Dielectrophoretic manipulation of particles in a modified microfluidic H filter with multi-insulating blocks

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The conventional microfluidic H filter is modified with multi-insulating blocks to achieve a flow-through manipulation and separation of microparticles. The device transports particles by exploiting electro-osmosis and electrophoresis, and manipulates particles by utilizing dielectrophoresis (DEP). Polydimethylsiloxane (PDMS) blocks fabricated in the main channel of the PDMS H filter induce a nonuniform electric field, which exerts a negative DEP force on the particles. The use of multi-insulating blocks not only enhances the DEP force generated, but it also increases the controllability of the motion of the particles, facilitating their manipulation and separation. Experiments were conducted to demonstrate the controlled flow direction of particles by adjusting the applied voltages and the separation of particles by size under two different input conditions, namely (i) a dc electric field mode and (ii) a combined ac and dc field mode. Numerical simulations elucidate the electrokinetic and hydrodynamic forces acting on a particle, with theoretically predicted particle trajectories in good agreement with those observed experimentally. In addition, the flow field was obtained experimentally with fluorescent tracer particles using the microparticle image velocimetry (μ -PIV) technique. © 2008 American Institute of Physics. [DOI: [10.1063/1.2973661](https://doi.org/10.1063/1.2973661)]

I. INTRODUCTION

With the advancement of microfabrication technologies, recent developments in biological and chemical analyses seek to integrate the complicated process of separation and sorting of particles and cells onto a chip, leading to the well-known lab-on-a-chip (LOC) technology.¹ Numerous particle manipulation and separation techniques have been developed, such as flow field fractionation (FFF), optical tweezers, fluorescence activated cell sorter (FACS), electrophoresis (EP), and dielectrophoresis (DEP). While optical tweezers² and FACS³ require sophisticated peripheral equipment and/or modification on the surface of the samples for separation, FFF^{4,5} frequently encounters difficulties associated with the overlapping zone of particle samples due to band broadening. In addition, as separation using EP is promising only for charged particles and molecules such as proteins and DNA in a uniform electric field, DEP, originally termed by Pohl,^{6,7} has been widely used for manipulating and separating biological cells such as live/dead yeast cells,⁸ bovine red blood cells,⁹ bacteria,¹⁰ and DNA¹¹ in a nonuniform electric field.

The DEP-based techniques widely used in microscaled devices may be classified as the conventional DEP (cDEP),¹² traveling wave DEP (twDEP),^{13,14} moving dielectrophoresis (mDEP),⁸ and insulator-based DEP (iDEP).^{15–17} DEP employs a spatially nonuniform electric field to induce a dipole in a particle, resulting in the unbalanced forces on each pole. Thus, the net effect on the dipole gives rise to a dielectrophoretic force.¹² As cDEP utilizes metallic electrodes with ac voltages to manipulate the particles of different types toward either the high or low electric field

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FIG. 1. (a) Structure and dimensions of the modified H filter, (b) SEM image, and (c) the modified H-filter chip.

region, and uses a pressure-driven flow to elude the target particles, the flow-through separation of both types of particles is difficult to achieve.¹³ Although twDEP offers continuous separation of particles, it requires metallic electrodes and a multichannel voltage power supply with phase-adjusting functions.¹⁴ mDEP could separate particles for a stationary fluid with a single channel voltage supplied, however, the requirement of metallic electrodes remained.⁸ In contrast, by placing external electrodes into reservoirs, iDEP is able to separate particles in a simple channel with insulating posts. Using the iDEP concept, Lapizco-Encinas *et al.*¹⁵ achieved separation of two species of living bacteria in water, but their device could not provide flow-through separation of these species. As a result, the collection of the target particles is not efficient. Kang *et al.* fabricated a kernel block inside a microchannel for separating polystyrene particles¹⁶ and red blood cells¹⁷ by size. However, the device requires a multichannel dc voltage power supply.

The commercially available microfluidic H filter offers continuous extraction and separation of analytes using mass diffusion as a driving force and its platform also facilitates easy collection of analytes.^{18,19} However, the separation function of this device can suffer low efficiency and even fail, especially for large sized particles such as cells whose mass diffusivity is very low and the difference of mass diffusivities between cells is not significant.

We report here a modified polydimethylsiloxane (PDMS)-based H-filter platform with multi-insulating blocks. Manipulation of flowing particles and size-based continuous separation of particles are demonstrated using such a modified H-filter device operated by only a single channel dc power supply. Specifically, we show that the use of multi-insulating blocks can enhance the dielectrophoretic force effect on the particles, which not only can improve the manipulation and separation of particles but also can confine the particles in a small region so as to facilitate the necessary functions of the microfluidic device. Subsequently, by employing a single power supply that can simultaneously produce both ac and dc electric field, we demonstrate that while the particles can be transported by the dc voltage, the combined ac and dc electric field allows for the controlled manipulation of the particles. This provides yet another parameter, namely the ac voltage, for controlled manipulation and separation of particles. More importantly, this leads to a reduction of the threshold dc voltage for directing and separating particles in the H-filter device.

II. MATERIALS AND METHODS

Figure 1(a) depicts the structure and dimensions (in millimeters) of the modified H filter. All channels have a depth of 60 μm . The patterned PDMS microfluidic channels were fabricated using the standard photo- and soft-lithography techniques,²⁰ and Fig. 1(b) shows an image of the fabricated H-filter channels by scanning electron microscopy (SEM). After four reservoirs were punctured for inlets and outlets, the patterned PDMS and a glass slide were irreversibly bonded through an oxygen plasma treatment. A photograph of the entire device is shown in Fig. 1(c). As reported in the literature, the hydrophobicity and surface charges of the PDMS channel walls changed with time (in terms of hours). The bonded channel was left overnight to obtain stable

operation conditions,²¹ such that the PDMS walls exhibited hydrophobic tendency and a decrease in the zeta potential.²¹ As a result, during the operation of the device, the particle EP is stronger than the fluid electro-osmotic force (EOF).

Latex polystyrene microspheres (Duke Scientific, USA) of 5, 10, and 15 μm in diameter were selected in our experiments, as their sizes are comparable to biological cells such as yeast and red/white cells. The number concentration of particles in deionized (DI) water purchased from the supplier was approximately $10^7/\text{ml}$. Prior to experiment, the particle dispersion was diluted in a 10^{-3} M NaHCO_3 buffer with a volumetric ratio of 1:10. This particle-buffer mixture was introduced at inlet C [see Fig. 1(a)] while other reservoirs were filled with the buffer solution only. After electrodes were placed in the four reservoirs (but before the application of an electric field), the buffer level in each reservoir was balanced using a 50 μl precision syringe until no noticeable particle motion was observed under a microscope. The device was operated under various voltages produced by either one of the two different power sources: (i) In the dc electric field mode operation, a dc power supply (Stanford Research, USA); and (ii) in the combined ac and dc field mode operation, a function generator (Agilent 33250A, USA) was connected with a customized high voltage amplifier (Optrobo, Singapore), with the output signal monitored by an oscilloscope (CombiScope[®] HM 1008, USA). Particle motion was captured at a rate of 15 frames per second (fps) through a charge-coupled device (CCD) camera (Sensovation, Germany, image resolution of 696×520 pixels) attached to a microscope (Zeiss, Germany) under a $5\times$ objective lens. Since an image contains many particles, only one representative particle was selected for analysis, and its trajectory was constructed using image processing software (ImagePro[®] Plus).

In addition, the microparticle image velocimetry ($\mu\text{-PIV}$) technique was used to measure the velocity of the 0.93 μm fluorescent tracer particles (Duke Scientific). The fluorescent particles have a maximum excitation wavelength of 540 nm and a maximum emission wavelength of 610 nm. The original concentration 2×10^9 particle/ml in DI water was diluted with 10^{-3} M NaHCO_3 buffer solution in a volumetric ratio of 1:10. A mercury lamp (mbq 52 ac, Zeiss) connected to the fluorescence microscope (Zeiss) with an appropriated filter set (excitation of 546 nm and emission greater than 590 nm) was used for fluorescence excitation to illuminate the fluorescent tracer particles. Two sequentially captured images were then evaluated with the PIVview software (Piv-Tec GmbH) to obtain the velocity field of the tracer particles.

III. MODELING OF PARTICLE'S MOTION

In the modified PDMS microfluidic H filter, the motion of a charged particle in an electrolyte solution subjected to an externally applied electric field can be approximated to a two-dimensional model. The following assumptions are made: (i) Sedimentation due to particle gravity is negligible as the density of particle (1.05 g/cm^3) is close to that of the solution, (ii) the zeta potential values of particles and channel walls are constant, (iii) the electrical and thermophysical properties (such as electrical permittivity and viscosity) of the buffer are constant due to negligible Joule heating effect in a PDMS/glass channel filled by low concentration (1 mM) NaHCO_3 ,²² (iv) the electric field distribution in the modified H filter is two-dimensional because both top and bottom wall are electrically insulating, and (v) the coupled particle-channel interactions are not included.²³ Therefore, the equation of the particles trajectory can be described by Newton's second law in two dimensions,

$$\sum \vec{F} = m_p \frac{d^2 \vec{r}}{dt^2}, \quad (1)$$

where the position of a particles center is denoted by a vector $\vec{r} = x_p \vec{i} + y_p \vec{j}$, $m_p = (4/3)\pi a^3 \rho_p$ is the particle's mass, a and ρ_p are the particle's radius and density, respectively, and $\sum \vec{F}$ represents the net external forces exerting on the charged particle. By applying a voltage, an electric field over the whole channel can be generated as schematically illustrated in Fig. 2(a). Near the insulating block's corner, the physical forces exerting on a charged particle are attributed to electrophoresis (EP), electro-osmosis (EO), hydrodynamics, and DEP as illustrated in Fig. 2(b). It is further

FIG. 2. Schematic diagrams of (a) electric field lines in the modified H filter and (b) physical forces on a particle passing a block's corner.

assumed that these forces on a particle can be decoupled.²⁴ Since the walls and particle are usually negatively charged, the direction of the EOF and EP forces on the particle is opposite. Figure 2(b) shows the situation where EP is stronger than EOF, resulting in particle transport against EOF toward the positive electrode.

A. Electrophoretic force

Under the assumptions of a thin electric double layer (EDL) of particles and no retardation effect, the electrophoretic force acting on a charged particle is given by²⁴

$$\vec{F}_{EP} = 6\pi a \epsilon_m \zeta_p \vec{E}, \quad (2)$$

where ϵ_m is the electrical permittivity of the buffer. In the absence of EOF, the negatively charged particle with a zeta potential ζ_p under an electric field $\vec{E}$ is induced toward the positive electrode.

B. Hydrodynamic and electro-osmotic force

As a charged particle travels in the microfluidic H filter with a velocity of $d\vec{r}_p/dt$, it experiences hydrodynamic drag that retards its motion according to Stokes' law, expressed as²⁴

$$\vec{F}_{drag} = -6\pi\eta a \left(\frac{d\vec{r}_p}{dt} - \vec{U}_{EOF} \right). \quad (3)$$

With the assumption of a thin EDL of the channel walls, the EOF velocity can be estimated from Smoluchowski's equation, $\vec{U}_{EOF} = -(\epsilon_m \zeta_w / \eta) \vec{E}$, where η is the dynamic viscosity of the buffer, and ζ_w is the zeta potential of the channel wall.

C. Dielectrophoretic force

When a particle is suspended in a dielectric medium under an applied spatially nonuniform electric field, both the particle and medium are polarized, and a dipole on the particle is induced. Each pole undergoes unequal electric forces resulting in a net (or DEP) force on the particle. Using the dipole moment method, the time-average DEP force exerting on a spherical particle is given by²⁵

$$\langle \vec{F}_{DEP} \rangle = \pi \epsilon_m a^3 \text{Re}[\underline{K}(\omega)] \nabla |\vec{E}|^2, \quad (4)$$

where $\langle \rangle$ denotes the time average, and $\underline{K}(\omega) = (\epsilon_p - \epsilon_m) / (\epsilon_p + 2\epsilon_m)$ is known as the Clausius-Mossotti (CM) factor; $\epsilon = \epsilon' - i\sigma/\omega$ is a complex permittivity, where ϵ' is a permittivity, σ is an electrical conductivity, $i = \sqrt{-1}$, and $\omega = 2\pi f$ is the angular frequency. $\text{Re}[\]$ denotes the real part of the CM factor ranging from -0.5 to $+1$. When the particle is more polarized than the suspending

TABLE I. Boundary conditions for the Laplace equation and the Navier-Stokes equation.

Governing equation	Boundary conditions		
Laplace	Along the walls $\vec{E} \cdot \vec{n} = 0$	Inlet <i>C</i> and <i>D</i> $\phi = 0$	Outlet <i>A</i> and <i>B</i> $\phi = V_{DC}$ or $\phi = V_{DC} + V_0 \sin(\omega t)$
Navier Stokes	$\vec{u} \cdot \vec{t} = (\epsilon_m \zeta_w / \eta) \vec{E} \cdot \vec{t}$		$P = 0$

medium, $\text{Re}[\underline{K}(\omega)]$ is positive and the positive DEP will direct the particle toward the region of high electric field. If the particle is less polarized than the medium, $\text{Re}[\underline{K}(\omega)]$ becomes negative and the negative DEP force will repel the particle to the region of low electric field. Since the channel walls in the modified H filter are insulating, the total current is conserved in any cross-sectional area of the channel. When the area is considerably reduced at the blocks' gap, the electric field becomes high. The maximum gradient of the electric field occurs at the blocks' corners, yielding the greatest DEP force. Specifically, under operation of the dc field mode where $f=0$, the CM factor reduces to $\underline{K}(\omega) = (\sigma_p - \sigma_m) / (\sigma_p + 2\sigma_m)$. For an electrically insulating particle whose conductivity is negligible compared to the buffer's conductivity, the CM factor becomes $-\frac{1}{2}$. Therefore, the particle experiences a negative DEP force that repels the particle away from the blocks' corners. The DEP force in Eq. (4) becomes

$$\langle \vec{F}_{\text{DEP}} \rangle = -\frac{1}{2} \pi \epsilon_m a^3 \nabla |\vec{E}|^2. \quad (5)$$

Substituting the expressions of the EP, hydrodynamic, EOF, and DEP forces into Eq. (1), we obtain the equation governing the particle's trajectory as

$$6\pi\eta a \vec{U}_{\text{EOF}} + 6\pi a \epsilon_m \zeta_p \vec{E} + (-1/2) \pi \epsilon_m a^3 \nabla |\vec{E}|^2 - 6\pi\eta a \frac{d\vec{r}_p}{dt} = m_p \frac{d^2 \vec{r}_p}{dt^2}. \quad (6)$$

To solve Eq. (6), the electric and EOF velocity fields are required. The typical thickness of an EDL is in the range of 1 to 100 nm; while the characteristic hydraulic diameter of microfluidic channels is of order 10 to 100 μm . With such a large difference, the electrical potential distribution and the EOF velocity profile within the EDL region become insignificant. Thus, the electrical potential distribution can be governed by Laplace's equation,²⁴ expressed as

$$\nabla^2 \phi = 0. \quad (7)$$

Furthermore, under such a thin EDL assumption, the EOF can be considered to be induced by a moving wall with a slip velocity, and thus the EOF velocity field is governed by the Navier-Stokes equation and the continuity equation, respectively expressed as

$$\rho \left(\frac{\partial \vec{u}}{\partial t} + \vec{u} \cdot \nabla \vec{u} \right) = -\nabla p + \eta \nabla^2 \vec{u}, \quad (8a)$$

$$\nabla \cdot \vec{u} = 0 \quad (8b)$$

The boundary conditions for Eqs. (7), (8a), and (8b) are summarized in Table I. For Laplace's equation, the electric field normal to the wall must be zero since all channel walls are insulating. The symbol $\vec{n}$ is the unit vector whose direction is perpendicular to the insulating walls. To transport the negatively charged particle from inlet C to either outlet A or B, a voltage of V_{DC} is applied at outlets A and B while inlets C and D are electrically grounded. In the combined ac and dc field mode, V_0 is the ac output voltage and V_{dc} is the dc component. As for the Navier-Stokes equation, all channel walls are set to have a slip velocity according to the Smoluchowski equation. The unit vector $\vec{t}$ is tangential to the insulating walls. In the electrokinetic flow, there is no external

FIG. 3. Effect of voltage on experimental trajectories for (a) the 10 μm particle and (b) the 15 μm particle. Comparison of the simulation and experimental results of (c) the 10 μm particle and (d) the 15 μm particle.

pressure applied at any reservoirs. With the stated boundary conditions, the electric and velocity fields were numerically solved using COMSOL MultiphysicsTM. A grid-independent study was also performed.

IV. RESULTS AND DISCUSSION

In the simulations, the zeta potential of hydrophobic PDMS walls at room temperature was set to -23.5 mV.²¹ The zeta potential values of the 5, 10, and 15 μm particles suspended in 1 mM NaHCO_3 buffer solution were chosen as -55.1 , -71.6 , and -93.1 mV, respectively. The buffer solution's density, dynamic viscosity, and electrical permittivity were 1000 kg m^{-3} , 9×10^{-4} kg m^{-1} s $^{-1}$ and 6.9×10^{-10} C V $^{-1}$ m $^{-1}$, respectively.

A. Effect of applied voltage

Experimental trajectories of the 10 and 15 μm particles under the effect of various applied dc voltages ranging from 60 to 140 V are presented in Figs. 3(a) and 3(b). Starting from arbitrary locations, when passing a block the particles were pushed away from the block where the electric field was high and thus the DEP force was large, and finally the particles were found to be confined area in a small region of the gap between the block and the channel wall. Depending on the applied voltage, the particles can be directed into either the upper or lower reservoir.

According to Eq. (6), the EP and EOF forces are linearly proportional to the electric field strength $\vec{E}$. However, the magnitude of the DEP force depends on the gradient of the square of electric field, $\nabla|\vec{E}|^2$. An increase of applied voltage causes the particle to move faster, and more importantly results in a greater repulsive DEP force that pushes the particle away from the block. Hence, the particles trajectory can be shifted into the upper reservoir once the applied voltage reaches a threshold voltage V_{Th} . As shown in Figs. 3(a) and 3(b), under the experimental

FIG. 4. Experimental results of separation achieved by 85 V for (a) the 5 and 15 μm particles and (b) the 10 and 15 μm particles. Comparison of the simulation and experimental results for (c) the 5 and 15 μm particles and (d) the 10 and 15 μm particles.

conditions reported in this study, the threshold voltages V_{Th} for the 10 and 15 μm particles were 120 and 85 V, respectively. For voltages above V_{Th} , the particles were transported towards the upper reservoir. As suggested by Eq. (5), the 15 μm particle required a lower threshold voltage since it acquired larger DEP force compared to the 10 μm particle under the same applied voltage.

Figures 3(c) and 3(d) show reasonable agreement between experimental (dotted lines) and simulated (solid lines) results. In the simulations, the initial locations of the particles were selected to be the same as those determined from the experiments.

B. Size-dependent separation of particles

According to Eq. (4), the DEP force is proportional to the cubic power of a particles radius. Particles of different sizes will thus experience a significant difference in the DEP force. Such mechanism was utilized in the modified H filter to perform the separation of particles by size, which may have a potential in separating cells of different sizes for biomedical applications. For demonstration purposes, a mixture of two sized particles (e.g., 5 and 15 μm) was placed in the reservoir C of the H filter while an equal dc voltage was imposed to the two reservoirs C and D. Making use of the experimental findings presented in Figs. 3(a) and 3(b), 85 V was applied. Such a voltage reaches the threshold voltage of the 15 μm particles, but is still below the threshold voltage of the 5 μm particles. As shown in Fig. 4(a), the 15 μm particles were successfully separated from the 5 μm particles. Another set of experiments was also conducted in the modified H filter to separate the 15 μm particles from the 10 μm particles, and the separation was achieved using the same voltage [see Fig. 4(b)]. These experimental observations are in good agreement with simulations, as shown in Figs. 4(c) and 4(d).

FIG. 5. Effect of ac voltage on the trajectories of (a) the $10\ \mu\text{m}$ particle and (b) the $15\ \mu\text{m}$ particle. Comparison of simulation and experimental results of (c) the $10\ \mu\text{m}$ particle and (d) the $15\ \mu\text{m}$ particle.

C. Reduction of the threshold voltage

As demonstrated in the previous section, a rather high applied voltage is required so as to intensify the repulsive DEP force for deflecting the particle's trajectory. High voltage, however, not only may produce Joule heating effects, but also can generate bubbles due to electrolysis. Furthermore, in biomedical applications, high voltage could also potentially jeopardize those cells sensitive to high voltage. To reduce the applied voltage, the utilization of a combined dc and ac electric field is proposed. Moreover, the ac component presents an added parameter for the control of the particle motion.

Figures 5(a) and 5(b) show the experimental investigation of the $10\ \mu\text{m}$ and $15\ \mu\text{m}$ particles' trajectories under combined dc and ac (at 2 kHz) voltages, and the experimental and simulation results are compared in Figs. 5(c) and 5(d). The results indicated that the threshold voltage for the $10\ \mu\text{m}$ and $15\ \mu\text{m}$ particles was reduced. For example, the required threshold voltage for the $10\ \mu\text{m}$ particles was decreased from 120 V in the dc field mode to $90+20\sin(\omega t)$ V in the combined dc and ac field mode, and correspondingly the electric field strength at the blocks' gap is lowered from 580 to 532 V/cm. Similarly, for the $15\ \mu\text{m}$ particles, the threshold voltage was reduced from 85 V to $50+20\sin(\omega t)$ V. Experimental measurements and observations were supported by theoretical modeling. Lee *et al.* reported that the electric field strength required for cell lysis was 21 kV/cm at a frequency of 2 MHz for both yeast cells and *E. coli* in a microchannel.²⁶ However, the electric field strength in our device was only 530 V/cm through the application of $90+20\sin(\omega t)$ V. Therefore, the combined dc and ac electric field mode proposed in our technique will be useful and safe in applications of living cell separation.

Scaling analysis suggests that the characteristic response time of a charged particle to an applied electric field is of order²⁷

FIG. 6. EOF velocity field by (a) numerical simulation and (b) μ -PIV measurement.

$$\tau_{ep} = O(a^2 \rho_p / \eta). \quad (9)$$

Using $10 \mu\text{m}$ in diameter and 1050 kg/m^3 in density, we can obtain a particle electrophoretic response time of order $\tau_{ep} = O(10^{-5})\text{s}$. In the same fashion, the characteristic response time of an EOF to electric field can be estimated from²⁷

$$\tau_{eo} = O(D_h^2 / \eta). \quad (10)$$

For a typical microchannel of $100 \mu\text{m}$ in hydraulic diameter D_h , we can find the EOF relaxation time of order $\tau_{eo} = O(10^{-2})\text{s}$. Since 2 kHz was used in the experiment, the particle EP should be able to follow the applied ac field component but not the liquid EOF. In other words, the ac component of the EOF is suppressed. However, as our CCD camera could only record images at

a rate of 15 fps, a steady particle motion still was observed during the experiment, but a change in particle velocity due to ac electric field was noted.

D. Further discussion

The experimentally observed particle trajectories were in reasonably good agreement with the theoretical predictions. However, in the simulation, the zeta potential values of the 5, 10, and 15 μm particles suspended in 1 mM NaHCO_3 buffer solution were chosen as -55.1 , -71.6 , and -93.1 mV, respectively. Obviously, such dependency of the particle zeta potential on size does not comply with the conventional electrokinetic theory. Hence, the particle zeta potential can only be considered as a fitting parameter in the model prediction. It should be pointed out that several factors were not included in the model presented in this study, and they include (i) pressure head built up due to unequal solution levels at the reservoirs, (ii) hydrodynamic and electrokinetic interactions between the particle and the channel wall, (iii) heterogeneous electrokinetic effects such as discrete surface charges and nonuniform zeta potential, and (iv) roughness and inclined edges of the channel walls. Therefore, further improvement of the theoretical model is required in our future studies.

E. Electro-osmotic flow field

Subjected to the boundary conditions (Table I), the numerically simulated EOF velocity field induced by 50 V performed using COMSOL MultiphysicsTM is shown in Fig. 6(a). Since outlets A and B are assigned with a higher voltage than inlets C and D, two EOF streams transport from outlets to inlets. The EOF velocity profiles at channels linked from outlets A and B exhibit plug-like flow but are distorted at the T junction. The EOF velocities satisfying the conservation of mass are increased when passing the blocks' gaps.

In addition, the velocity measurement of the 0.93 μm fluorescent tracer particles subjected to 50 V was performed using the μ -PIV technique, and the μ -PIV results are presented in Fig. 6(b). A qualitative comparison between simulations and the experimental results indicates reasonable agreement in the EOF flow pattern. The μ -PIV measurement showed that the tracer particles moved along the same direction of the EOF streams. This implies that the tracer particles have a lower electrical zeta potential compared to the channel walls. As both the channel walls and the particles were negatively charged, EOF was opposite to EP. Therefore, the magnitude of the velocity field obtained from the μ -PIV measurement was smaller than that from the simulation. In addition, the tracer particles' DEP was not considered in the simulation, and it also contributed to the discrepancy. However, due to limitation of the frame speed of our CCD camera, the μ -PIV measurement of the EOF field was not performed for the combined dc and ac field mode.²⁷

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