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A Dielectrophoresis Microfluidic Device for Trapping Bioparticles at Low Voltage and Frequency

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Abstract

Purpose: The necessity for precise manipulation of bioparticles has greatly increased in the fields of bioscience, biomedical, and environmental monitoring. Dielectrophoresis (DEP) is considered to be an ideal technique to manipulate bioparticles. The objective of this study is to develop a DEP microfluidic device that can trap fluorescent beads, which mimic bioparticles, at the low voltage and frequency of the sinusoidal signal supplied to the microfluidic device. **Methods:** A DEP microfluidic device, which is composed of polydimethylsiloxane (PDMS) channels and interdigitated electrode networks, is fabricated to trap fluorescent beads. The geometry of the interdigitated electrodes is determined through computational simulation. To determine the optimum voltage and frequency of the sinusoidal signal supplied to the device, the experiments of trapping beads are conducted at various combinations of voltage and frequency. The performance of the DEP microfluidic device is evaluated by investigating the correlation between fluorescent intensities and bead concentrations. **Results:** The optimum ratio of the widths between the negative and positive electrodes was 1:4 (20:80 μm) at a gap of 20 μm between the two electrodes. The DEP electrode networks were fabricated based on this geometry and used for the bead trapping experiments. The optimum voltage and frequency of the supplied signal for trapping fluorescent beads were 15 V and 5 kHz, respectively. The fluorescent intensity of the trapped beads increased linearly as the bead concentration increased. The coefficient of determination (R^2) between the fluorescent intensity and the bead concentration was 0.989. **Conclusions:** It is concluded that the microfluidic device developed in this study is promising for trapping bioparticles, such as a cell or virus, if they are conjugated to beads, and their concentration is quantified.

Keywords: Bead manipulation, Bioparticle, Dielectrophoresis (DEP), Fluorescent intensity, Microfluidic device

Introduction

Microfluidics deals with the control and manipulation of fluids in miniaturized systems. The field of microfluidics has grown rapidly during the last decade. Its numerous applications are found in biomedical, diagnostics, chemical analysis, and environment monitoring (Sajeesh and Sen, 2014). Recently, the necessity for precise manipulation of bioparticles and biological cells has increased (Qiu et al., 2014).

To manipulate particles, various techniques have been developed in microfluidics, such as optical, magnetic, and dielectrophoresis (DEP) techniques. Optical methods have the advantages of strong controllability and low damage to targets and the drawbacks of high cost, low efficiency, and hard operation. The characteristics of magnetic techniques are similar to those of optical methods, except for low cost. DEP is considered to be a suitable technique for bioparticle manipulation with the advantages of precise control, high efficiency, easy operation, and low damage to biomaterials (Qian et al., 2014).

Chen et al. (2013) trapped a single cell and measured the impedance of the cell by using a parallel-plate chip. An electrode array was designed to trap a single cell in the highest electric field. The cell was trapped using negative

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DEP with a 10 V_{pp}/50 kHz signal. Das et al. (2014) studied a dielectrophoretic microdevice for continuous manipulation and trapping of a polystyrene bead and HeLa cell in a microfluidic channel. Both positive DEP and negative DEP were used to trap the particles. Polystyrene beads always responded as negative DEP up to a frequency of 1 MHz, whereas HeLa cells responded as negative DEP up to a frequency of 400 kHz, and then they experienced positive DEP up to a frequency of 1 MHz.

Most of the previous studies regarding manipulation of particles by using DEP used a relatively high voltage and frequency. This can cause some disturbance during device operation. Therefore, the objective of this study is to develop a DEP microfluidic device for trapping beads, which mimic bioparticles, at the low voltage and frequency of the sinusoidal signal supplied to the microfluidic device. We designed interdigitated electrode networks that can impose high DEP forces on the beads, resulting in trapping of the beads.

Materials and Methods

Theory of dielectrophoresis

When a dielectric particle is in an electric field, electrical charges are induced on the particle. In a uniform electric field (Figure 1(a)), the Columbic forces in the positive and negative charges are equal. This leads to zero net force imposed on the particle. When an electric field is non-uniform (Figure 1(b)), the Columbic forces are unequal, resulting in a net force imposed on the particle. The particle can be moved by the imposed force, and such a motion is called DEP (Khoshmanesh et al., 2011). The DEP force (F_{DEP}) is determined using Eq. (1).

$$F_{DEP} = 2\pi\varepsilon_m r^3 \, Re\{f_{CM}\} \nabla |E|^2 \tag{1}$$

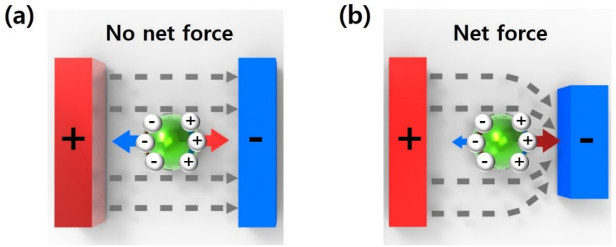


Figure 1. Dielectrophoresis force: (a) in a uniform electric field and (b) in a non-uniform electric field.

where ε_m is the permittivity of the medium, r is the radius of the particle, $Re\{f_{CM}\}$ is the real part of the Clausius–Mossotti (CM) factor, and E^2 is the root-mean-square value of the electric field. The CM factor (f_{CM}) is derived from Eqs. (2)-(4).

$$f_{CM} = \frac{\varepsilon_P^* - \varepsilon_m^*}{\varepsilon_P^* + 2\varepsilon_m^*} \tag{2}$$

$$\varepsilon_m^* = \varepsilon_m - \frac{j\sigma_m}{\omega} \tag{3}$$

$$\varepsilon_p^* = \varepsilon_p - \frac{j\sigma_p}{\omega} \tag{4}$$

where ε_m, ε_p and σ_m, σ_p are the permittivity of the medium and the particle and the conductivity of the medium and the particle, respectively, and ω is the angular frequency. When $\varepsilon_p^* > \varepsilon_m^*$, the CM factor is positive, and the particles move toward the maximum of the electric field; this is called positive DEP. In contrast, negative DEP occurs when $\varepsilon_m^* > \varepsilon_p^*$; the particles move toward the minimum of the electric field (Hunt et al., 2008). As shown in Eq. (1), the DEP force is determined by the permittivity of the medium (ε_m), the radius of the particle (r), the CM factor (f_{CM}), which is dependent on frequency, and the gradient of the root-mean-square value of electric field. When the movement of particles is controlled using the DEP, the size of the particles and the permittivity of the medium are constant. Therefore, the particle motion can be determined by the magnitude and frequency of the signal supplied to generate an electric field.

Design of the microfluidic device

A microfluidic device was designed for experiments as shown in Figure 2. It consisted of two components: a microfluidic channel and an electrode array. Three inlets, one outlet, and a detection area were constructed in the microfluidic channel. The width and height of the channel were 500 μm and 30 μm, respectively.

To manipulate particles flowing in a microfluidic channel by using the DEP force, the DEP force should be larger than the forces imposed on the particle, such as the hydrodynamic force and the gravitation force. As shown in Eq. (1), the DEP force can be increased by supplying high voltage, resulting in a high electric field. However, the high voltage can cause Joule heating in the device and

electrolysis of the medium. This may lead to bubble formation, and thus, disturb the operation of the device. As an alternative way of strengthening the electric field without increasing supply voltage, a change of electrode dimension can be considered. We designed interdigitated electrodes with a rectangular shape. The gap between the adjacent electrode edges was 20 μm. The ratio of the width of the negative electrode to that of the positive electrode varied from 1:1 (20:20 μm), 1:2 (20:40 μm), 1:3 (20:60 μm), and 1:4 (20:80 μm). The electric field was numerically simulated at four different ratios of electrode width and three different heights (5, 10, and 15 μm) above the electrode surface by using COMSOL Multiphysics (ver 4.2, COMSOL Inc., USA).

Fabrication of microfluidic channel and electrode

The microfluidic channel was fabricated on a silicon wafer by using conventional photolithographic techniques with polydimethylsiloxane (PDMS) (Figure 3). To fabricate the DEP electrode, Cr and Au with thicknesses of 30 and 150 nm, respectively, were deposited on a clean glass slide. SU-8 photoresist was spin-coated with a thickness of 30 μm on the Cr/Au surface. Through the process of developing and etching, the electrode was fabricated. The PDMS channel and the electrode deposited on the glass slide were bonded together by oxygen plasma treatment. Electrical wires were soldered to the electrode pads to supply the voltage signal.

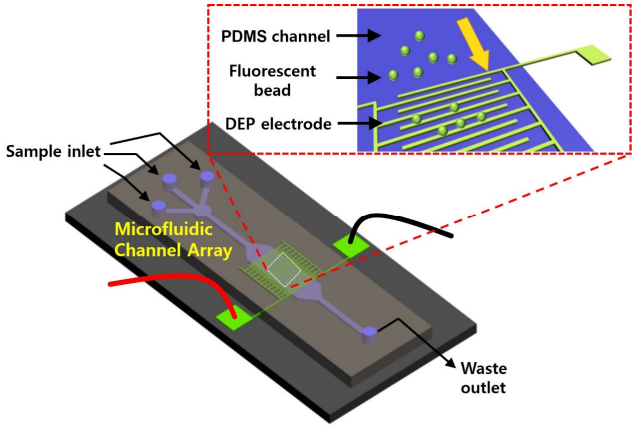


Figure 2. Schematic of the microfluidic device.

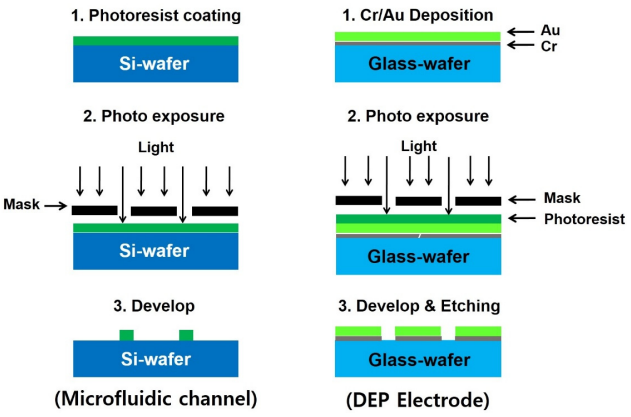


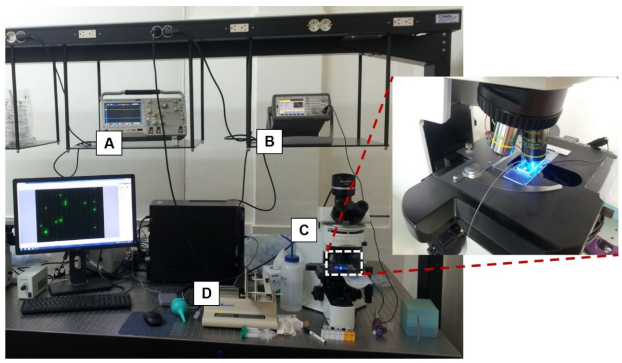
Figure 3. Fabrication procedure for the microfluidic channel and the DEP electrode.

Particle sample preparation

To test the performance of the microfluidic device for trapping particles, a 5 μm green fluorescent polystyrene bead (G0500, Thermo scientific, USA) with the concentration of 1.05 g cm^{-3} at stock solution was prepared. For the experiments, the bead was diluted into 5 levels of concentration: 0.00328, 0.00656, 0.0131, 0.0265, and 0.0525 g cm^{-3}.

Experimental setup

The sinusoidal voltage was supplied to the device by a function generator (33521A, Agilent, USA) and monitored by an oscilloscope (Tektronix MSO 2012, Tektronix, USA) (Figure 4). The bead samples were injected into the microfluidic channel by using a syringe pump (781630, kdScientific, USA) at a flow rate of 50 L h^{-1}. The flow motion of the beads were observed through a fluorescent microscope (BX-51, Olympus, Japan) equipped with a green color filter. The images of the bead motion in the microfluidic channel were captured using a charge coupled device (CCD) camera (DP73, Olympus, Japan).



A: Oscilloscope B: Function generator C: Fluorescent microscope D: Syringe pump

Figure 4. Experimental setup.

Experimental method

To determine the optimum voltage and frequency of the sinusoidal signal supplied to the microfluidic device, the experiments for trapping fluorescent bead samples were conducted at three combinations of voltage and frequency: 10 V at 5 kHz, 15 V at 5 kHz, and 15 V at 10 MHz. The voltage and frequency having the greatest trapping performance were used as the operating condition of the device.

The efficiency of the microfluidic device for trapping bead samples was investigated. The fluorescent bead samples were continuously injected into the microfluidic channel. The beads were trapped for 2 min by using the device, and then the image was taken. The fluorescent intensity of the captured images was analyzed using an image analysis software (ImageJ, NIH, USA). These experiments were repeated at five levels of bead concentrations: 0.00328, 0.00656, 0.0131, 0.0265, and 0.0525 g cm^{-3}. The correlation between the fluorescent intensity and the bead concentration was examined.

Results and Discussion

Simulation of electric field

The gradient of the electric field on the interdigitated electrode networks was simulated at different widths of positive electrodes with a constant width of negative electrodes and different heights above the electrode surface. The simulation results are shown in Figure 6. The gradient of the electric field drastically decreased as the height above the electrode surface increased. This indicates

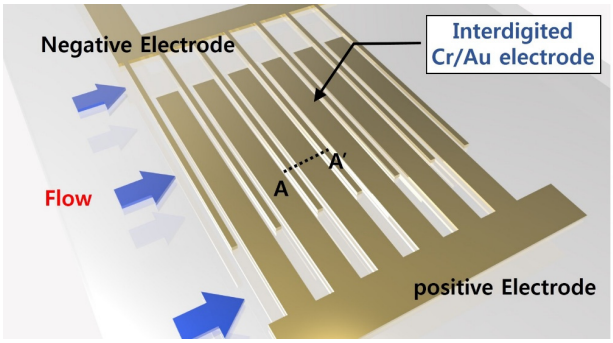


Figure 5. Schematic of electrode.

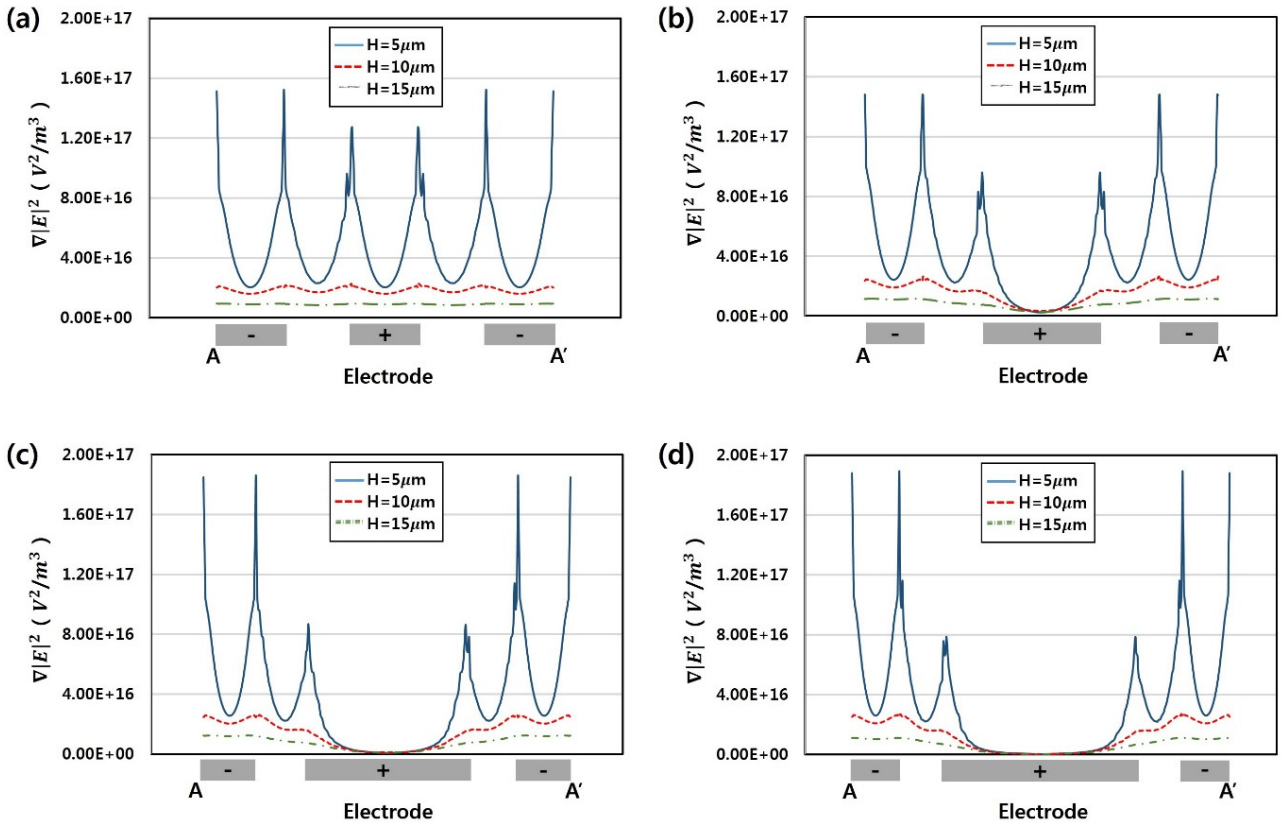


Figure 6. Gradient of electric field square in the channel at different heights (H) above the electrode surface and different width ratios between the negative and positive electrode: (a) 1:1 (20:20 μm), (b) 1:2 (20:40 μm), (c) 1:3 (20:60 μm), and (d) 1:4 (20:80 μm).

that the DEP force imposed on the beads flowing at high heights will not be sufficient to trap the beads.

When the ratio of the widths between the negative and positive electrodes was 1:1 (Figure 6(a)), the gradients of the electric field at the edge of the two electrodes were at a similar level. The net DEP force between the two electrodes, which was the difference in magnitude of the electric field gradient between the edge of the negative electrode and the edge of the positive electrode, was very low so that the flowing beads could not be trapped. As the ratio of the widths increased, the net DEP force became greater, and this allowed the beads on the edges of the electrodes to be trapped. The magnitude of the electric field gradient at ratios higher than 1:4 was not changed much (the results were not shown here). Therefore, it was believed that the optimum ratio of the widths between the negative and positive electrodes was 1:4 (20:80 μm) at the 20 μm gap between the two electrodes. The DEP electrode networks were fabricated based on this dimension and used for the experiments of bead trapping.

Bead trapping at various combinations of voltage and frequency

The performance of the microfluidic device for trapping beads was examined at the different voltages and frequencies of a supplied sinusoidal signal. The beads flowing in the microfluidic channel were not trapped when a sinusoidal signal of 10 V and 5 kHz was supplied (Figure 7(a)). The DEP force generated by the supplied voltage might not be enough to trap the beads. As the voltage increased to 15 V with the same frequency (Figure 7(b)), the beads were securely trapped at the edge of the negative electrodes. When the voltage signal was turned off, the beads were released and then came back to free motion. However, when the frequency increased to 10 MHz at the same voltage of 15 V, most of the beads were not trapped

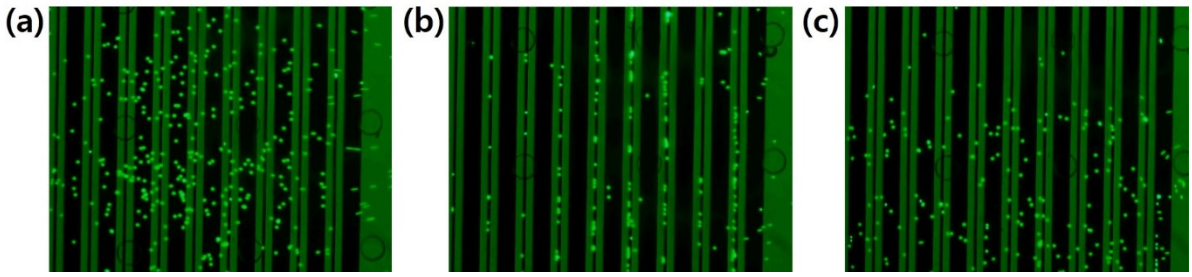


Figure 7. Fluorescent images of bead trapping at different combinations of voltage and frequency of the supplied sinusoidal signal: (a) 10 V at 5 kHz, (b) 15 V at 5 kHz, and (c) 15 V at 10 MHz.

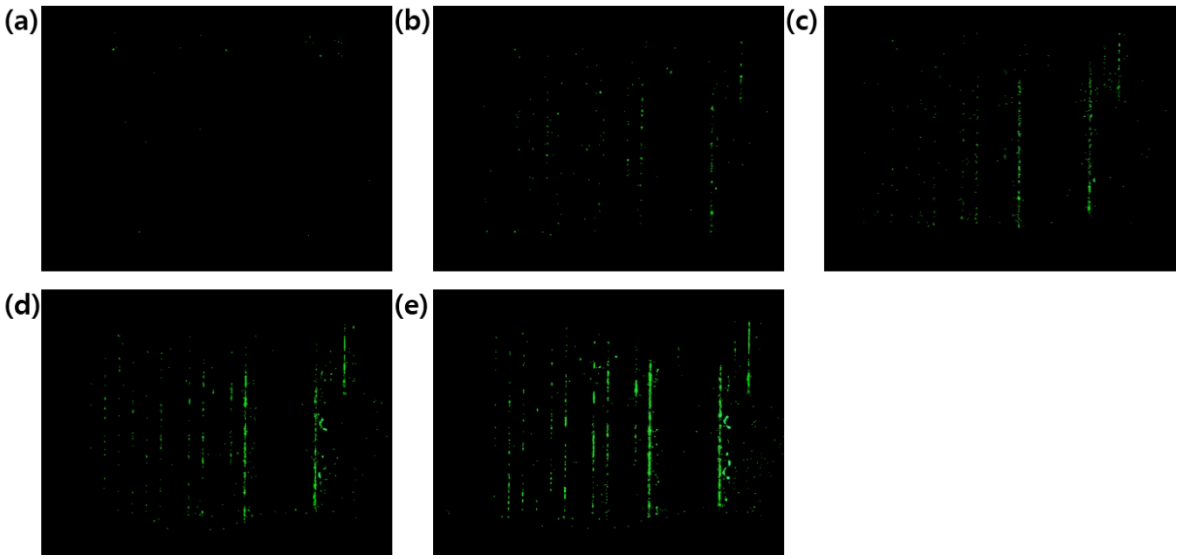


Figure 8. Fluorescent images of bead trapping at different bead concentrations: (a) 0.00328 g cm^{-3}, (b) 0.00656 g cm^{-3}, (c) 0.0131 g cm^{-3}, (d) 0.0265 g cm^{-3}, and (e) 0.0525 g cm^{-3}.

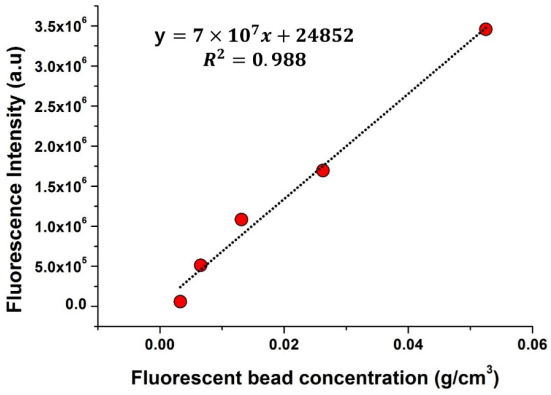


Figure 9. Correlation between fluorescent intensity and fluorescent bead concentration.

(Figure 7(c)). Therefore, the optimum voltage and frequency of the supplied sinusoidal signal to trap the bead samples were 15 V and 5 kHz, respectively.

Bead trapping at different bead concentrations

The fluorescent images of trapped beads at different bead concentrations are shown in Figure 8. As the bead concentration increased, the green colored portion in the images, depicting the portion that was occupied by fluorescent beads, became larger. When the beads were trapped, they were aligned in a straight line at the edges of the electrodes. The coefficient of determination between the fluorescent intensity and the bead concentration was 0.989, as shown in Figure 9. From these results, it is concluded that the microfluidic device developed in this study can trap bioparticles such as a cell or virus, if they are conjugated to beads, and then quantify their concentration.

Conclusions

A DEP microfluidic device, which is capable of trapping fluorescent beads and quantifying their concentrations in a continuous mode, has been demonstrated. The optimum width ratio between the negative and positive electrodes that could impose the highest DEP force on the beads was 1:4 (20:80 μm) at the 20 μm gap between the two electrodes. The voltage and frequency of the sinusoidal signal supplied to the device for trapping the beads were 15 V and 5 kHz, respectively. The fluorescent intensities measured at five levels of bead concentrations were correlated well with the bead concentrations (R^2=0.989). Therefore, it can be concluded that the DEP microfluidic device proposed in this study is promising for trapping bioparticles such as a cell or virus, if they are conjugated to beads, and then quantifying their concentration.

Conflict of Interest

The authors have no conflicting financial or other interests.

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