PCB) pin connectors, and a holder. (b) The microchannel has three inlets, three outlets, and a main flow channel. (c) The schematic diagram of the main flow channel.

FIG. 2. Micro computed tomography (μ CT) images of the 3-DIMES with square cross-sections.

electrode-integrated positions in the microchannel. The length of a rectangular cutting matches the span of the electrodes in the quartz substrate at each side of the microchannel. Step-3, platinum vapour deposition to make platinum electrodes is carried out while keeping the substrate in intimate contact with shadow-mask. Step-4, the mask is removed after precise vapour deposition on substrate. Step-5, another quartz substrate is overlapped to the substrate having vapour deposition. Step-6, the two quartz substrates are then compressed so that the platinum deposition is equally embedded in both of these substrates. The depth of the platinum deposition in each substrate determines the thickness t of an electrode. Step-7, step-2 to step-6 are repeated to make four electrode layers within five quartz substrates. Step-8, the microchannel is made by drilling the component made in step-7. Step-9, additional substrate layers are added to cover the microchannel. Step-10, three inlet and three outlet holes are made by drilling the component made in step-9. Step-11, copper terminal is fixed in the terminal of the platinum electrodes. The copper terminals connect the platinum electrodes to PCB.

One significant application of the fabricated 3-DIMES is to manipulate the cells in 3 dimensions. The specific operation is like this: with different combinations of the excitation multilayered electrodes, different types of electric fields are generated,²⁵ correspond to various electrokinetic forces in 2 dimensions; furthermore, 5 cross-sections are used to manipulate the cells in the 3rd dimension. In the present experiment, as a first step to understand the particles' movement in high conductive fluid and to estimate the dominant forces exerting on the particles in the 3-DIMES, 2 electrodes are excited to understand the particles' movement in the microchannel, which provides a fundamental test for 3D manipulation in the next study.

III. EXPERIMENTAL EVALUATION OF THE 3-DIMES

A. Experimental set-up

The experimental setup is shown in Figure 4(a) which consists of 3-DIMES, an optical microscope (Eclipse LV100D; Nikon Instruments, Japan), a high-speed charge-coupled device

FIG. 3. Fabrication process of the 3-DIMES with integrated multilayered electrodes.

(CCD) camera (Fastcam SA3; Photron K.K., Japan), a halogen lamp, a function generator, and a PC (Personal Computer). The x -component of the coordinate is opposite to the gravity direction, the y -component and the z -component represent the two directions of the plane (y - z plane) parallel to the ground. The 3-DIMES was placed along the gravity direction to make the main flow channel along the opposite direction of the x -component to eliminate the gravity influence on the particles' movement in the y - z plane in the cross-sections of the main flow channel. The optical microscope was equipped with a chrome-free (CF) infinity corrected objective lens (EPI SLWD; Nikon Instruments, Japan) with 20 magnifications. The CCD camera was used to take the images of the particles' movement inside the 3-DIMES through the microscope and the objective lens. The volume illumination of the particle fields was carried out using a halogen lamp that was embedded in the microscope. The function generator (33220A Function/Arbitrary Waveform Generator; Agilent Technologies, USA) was used for the supply of the AC electric field.

As shown in Figures 4(b) and 4(c), the 3-D scope between the 2 electrodes was used for the analysis of particles' movement. One of the two electrodes (red one) is used for current injection, and another (black one) for the grounding. The origin of the coordinate O is at the bottom left corner of the electrode with current injection. The direction along the channel was termed as vertical length L_x represented by x -component. The direction from electrodes toward the opposite side of the cross-sections was termed as horizontal length L_y represented by y -component. $L_x = 200 \mu\text{m}$, $L_y = 550 \mu\text{m}$, and $L_z = 100 \mu\text{m}$; 10 measurement planes are focused to capture the particle images between $z = 0 \mu\text{m}$ and $z = 100 \mu\text{m}$ at the increment of $10 \mu\text{m}$ by axially translating the objective lens along the z -component. In the experimental results, the x and y -components' displacements are normalized as x/L_x , y/L_y , and z/L_z on the basis of the electrode length.

FIG. 4. Experimental setup. (a) The 3-DIMES was placed along the gravity direction to make the main flow channel along the opposite direction of the x -component to eliminate the gravity influence on the particles' movement in the y - z plane in the cross-sections of the main flow channel; (b) one of the two electrodes (red one) is used for current injection, and another (black one) for the grounding; (c) observed cross-sections by optical microscope at different depths of y/L_y and z/L_z .

B. Experimental conditions and methods

The particles' movement velocity in two conductivity different types of fluids in the optical transparent 3-DIMES was quantitatively measured to evaluate the dominant electrokinetic forces. The specifications of dielectric particles are shown in Table I. The influence of the buoyancy and gravity on the particles is neglected, since the similar densities of the particles and the fluid. The Polystyrene particles were mixed separately with a volume concentration of 0.5% with the deionized water as the low conductivity fluid and PBS as the high conductivity fluid, as shown in Table I. The applied AC electric voltage with sinusoidal-wave peak to peak is $V_{pp} = 10$ V with frequency $\omega/(2\pi) = 10$ MHz supplied by a function generator.

The vision depth of focus δz in measurement planes of the microscope objective lens was decided from diffraction, geometrical effects, and particles' size by²⁶

$$\delta_z = \frac{3\eta\lambda}{N_A^2} + \frac{2.16d_p}{\frac{N_A}{\eta}} + d_p, \quad (1)$$

where η represents the refractive index of the fluid, N_A represents the numerical aperture of the lens, λ is the wavelength of light in a vacuum being imaged by the optical system, and d_p is the particle diameter. Similarly, the depth of field δ_z of microscope objective lens was $10 \mu\text{m}$ obtained by Eq. (1) for $\eta = 1.33$, $\lambda = 532 \text{ nm}$, $d_p = 3 \mu\text{m}$, and $N_A = 0.55$. On the basis of this depth, 10 measurement planes were focused to capture the particle images between $z/L_z = 0 \mu\text{m}$ and $z/L_z = 1$ at the increment of $10 \mu\text{m}$ by axially translating the objective lens along the z -component.

The particle velocities $\mathbf{u}_p$ were measured by cross correlation algorithm of PIV technique.^{29–31} The particle images in the flow field are taken sequentially at the rate of 60 frames per second by the CCD camera, the shutter speed is $1/1000$ s, the time-mean velocity vectors $\mathbf{u}_p$ were obtained by processing the 20 successive particle images.³⁰ Resolution of each image was 1024×1024 pixels.

TABLE I. Summary of data for experiments and calculations.

Parameter	Data	Meaning
d_p	$3 \mu\text{m}$	Particle diameter
μ_w	$1.00 \times 10^{-3} \text{ Pa s}$	Water viscosity at 20°C
μ_m	$1.09 \times 10^{-3} \text{ Pa s}$ (Ref. 19)	PBS viscosity at 20°C
V	10 V	Applied voltage from AC source
Φ	1.0 V	Effective potential between electrodes
Φ_{RMS}	0.35 V	Root mean square of the effective potential
$K(\omega)_w$	-0.42	Clausius-Mossotti factor of water
$K(\omega)_m$	-0.225	Clausius-Mossotti factor of PBS
σ_w	$5.5 \times 10^{-5} \text{ S/m}$	Electric conductivity of water
σ_m	1.7 S/m	Electric conductivity of PBS
σ_p	$3.7 \times 10^{-4} \text{ S/m}$	Electric conductivity of particles
ρ_w	$1.0 \times 10^3 \text{ kg/m}^3$	Water density
ρ_m	$1.05 \times 10^3 \text{ kg/m}^3$	PBS density
ρ_p	$1.05 \times 10^3 \text{ kg/m}^3$	Particle density
φ_p	0.5%	Particle volume concentration
ϵ_0	$8.85 \times 10^{-12} \text{ F/m}$	Vacuum permittivity
ϵ_w	$78\epsilon_0$	Water permittivity
ϵ_m	$60\epsilon_0$	PBS permittivity
ϵ_p	$2.2\epsilon_0$	Particle permittivity
β	$0.207 \times 10^{-3} \text{ K}^{-1}$ (Ref. 27)	Thermal expansion coefficient
c_p	$4.3 \times 10^3 \text{ J/(kg K)}$	Specific heat capacitance
ζ_w	-10 mV	Zeta potential of water
ζ_m	-60 mV (Ref. 28)	Zeta potential of PBS
k	0.58 W/(m K)	Thermal conductivity

C. Experimental results

1. Particles' movement in low conductivity fluid

The time mean velocity vectors of the particles' movement $\mathbf{u}_p (= \mathbf{u}_{px} + \mathbf{u}_{py})$ in x' - y' plane at various z/L_z between the two electrodes in the case of deionized water are shown in Figure 5. The red line represents the electrode with current injection, and the other electrode for the grounding is not seen in this figure. The time-mean velocity vectors $\mathbf{u}_p$ up to the length $y/L_y = 0.6$ are shown because the particles' movement beyond that position was sufficiently small. Most of the time-mean velocity vectors $\mathbf{u}_p$ point toward y -component from the electrodes, and high intensity velocity vectors among them are induced near the electrode. So far as depth is concerned, it is observed that the y -component intensity is higher near the current injection electrode (i.e., $z/L_z = 0.0$ or 0.2), while it is lower as the depth is increased in the direction toward ground electrodes (i.e., $z/L_z = 0.8$). These observations are also consistent with the common understanding that the higher force region should be nearer to the current injection electrode. These results are explained as that the particles are driven away from the electrode due to the DEP force F_{DEP} which is the dominant force. The other three velocities due to electrokinetics forces are quite small in low conductive fluid, which is explained in Sec. III D.

2. Particles' movement in high conductivity fluid

The time mean velocity vectors of the particles $\mathbf{u}_p$ in x' - y' plane at various z/L_z between the two electrodes in the case of PBS are shown in Figure 6. As shown in the figure, the velocity vectors indicate positive x -component flow at all z/L_z . However, the particles' movement pattern in terms of y -component is different at various z/L_z as well as x positions. At $z/L_z = 0$,

FIG. 5. Time mean velocity vectors $\mathbf{u}_p$ obtained at x' - y' plane at various depth z/L_z in Fig. 4(c) in the case of deionized water.

FIG. 6. Time-mean velocity vectors $\mathbf{u}_p$ measured at x' - y' plane at various depth z/L_z in Fig. 4(c) in the case of PBS.

the high intensity region of the velocity vectors is induced near the center of the electrode at $x/L_x = 0.5$ and towards the electrode. Additionally, it is understood from the particles' movement pattern that the particles' movement in this region had components in z -component too because there is discontinuity of the mass conservation of the flow. For example, the flow regime is gradually progressing from $x/L_x \leq 0.5$ to $x/L_x \geq 0.5$ as the z position shifted from $z/L_z = 0.0$ to $z/L_z = 0.5$. Additionally, at the z positions from $z/L_z = 0.0$ to $z/L_z = 0.5$, the velocity vortex appears due to the electroosmotic force. On the basis of the conservation of the mass of the flow, this gradual shifting of the flow regime indicates the particles' movement in the z -component, thus revealing the three dimensional flow structures between the electrodes.

Figure 7 shows the spatial distribution of the direction and intensity of the x and y components of the velocities. Though the x -component of velocity is always upward ($+x$; i.e., opposite to the gravitational field), the intensity is lower at $x/L_x = 0.5$ than that at the edges of electrode. Similarly, the range of the spatial change of overall x -component intensity is higher at the y position away from the electrodes (Figure 7(a)) than in the y position near the electrodes (Figure 7(b)). This is in line with the expectation that the DEP force near the electrodes contributes to the intensity of the y -component of the velocity, as observed in the case of deionized water, and influences the x -component velocities in the electrode vicinity.

The y -component of the velocity gradually decreases as depth z/L_z is increased. The intensity is higher around the midpoint of the electrodes ($x/L_x = 0.5$) than at the edge of the electrodes ($x/L_x = 0.0$ or 1.0). It is strong to moderately positive ($+y$; i.e., towards the electrodes) before $x/L_x = 0.5$ and strong to moderately negative ($-y$; i.e., from the electrodes) after $x/L_x = 0.5$. The velocity patterns are similar at two different y positions though the

FIG. 7. x and y -components of the time-mean velocity vectors u_{px} and u_{py} in the case of PBS. (a) The time-mean velocity vectors u_{px} measured at $y/L_y = 0.3$ of x'' - z'' plane at various depth z/L_z . (b) The time-mean velocity vectors u_{px} measured at $y/L_y = 0.6$ of x'' - z'' plane at various depth z/L_z . (c) The time-mean velocity vectors u_{py} measured at $y/L_y = 0.3$ of x'' - z'' plane at various depth z/L_z . (d) The time-mean velocity vectors u_{py} measured at $y/L_y = 0.6$ of x'' - z'' plane at various depth z/L_z . (e) and (f) The comparison of u_{px} and u_{py} at various depth y/L_y and z/L_z .

intensity is higher, as expected due to strong DEP force, at the y position nearer to the electrode (Fig. 7(c)).

The spatial distribution of u_{px} and u_{py} is compared in Figures 7(e) and 7(f). The y -component has lower values at deeper z/L_z . In contrast, the overall u_{px} has higher values at deeper z/L_z . This indicates the continuity of mass flow in three-dimensional spaces and supports our earlier understanding that the three-dimensional flow exists between the electrodes if analyzed from the perspective of continuity of mass flow. This is particularly important because it is hard to decipher continuity if just observed in two-dimensional plane at certain z/L_z .

In order to fully understand the particles' movement observed in the experiments, even to clarify the dominant force exerting on the particles, the theoretical discussion of the particle and fluid movement by electrokinetics is conducted next.

FIG. 8. Theoretical explanation of the experimental results of 3-D particle velocities for water and PBS. (a) Theoretical comparison of velocities due to different electrokinetic forces. (b) and (c) Qualitative analysis of particles' movement in x' - y' and x'' - z'' plane, dominant velocities are determined by the theoretical calculations in Table II as u_{TB} and u_{ET} .

D. Discussion of the velocities due to dominant electrokinetic forces

The theoretical magnitude of the velocities is summarized in Table II of Appendix, and the theoretical comparisons among different kinetic forces are drawn in Figure 8(a). The velocities due to thermal buoyancy F_{TB} and electrothermal force F_{ET} are much higher than the other two forces F_{DEP} and F_{EO} . The velocities of the fluids' and particles' movement are quantitatively drawn in Figures 8(b) and 8(c).

The particles' movement in three different directions (i.e., $+x$, $+y$, and $-y$ directions) is broadly explained in terms of four different forces. The velocity u_{TB} for PBS induced by thermal buoyancy F_{TB} directs the particles toward the opposite direction of gravity, while the velocity u_{TB} is quite small even negligible for water due to the low conductivity σ_w . Hence, this force contributes to the $+x$ component of the velocities in our case. Hence, the remaining flow toward the electrode ($-y$ direction) is due to u_{ET} induced by electrothermal force F_{ET} . For example, if the thermal buoyancy F_{TB} is the sole cause of the particles' movement in the $+x$ directions, the x -component velocity should have gradually increased or be at least same as x position increases from $x/L_x = 0.0$ to $x/L_x = 1.0$. However, the discontinuity in the pattern of the x -component near $x/L_x = 0.5$ reveals the particles' movement towards the y direction and z direction. This movement is possible from u_{TB} and u_{ET} , comparatively, which are too small in low conductive fluid as in Figure 5. Furthermore, the velocity vortex appears at the two edges of the electrode due to u_{EO} induced by the electroosmotic force F_{EO} . Near the electrode ($r < 25 \mu\text{m}$), the velocity is higher than other places, because of the higher voltage and the effect of u_{DEP} induced by the DEP force F_{DEP} in the vicinity of the electrode. In this way, the velocity vector patterns show characteristic three dimensional particles' movement structure between the pair of the electrodes in PBS. These particles' movement structure is possible by the combination of u_{DEP} , u_{TB} , u_{ET} , and u_{EO} . These characteristics of 3-D particles' movement from the experiments suggest that the 3-DIMES with PBS as high conductive fluid helps to understand the three-dimensional flow structures for cell manipulation in biomedical applications.

IV. CONCLUSIONS

A 3-DIMES has been developed in order to understand the particles' movement in the microchannel using electrokinetic forces. The main results are concluded as follows.

- (1) A 3-DIMES was successfully fabricated with MEMS technique which has three inlets, three outlets, and a microchannel with integrated multilayered electrodes. The microchannel has 5 square cross-sections, each cross-section has 8 electrodes with 4 electrodes embedded in the 2 opposite sides of the cross-section and the other 2 opposite sides realize the optical transparency. This 3-DIMES is applicable to study the particles' movement with AC electrokinetics and PIV technique.
- (2) The velocities of particles' movement in the 3-DIMES were measured 3 dimensionally at various depths by axially translating the objective lens with PIV technique. The particles' movement due to AC electrokinetics forces is fluid conductivity-dependent which is quite small when the fluid conductivity is low, and vice versa.
- (3) The particle velocities induced by electrokinetics forces, which are DEP force, thermal buoyancy, electrothermal force, and electroosmotic force, were discussed theoretically from a point view of magnitude and directions. The thermal buoyancy and the electrothermal force are found as the dominant forces exerting on the particles in the 3-DIMES from the theoretical calculations for high conductive fluid.
- (4) The characteristics of the particles' movement from the experimental results were explained with the theoretical calculation results. The velocity towards to the electrode along the opposite gravity is much higher than those along other directions due to the dominant forces which are thermal buoyancy opposite the gravity and electrothermal force toward to the electrode. The velocity vortex is formed at the 2 edges of the electrodes because of the electroosmotic

force. The DEP force becomes quite small even negligible when the distance is more than $25\text{ }\mu\text{m}$.

The 3-DIMES has great ability to generate systematic three dimensional flow structures using the switching combination of the electrodes to control the cells or the particles in the microchannel and offers effective microchannel/electrode arrangement to study the complex nature of the particle/cell manipulation in microfluidics. Though further investigation of various forces acting together is required, the developed system is an effective platform to shed the light on the study of the various phenomena of the particles' movement under AC electric field in the microchannel. This ultimately results to the effective and high throughput cell manipulation technology for biomedical applications.

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NOMENCLATURE

c	specific heat capacitance [J/(kg K)]
d	particle diameter [m]
E	electric field strength [V/m]
g	gravity acceleration [kg m/s^2]
k	thermal conductivity [W/(m K)]
r	distance from electrode [m]
u	velocity [m/s]

Greek symbols

β	coefficient of thermal-expansion [1/K]
ε	permittivity [F/m]
ε_0	vacuum permittivity [F/m]
ζ	zeta potential [V]
μ	viscosity [Pa s]
ρ	density [kg/m^3]
σ	electric conductivity [S/m]
Φ	effective potential on electrodes [V]
ω	angular frequency [rad/s]
V	applied voltage [V]

Subscripts

DEP	dielectrophoresis
e	electric charge
EO	electroosmosis
ET	electrothermal flow
f	fluid
m	PBS
p	particle
RMS	value of root mean square
TB	thermal buoyancy
w	water

TABLE II. Summary of the velocities due to different electrokinetic forces in the fluids of water and PBS.

Electrokinetic forces	Magnitude estimation of velocity	Calculation coefficient α	Direction
Dielectrophoretic force (F_{DEP})	$u_{\text{DEP}} = \alpha r^{-3}$	Water: 3.02×10^{-21} PBS: 1.16×10^{-21}	Negative DEP force, the particles are driven away from the electrode
Thermal buoyancy (F_{TB})	$u_{\text{TB}} = \alpha r^{0.5}$	Water: 3.46×10^{-5} PBS: 6.0×10^{-3}	The fluid is driven away from the electrode to the opposite gravity
Electrothermal force (F_{ET})	$u_{\text{ET}} = \alpha r^{-1}$	Water: 4.02×10^{-9} PBS: 3.28×10^{-9}	The fluid is driven toward the electrode
Electroosmotic force (F_{EO})	$u_{\text{EO}} = \alpha r^{-1}$	Water: 3.6×10^{-11} PBS: 7.8×10^{-12}	The velocity vortex appears at the two edges of the electrode

APPENDIX: CALCULATION OF VELOCITIES DUE TO ELECTROKINETIC FORCES

The particles' movement in the 3-DIMES is determined by the four electrokinetic forces which are DEP force (F_{DEP}), thermal buoyancy F_{TB} , electrothermal force F_{ET} , and electroosmotic force F_{EO} , corresponding to 4 velocities which are the particle velocity induced by DEP force u_{DEP} , the fluid velocity induced by thermal buoyancy u_{TB} , the fluid velocity induced by electrothermal force u_{ET} , and the fluid velocity induced by electroosmotic force u_{EO} , as shown in Figure 9. O' is the centre point of the 2 electrodes in y - z plane, r is the distance away from O' . The direction along r from O' is defined as plus. Because the total force F_{Total} exerting on the particle includes F_{DEP} and the Stokes' forces due to the fluid flow velocity (u_{TB} , u_{ET} , u_{EO} , and u_p), F_{Total} is expressed by

$$F_{\text{Total}} = F_{\text{DEP}} + 3\pi\mu d_p(u_{\text{TB}} + u_{\text{ET}} + u_{\text{EO}}) - 3\pi\mu d_p u_p. \quad (\text{A1})$$

The theoretical analysis of these 4 velocities is conducted later.

1. Particle velocity u_{DEP} induced by DEP force F_{DEP}

The particle velocity u_{DEP} induced by DEP force F_{DEP} is calculated by assuming that the particle only experiences DEP force in a laminar fluid flow with low Reynolds number $\text{Re} < 1$.³² The direction of F_{DEP} is determined by the positive or negative value of $\text{Re}[K(\omega)]$.¹² Considering that $|E_{\text{RMS}}| = \Phi_{\text{RMS}}/\pi r$ with the effective potential between the two electrodes and particle distance r from the center O' of the two electrodes' surfaces as described in Figure 9, the magnitude of the particle velocity u_{DEP} is given by

FIG. 9. Particle and fluid movement by electrokinetics under AC electric field, corresponding to 4 kinds of velocities.

$$u_{DEP} = \frac{d_p^2 \epsilon_f \text{Re}[K(\omega)] \Phi_{\text{RMS}}^2}{12\pi^2 \mu r^3}. \quad (\text{A2})$$

Because a large voltage drop from the applied voltage V to the effective potential Φ between the two electrodes, $\Phi_{\text{RMS}} = 0.35$ V is estimated assuming that the particles only experience DEP force in the experimental results in Figure 5. After using the data in Table I, the magnitude of particle velocity in the fluids from the experimental conditions is calculated. Negative F_{DEP} exerting on the particles in both fluids which means the particles are driven away from the electrode or toward the regions of low electric field strength due to F_{DEP} under non-uniform electric field. The magnitude of the particle velocity u_{DEP} is inversely proportional to the third power of the distance r from the electrode center O' .

2. Fluid velocity u_{TB} due to thermal buoyancy F_{TB}

The fluid velocity u_{TB} induced by thermal buoyancy force is governed by Navier-Stokes equation combining with the buoyant effect generated from the local fluid temperature change δT .³³ The fluid velocity u_{TB} is expressed in Navier-Stokes equation under the assumption of neglecting the pressure gradient term and the viscosity term, considering the convection term

$$(\mathbf{u}_{\text{TB}} \cdot \nabla) \mathbf{u}_{\text{TB}} = -\beta \delta T \mathbf{g}. \quad (\text{A3})$$

From the left term of Equation (A3), the magnitude of the velocity u_{TB} is approximately estimated considering the vertical distance r in Figure 9.³⁴ Under the experimental condition, using the data in Table I, the velocity induced by thermal buoyancy from Eq. (A3) is calculated.

3. Fluid velocity u_{ET} induced by electrothermal force F_{ET}

The fluid velocity u_{ET} induced by electrothermal force is governed by Navier-Stokes equation considering the electrothermal force per volume F_{ET} caused by the changes of conductivity and permittivity of the fluid under an inhomogeneous electric field E (Ref. 35)

$$\rho_f (\mathbf{u}_{\text{ET}} \cdot \nabla) \mathbf{u}_{\text{ET}} - \mu \nabla^2 \mathbf{u}_{\text{ET}} = F_{\text{ET}}. \quad (\text{A4})$$

F_{ET} consisting of Coulomb force and dielectric force of the fluid.^{33,36} In consequence, the velocity induced by electrothermal force is calculated as in Table II.

4. Fluid velocity u_{EO} induced by electroosmotic force F_{EO}

The fluid velocity u_{EO} induced by electroosmotic force F_{EO} is generated in the EDL near the wall of the 3-DIMES, which is governed by the balance between the Coulomb force and the viscosity force in the fluid after assuming that the unsteady term, the convection term, and the pressure gradient of incompressible Navier-Stokes equation are neglected because of quite low Reynolds number of u_{EO} in the 3-DIMES, Helmholtz-Smoluchowski equation is obtained as

$$u_{\text{EO}} = \frac{\epsilon_f E \Phi}{\mu}. \quad (\text{A5})$$

Using the data in Table I, the velocity is calculated. Since the direction of the AC electroosmotic force is independent of the electric field direction, the AC electroosmotic results in a unidirectional flow. The magnitude of the u_{EO} is quite small comparing to other velocities. However, the velocity vortex is predicted to appear at the two edges of the electrode under the present experimental conditions based on the experimental results of this paper and other researchers, such as Liu *et al.* (2015).¹⁷

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