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AC-dielectrophoretic characterization and separation of submicron and micron particles using sidewall AgPDMS electrodes

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AC-dielectrophoretic characterization and separation of submicron and micron particles using sidewall AgPDMS electrodes

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The recent development of microfluidic “lab on a chip” devices requires the need to continuously separate submicron particles. Here, we present a PDMS microfluidic device with sidewall conducting PDMS (AgPDMS) composite electrodes capable of separating submicron particles in hydrodynamic flow. In particular, the device can service dual functions. First, the AgPDMS composite electrodes embedded in a sidewall of the device channel allow for performing AC-dielectrophoresis (DEP) characterization through direct microscopic observation of particle behavior. Characterization experiments are carried out for numerous parameters including particle size, medium conductivity, and AC field frequency to reveal important dielectrophoretic DEP information in terms of the crossover frequency and positive/negative DEP behavior under specific frequencies. Second, the device offers an advantage that sidewall AgPDMS composite electrodes can produce strong DEP effects throughout the entire channel height, and thus the robustness of the on-chip particle separation is demonstrated for continuous separation in a flowing mixture of 0.5 and 5 μm particles with 100% separation efficiency.


I. INTRODUCTION

In clinical and biotechnological applications, most biosamples are complex because they contain various bio-components such as red and white blood cells, platelets, bacteria, and virus. These biosamples largely vary from micrometer to sub-micrometer in size and usually possess different electrical properties (e.g., conductivity and permittivity). These electrical properties can lead to their different polarizabilities under an externally applied electric field. In biomicrofluidic technologies, dielectrophoresis (DEP) has become one of the promising techniques for characterizing and separating particles and cells based on their polarizability interacting with a spatially nonuniform electric field. When a particle is more polarizable than its suspending medium, a positive DEP (or pDEP) force attracts the particle to high electric field region. When a particle is less polarizable than its suspending medium, a negative (or nDEP) force repels the particle away from high electric field region.

Conventionally, DEP techniques usually utilize planar microelectrodes to induce electric field gradients which produce DEP force acting on samples. Krishnan et al. developed a planar microelectrode array coated with porous hydrogel layers for separating 60-nm DNA-derivatized nanoparticles (or 0.2 μm submicron particles) from 10 μm microparticles based on their different polarizabilities in stagnant physiological media with a high conductivity of 0.109-1.68 S/m. Similar studies provided more evidence that even in medium-to-high conductivity solutions, microparticles and cells tend to experience nDEP whereas nanoparticles and DNA still exhibit pDEP. This suggests a potential mechanism of separating...
submicron and micron particles based on their different polarizabilities in a physiological medium.

However, it is well known that the use of planar microelectrodes has a major drawback: the exponential decay of electric field with a distance from the electrode surface. As a result, the samples far from the electrode surface, especially submicron particles at the middle height of the channel, experience weak DEP force, and thus cannot be manipulated efficiently. This rationale could generally explain why the work presented by Krishnan et al. was not carried out in a continuous manner and the process usually required about 20 min to complete. To address this issue, several DEP devices have been reported to generate three-dimensional (3D) electric field by using 3D or sidewall metallic electrodes such as double planar electrodes, heavy doped silicon, pyrolyzed SU-8 photoresist, and electroplated gold. Nevertheless, because their fabrication materials are mainly glass and silicon, liquid leakage is a problem, leading to complicated device assemblies.

We have recently developed a PDMS-based AC-DEP device with conducting PDMS composites as sidewall electrodes for sorting and continuous separation of micro-sized particles (ranging from 5 \(\mu\)m to 15 \(\mu\)m in diameter) by size using repulsive nDEP force. The conducting PDMS composites made by mixing silver (Ag) particle powder with pure PDMS gel can be seamlessly integrated along the sidewall of PDMS microchannels, thus completely avoiding liquid leakage. Unlike the planar electrodes, the conducting PDMS (or AgPDMS) electrodes embedded in a channel sidewall allows for producing uniform electric field throughout the entire channel height and simultaneously generating electric field gradients along the lateral direction of the channel. As such, the device not only yields prominent lateral DEP forces over the separation channel for effective separation but also allows for straightforwardly visualizing particle motion, thereby for DEP characterization. However, as DEP force is scaled to the cubic power of particle size, the magnitude of DEP force is significantly reduced for dealing with submicron particles in a complex fluid.

To enhance the efficiency in separation, this paper reports continuous separation of submicron and micron-sized particles based on their different polarizabilities in a PDMS microfluidic device using 3D AC-DEP generated by sidewall AgPDMS electrodes. We first will take advantage of the fabricated sidewall electrodes to characterize different DEP behavior of 0.5 and 5 \(\mu\)m fluorescent latex particles in various concentrations of NaHCO₃ buffer solutions. Meanwhile, electric field and DEP force will be numerically computed to illustrate the device separation principle. Finally, based on the characterization results and numerical calculations, appropriate AC voltage and frequency will be chosen for the demonstration of continuous separation of 0.5 and 5 \(\mu\)m fluorescent latex particles in the same PDMS device.

II. MATERIALS AND METHODS

A. Chip design and fabrication

Fig. 1(a) shows a PDMS-fabricated microfluidic device, seamlessly integrated with AgPDMS electrodes. The microfluidic device mainly consists of a 200 \(\mu\)m wide and 1400 \(\mu\)m long separation channel (Fig. 1(b)) together with four branch channels connected to two inlets (A and B) and two outlets (C and D). All the channels have the same height of 40 \(\mu\)m. The widths of branch channels A, B, C, and D are 100, 115, 50, and 170 \(\mu\)m, respectively. Four 100 \(\mu\)m wide AgPDMS electrodes, spaced 100 \(\mu\)m apart, are fabricated along one side of the PDMS separation channel. Inlet A is fabricated for supplying particle suspensions and inlet B is for supplying buffer solution used for generating hydrodynamic focusing effect while outlets C and D are for collecting submicron and micron particles, respectively. Each reservoir at the inlets and outlets has 6 mm in diameter and 4 mm in height.

The device was fabricated using the same fabrication procedures as reported in our previous work. In brief, for fabricating PDMS microfluidic channels, a PDMS replica molding was obtained from a 40 \(\mu\)m thick SU-8 patterned silicon wafer. To achieve cavities for housing AgPDMS electrodes, a 40-45 \(\mu\)m thick AZ9260 photoresist was double-coated and patterned through an UV exposure on top of the patterned SU-8 photoresist. Conducting (AgPDMS) paste...
was synthesized by adding 1 μm Ag particles to PDMS gel at a ratio of 85% w/w, above which the paste could be too powdery. The electrical conductivity of the cured AgPDMS electrodes is about \( \frac{1}{10^4} \) S/m. The synthesized AgPDMS paste was added and cured in the patterned AZ9260 photoresist. The AZ9260 layer was then removed from the wafer using acetone, followed by ethanol and DI water, finally leaving the cured AgPDMS electrodes with the SU-8 mold. PDMS gel was poured over the SU-8 mold and AgPDMS electrodes so that the cured PDMS replica was able to form channels from the SU-8 mold and to bind with the AgPDMS electrodes. The PDMS were peeled, then punched for inlets and outlets, and finally assembled with a glass slide cover using an oxygen plasma treatment which provides an excellent approach to prevent liquid leakage. It is noted that during the UV exposure for creating the housing for AgPDMS electrodes, a slight UV overexposure happens, and thus a hemispherical \( 10^{-20} \) μm wide AgPDMS thin layer may appear on top of the PDMS microchannel as shown in Fig. 5. However, underneath this hemispherical shape, no AgPDMS electrodes can be formed because they are blocked by the patterned SU-8 mold.

B. Microfluidic device operation

The device operation employs a hydrodynamic focusing approach and both repulsive force (for micron particles) and attractive DEP force (for submicron particles) to continuously separate a binary mixture of submicron and micron particles as illustrated in Fig. 1(b). The operation begins by filling the microfluidic channels with NaHCO₃ buffer solution thoroughly through inlets or outlets. The particle mixture was injected into the reservoir of inlet A while inlet B contains a buffer solution flow stream to hydrodynamically focus the particle stream flowing from inlet A. Pressure-like suction flow (from right to left) was achieved by simultaneously removing liquid from outlets C and D. The total flow rate in the main channel determines the particle velocity and the time window in separation while the degree of particle focusing...
can be adjusted via the ratio of the flow rates in branches B and A. Since each reservoir at the inlets and outlets has 6 mm in diameter and 4 mm in height, it could provide sufficient 10-min volumetric capacity for steady suction flow. The flowing particles, focused near the sidewall AgPDMS electrodes by hydrodynamic focusing, experience strong DEP force. Micron particles (displayed as large empty dots) subject to repulsive DEP force (e.g., negative DEP or nDEP) are pushed away from the bottom electrodes and then are directed to outlet D whereas submicron particles (displayed as small solid dots) undergoing attractive DEP force (e.g., positive DEP or pDEP) are attracted to the electrode surface and thus can continue their path to outlet C.

C. Sample preparation

For DEP characterization, two sizes of fluorescent latex particle suspensions (0.5 \( \mu \)m and 5 \( \mu \)m in diameter, from Duke Scientific, USA) were prepared in various NaHCO\(_3\) buffer solutions with their electrical conductivities ranging from 11.7 to 500 \( \mu \)S/cm. 0.02 ml of 0.5 \( \mu \)m particle solution and 0.1 ml of 5 \( \mu \)m particle solution were added to 1 ml of a NaHCO\(_3\) solution. As for separation, a sample mixture was prepared by putting above-prepared 0.5 and 5 \( \mu \)m particle suspensions together, and then was homogenized with an ultrasonic bath. Prior to each separation experiment, a 5% bovine serum albumin (BSA) solution was used to coat the PDMS microchannels for \( \sim \)4 h to prevent particle adhesion, and then the PDMS channels were thoroughly washed with NaHCO\(_3\) solution to remove BSA.

D. Experimental setup

A function generator (Agilent, 33250A) connected with an amplifier (Piezo System, EPA-102) is used to generate desirable voltages, which are applied on individual AgPDMS electrode pads via four alligator clips. The AC output voltage monitored by an oscilloscope (HAMEG Instruments, HM 1008) is in the range of 20-30 V. An epifluorescence microscope (Zeiss, AxioStar Plus FL) with 5\( \times \) magnification connected to a CCD camera (Samba, EZ-140 c) is used to observe and record particle motion at 15 frames per second.

III. THEORY

DEP is referred to as the motion of a particle in a nonuniform electric field resulted from the interaction of a polarized dielectric particle in liquid solution with spatially nonuniform electric field.\(^{28}\) The direction and magnitude of DEP depend on the particle polarizability relative to the suspending medium, particle size, and the gradient of electric field. For a spherical particle of radius \( r \), the DEP force is expressed as\(^{29}\)

\[
F_{\text{DEP}} = 2\pi \varepsilon_m r^3 \text{Re}[K^*(\omega)] |\nabla|E_{rms}|^2
\]

where \( \varepsilon_m \) is the solution medium permittivity and \( |\nabla|E_{rms}|^2 \) represents the gradient of the square of the root-mean-square electric field. The Clausius-Mossotti (CM) factor \( K^*(\omega) \) is a function of the complex permittivities of the particle and the medium \( (\varepsilon_p^* \text{ and } \varepsilon_m^*) \) given by

\[
K^*(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*},
\]

where \( \varepsilon^* = \varepsilon - i\sigma/\omega \), \( \varepsilon \) is permittivity, \( \sigma \) is electrical conductivity, and \( \omega = 2\pi f \) with \( f \) representing the frequency of AC electric field. When a particle is more polarizable than the suspending medium \( (\text{Re}[K^*(\omega)] > 0) \), the particle is attracted to the high electric field region (pDEP). On the other hand, if a particle is less polarizable than the suspending medium \( (\text{Re}[K^*(\omega)] < 0) \), the particle is repelled away from the high electric field region (nDEP). At high frequencies (i.e., \( \omega \to \infty \)), the CM factor is mainly dependent on the permittivities of the particle and the medium. Conversely, at low frequencies (i.e., \( \omega \to 0 \)), the CM factor is...
dominated by the electrical conductivities of the particle and the medium. As presented in the work by O’Konski, for micron particles larger than 1 μm, the particle electrical conductivity is the sum of the particle bulk conductivity ($\sigma_{p,\text{bulk}}$) and the particle surface conductivity ($2K_s/r$), and is expressed as \[ \sigma_p = \sigma_{p,\text{bulk}} + 2K_s/r, \tag{3} \]

where $K_s$ is the surface conductance ranging from 0.2-2.1 nS. On the other hand, the electrical conductivity of smaller particles (<1 μm) is given by \[ \sigma_p = \sigma_{p,\text{bulk}} + 2K_{\text{Stern}}/r + 2K_{\text{Diff}}/r \tag{4} \]

where $K_{\text{Stern}}$ and $K_{\text{Diff}}$ are the Stern layer and diffuse layer conductance, respectively. In the limit of medium conductivity less than 1000 $\mu$S/cm, the magnitude of $K_{\text{Diff}}$ is typically two orders lower than $K_{\text{Stern}}$, and thus the conductivity of the Stern layer dominates the particle behavior. This means that Eq. (4) can be reduced to Eq. (3).

IV. RESULTS AND DISCUSSION

A. Numerical simulation of electric field and DEP force

The electric field and DEP force generated by the sidewall AgPDMS electrodes (shown in Fig. 1) are numerically simulated using the Conductive Media DC mode in COMSOL Multiphysics. In simulation, the electric potential $\phi$ in the microchannel is governed by Laplace’s equation with electric field denoted by $\mathbf{E} = \nabla \phi$. Two boundary conditions are set as follows. First, the electric field components normal to insulating walls are zero. Second, electric potentials of +10 V and −10 V are assigned at two adjacent electrodes. Furthermore, DEP force was computed using Eq. (1).

Fig. 2 shows the electric field distribution with local maxima at the edges of electrodes and local minima at the center of the electrodes and between the gap of the electrodes. Such variation in electric field strength causes highly spatial electric field gradients, thereby giving rise to strong DEP force in the vicinity of the electrodes. However, the magnitude of DEP forces rapidly decays as illustrated by the relatively shorter lengths of DEP arrows at further distance (in the y direction) from the electrodes. Thus, our present device integrates hydrodynamic focusing effects (illustrated in Fig. 1(b)) such that particle mixture stream can be confined to a region near the sidewall electrodes where strong DEP forces occur. For a particle which is less polarizable than the suspending medium, repulsive DEP force pushes it laterally from the electrodes (Fig. 2(a)). Inversely, a particle more polarizable than the suspending medium undergoes attractive DEP force and thus moves towards the electrodes (Fig. 2(b)). This is applied to all the particles no matter where they locate along the channel height (perpendicular to the x-y plane) because sidewall AgPDMS electrodes can produce uniform electric field throughout the entire channel height.

B. Characterization of AC-DEP behavior of particles

AC-DEP behavior of 0.5 and 5 μm fluorescent latex particles was characterized by investigating the effect of medium conductivity and particle size under applied AC electric field with frequencies ranging from 10 kHz to 80 MHz. The effect of medium conductivity on DEP behavior of particles was examined in two different NaHCO$_3$ solutions with their electrical conductivities as 11.7 and 500 $\mu$S/cm. Eq. (2) suggests that for a given particle size, the CM factor, determining the sign of DEP force, can be changed from positive to negative by adjusting the conductivity of suspending medium. Based on Eqs. (2) and (4), Re[$K'(\omega)$] versus electric field frequency calculated for a 0.5 μm particle (with its bulk conductivity of ~0 $\mu$S/cm, negligible $K_{\text{Diff}}$ of ~0 nS and $K_{\text{Stern}}$ of 2.1 nS) in 11.7 and 500 $\mu$S/cm NaHCO$_3$ solutions is plotted in Fig. 3(a). The permittivities of the particle and the medium used in the calculation are 2.5ε$_0$. 

\[ \text{[Ref]} \]
FIG. 2. Numerical simulation results of the electric field distributions induced by sidewall AgPDMS electrodes (shown by black segments) and DEP forces for (a) repulsive DEP of particles less polarizable than the particle suspension medium and (b) attractive DEP of particles more polarizable than the particle suspension medium. The length of arrows represents the magnitude of DEP forces.

FIG. 3. Effect of buffer solution conductivity on DEP behavior of 0.5 μm submicron particles: (a) the CM factor for 0.5 μm particles in 11.7 and 500 μS/cm NaHCO₃ solutions. It is also seen that the experimentally determined crossover frequency (3.5 MHz) is quite close to the calculated crossover frequency (3 MHz). In 11.7 μS/cm NaHCO₃ solution, the particles exhibit; (b) pDEP under 20 V at 1 MHz (after 3 min of imposing the AC field); and (c) nDEP under 20 V at 80 MHz (after ~17 s); (d) In 500 μS/cm NaHCO₃ solution, the particles undergo nDEP at 80 MHz (after 3 min); this repulsion profile occurs without any initial particle accumulation.
and 78\(\varepsilon_0\) with \(\varepsilon_0 \approx 8.854 \times 10^{-12} \text{ F·m}^{-1}\) being the vacuum permittivity. In line with such prediction, our experiments demonstrated that the 0.5 \(\mu\text{m}\) particles suspended in 11.7 \(\mu\text{S/cm}\) NaHCO\(_3\) solution exhibited pDEP under 20 V at 1 MHz (Fig. 3(b)) and nDEP at 80 MHz (Figs. 3(c) and 3(d)). Also, our device determined the crossover frequency of 3.5 MHz which is reasonably close to the calculated crossover frequency of 3 MHz predicted through the CM factor (Fig. 3(a)). In Fig. 3(b)), a tremendous amount of particles under pDEP was attracted to the edges of electrodes and also accumulated at the gap between electrodes after 3 min of imposing the AC field. The locations of particle accumulation are consistent with the simulated pDEP directions shown in Fig. 2(b). However, upon switching the frequency to 80 MHz (after \(\sim 17\) s), the accumulated particles experiencing nDEP force were instantly repelled from the electrodes (Fig. 3(c)), and again their motion was observed to follow the direction of nDEP force as shown in Fig. 2(a). Interestingly, by selecting the NaHCO\(_3\) solution medium with a higher conductivity (e.g., 500 \(\mu\text{S/cm}\)), the pDEP behavior was no longer happened and only nDEP behaviors (after 3 min) could be observed in the entire spectrum of frequencies as shown in Fig. 3(d). It was noted that this repulsion profile occurred without having any initial particle accumulation.

Furthermore, not only is the medium conductivity but also particle size critical in altering the frequency-dependent DEP behavior as described by the surface conductivity term in Eqs. (3) and (4). As shown in Fig. 4(a), the CM factor predicts that the 0.5 and 5 \(\mu\text{m}\) latex particles having their surface conductances of 2.1 nS\(^{32}\) and 1.2 nS\(^{31}\), respectively, in 80 \(\mu\text{S/cm}\) NaHCO\(_3\) solution, exhibit distinct DEP behaviors for frequencies less than 50 MHz. Under 20 V, the 0.5 \(\mu\text{m}\) particles were experimentally observed to experience pDEP at low frequency regions (e.g., 1 MHz in Fig. 4(b)) and nDEP at high frequency regions (e.g., 60 MHz in Fig. 4(c)). However, Fig. 4(a) shows for 5 \(\mu\text{m}\) particles, the surface conductivity term in Eq. (3) becomes small enough to result in a negative value of the CM factor within the entire frequency region.

FIG. 4. Effect of particle size on DEP behavior of dielectric latex particles: (a) the CM factor for 0.5 and 5 \(\mu\text{m}\) particles in 80 \(\mu\text{S/cm}\) NaHCO\(_3\) solution. Under 20 V, 0.5 \(\mu\text{m}\) particles experience (b) pDEP at 1 MHz and (c) nDEP at 60 MHz. 5 \(\mu\text{m}\) particles experience (d) nDEP at 60 MHz.
The resulting nDEP force repels 5 \( \mu \text{m} \) particles away from the electrodes at 60 MHz as demonstrated in Fig. 4(d). Moreover, a comparison of Fig. 4(c) and 4(d) shows that due to larger size, 5 \( \mu \text{m} \) particles underwent more prominent nDEP forces, and thus their final locations were found much farther away from the electrodes than those of 0.5 \( \mu \text{m} \) particles.

**C. Continuous separation of submicron (0.5 \( \mu \text{m} \)) and micron (5 \( \mu \text{m} \)) particles**

The DEP characterization results show that at 1 MHz, 0.5 and 5 \( \mu \text{m} \) particles in 80 \( \mu \text{S/cm} \) NaHCO3 solution undergo pDEP and nDEP, respectively, and such distinct DEP characteristic was used for continuous separation of these types of particles in the present microfluidic device. Fig. 5(a) shows that the present device can produce good hydrodynamic focusing effects to confine a flowing stream of 0.5 and 5 \( \mu \text{m} \) fluorescent latex particles near the AgPDMS electrodes. In the absence of electric field, the particle suspension flows to the lower outlet C. Under applied an AC voltage of 30 V at 1 MHz, 5 \( \mu \text{m} \) particles are repelled by nDEP force towards the upper branch D, and 0.5 \( \mu \text{m} \) particles, attracted by pDEP force, are transported towards the lower branch C. Good separation was observed after 14 s of imposing the AC field (Fig. 5(b)) and could last for 3 min (Fig. 5(c)). The time window for particles to achieve separation is estimated to be \( \sim 6.3 \) s which is determined from the known flow speed of 222 \( \mu \text{m/s} \) and channel length of 1400 \( \mu \text{m} \). This also can lead to an nDEP drift velocity of \( \sim 8 \mu \text{m/s} \). The device was observed to perform very well with the efficiency in separating 5 \( \mu \text{m} \) particles from the mixture of 0.5 and 5 \( \mu \text{m} \) particles as high as 100%. Nevertheless, it is noted that one can separate 0.5 and 5 \( \mu \text{m} \) particles using only nDEP in this device. For example, with an increase in the buffer conductivity to 500 \( \mu \text{S/cm} \), both particles experience nDEP as the values of Re\([K^*(\omega)]\) are \(-0.285\) for 0.5 \( \mu \text{m} \) particles and \(-0.485\) for 5 \( \mu \text{m} \) particles. Furthermore, because the difference in particle size is quite large here (10 times) and the nDEP force acting on the 5 \( \mu \text{m} \) particles is 3 orders larger than the 0.5 \( \mu \text{m} \) particles, the separation of these two particle sizes can therefore be readily realized.

![Image](https://example.com/image.png)

**FIG. 5.** Experimental images (acquired with a 10\( \times \) objective) for a flowing stream of 0.5 and 5 \( \mu \text{m} \) fluorescent latex particles near the device outlets C and D. (a) In the absence of electric field, hydrodynamic focusing effects can confine the particle mixture stream near the AgPDMS electrodes. (b) Good continuous separation of 5 \( \mu \text{m} \) particles from a 0.5 and 5 \( \mu \text{m} \) particle mixture stream is observed after 14 s of imposing an AC voltage of 30 V at 1 MHz. (c) An image shows good device performance after 3 min.
V. CONCLUDING REMARKS

We report the principle of operation and in-depth characterization of a PDMS microfluidic device with sidewall AgPDMS composite electrodes that induces DEP effect, which is capable of performing particle DEP characterization and continuous separation of submicron and micron particles. Since the fabricated AgPDMS composite electrodes are embedded in a sidewall of the device channel, the particle motion under DEP effects can be readily observed, and thus AC-DEP behavior of 0.5 and 5 \( \mu \)m fluorescent latex particles in 11.7, 80, and 500 \( \mu \)S/cm NaHCO\(_3\) buffer solutions was characterized by investigating the effect of medium conductivity and particle size under an applied AC electric field with frequencies ranging from 10 kHz to 80 MHz. The DEP characterization results provide crossover frequency and pDEP or nDEP behavior under specific frequencies. Continuous separation of particles by utilizing DEP polarity is achieved in the same device for 0.5 and 5 \( \mu \)m particles in 80 \( \mu \)S/cm NaHCO\(_3\) buffer solution under 30 V at 1 MHz. The device has another feature that sidewall AgPDMS composite electrodes can induce strong DEP effects throughout the entire channel height. As a result, the device yields 100% efficiency in continuously separating submicron and micron particles in hydrodynamic flow. These proof-of-concept experiments show the potential of these sidewall AgPDMS electrodes for characterization and separation of particles of various sizes or/and different polarizabilities in biomedical applications.

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