Experimental Study of Two-frequency Dielectrophoresis Effects on a Linear Electrode Array

Y. T. Zhang, Student member, IEEE, F. Bottausci, member, IEEE, N.C. MacDonald, Fellow, IEEE and I. Mezić

Abstract: Dielectrophoresis is a powerful tool for the manipulation of particles and biological cells. The magnitude and direction of the DEP force is determined by the comparative conductivity and permeability of the medium and the particle. Most previous work has focused on single frequency studies. In this paper, we present the work of two frequency dielectrophoresis effects on a linear electrode array. We show results where the advantage of applying two frequencies is to separate particles having similar dielectric properties. In addition, we report the first experimental data on breaking of DEP trapping zone by adding a low frequency signal to the main frequency signal. The phenomenon is due to a system disturbance by electro-hydrodynamic effect and has potential applications in DEP mixing and advanced control of particles.

I. INTRODUCTION

It is well known that when a dielectric particle is placed into a non-uniform electrical field, a force will be exerted on the particle due to the dipole moment. This force is called dielectrophoresis force, and has been applied extensively to trap or separate a variety of particles such as latex spheres at both micron and sub-micron scale [1-2], biopolymers like yeast cells [3], bacteria [4], DNA [5] and viruses [6]. According to dielectrophoresis theory [7], the time average DEP force on a spherical particle with the radius of \( a \) at given angular frequency \( \omega \) is described as

\[
F_{dep} = 2\pi a^3 \varepsilon_m \text{Re}[f_{CM}] V |E|^2
\]

where the Clausius-Mossotti factor \( f_{CM} \) represents the comparative polarizability of the particle and the medium and is given by

\[
f_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \left( \varepsilon^* = \varepsilon + \sigma \right) \quad \text{with} \quad \varepsilon
\]

\( p \) and \( m \) denote the particle and the suspending medium, respectively. From the above equation, it can be seen that the variation of frequency results in the DEP force’s change in both magnitude and direction. Most previous DEP work was focused on single frequency manipulation, and not much effort has been applied to dual or multi-frequency system [8]-[10].

Recently, Chang et al [11] developed a system theory on dielectrophoresis and predicted that two frequencies can be used to separate T-cells and red blood cells. In this paper, we experimentally show the separation of latex spheres using a two-frequency strategy. In addition, we first report the experimental observation of breaking the dielectrophoresis-trapping zone by applying a second low frequency to the system. The experimental results are in good agreement with theoretical predications.

II. DEVICE FABRICATION

The device used for this experiment is made of titanium and is fabricated using a novel titanium bulk micromachining technology [12]. In short, electrodes are patterned on the titanium substrate with an insulation layer. Then the titanium channel is adhesively bonded to the electrode substrate. Thus, a 24 linear electrode array is formed at the bottom of the channel, as illustrated at Fig.1. Electrodes are 20 micrometers wide with a pitch of 40 micrometers.

Fig.1 Schematic of the titanium DEP device

III. EXPERIMENTAL SETUP

A. Particle preparation
The particles are polystyrene fluorescence microspheres (Duke Scientific, Palo Alto, California, USA) with diameters in the range of 0.1 \( \mu m \) to 5 \( \mu m \) and a density of 1.05 \( \text{kg/m}^3 \). The microspheres contain dye incorporated in the polymer matrix producing a bright green color when excited by blue light. Before use, the beads are diluted to a low concentration in de-ionized water. The conductivity of the solution is measured by a Hanna Primo 5 conductivity meter (Automated Aquarium Systems, CA, USA).

B. Image capturing

All images are collected through an epi-fluorescence microscope (Model ECLIPSE E600FN, Nikon) with a Mercury Arc Lamp (HBO 100w/2, OSRAM) as the excitation source, and the illumination intensity is controlled by a power stabilizer (1962LTS, Opti-Quip). Visualizations are made using a Nikon 20x water immersion lens providing a working distance of 2 mm. A digital CCD camera (C7300, Hamamatsu) under software control (Digital Camera Control 4.0b17, Hamamatsu photonic Corporation) is used to record particle images.

C. Device assembly and operation protocol

![Device Assembly Image]

A Plexiglas fluid support stage is designed and fabricated to guide the fluid into the chip. Plexiglass is chosen for its transparency and electrical insulation properties. For this design, two aluminum tubes are glued onto the stage, and four threaded holes are used to clamp the chip. Rubber O-rings are used for sealing. Fluid is pumped into the tubing, until it reaches the central hole; the fluid then moves upward through the O-ring and enters the chip’s reservoir area and flows into the channel. This setup is easy to un-package, thus enabling the stage to be re-used for multiple analysis.

A 1mm thick PDMS lid is utilized to cover the channel and reservoirs to prevent evaporation while allowing optical detection. This sealing is not permanent therefore enabling convenient cleaning for reuse of the chip.

Electrical connection is accomplished using two card edge connectors (Digi-Key, Minnesota, USA) to connect the titanium chip and the electronic testing equipment such as function generator (Fig. 2).

During a typical experiment, samples are injected into the device using a PHD 2000 syringe pump (Harvard Apparatus, Massachusetts, USA) at a flow rate of 30 \( ul/\text{min} \). When the sample fills the channel, the flow is stopped. And flow is allowed to stabilize before the electric field is applied. A sinusoidal signal is applied with an 11MHz stabilized function generator (Wavetek, model 21). The signal is monitored by an oscilloscope (Tektronix TDS 3012).

D. Signal adding

Signal superposition is realized by a custom signal adder. Mathematically, when two sinusoidal signals with equal potentials are added, the sum potential doubles. For example, if two sinusoidal signals \( f_1 \) and \( f_2 \) where 
\[
 f_1 = 5 \sin(2\pi \times 10^4 t) \quad \text{and} \quad f_2 = 5 \sin(2\pi \times 10^7 t)
\]
are added, the amplitude of the combined signal is 20V pk-pk. The magnitude and direction of the dielectrophoresis force on the particle is a linear superposition of the DEP force of each of the frequencies.

III. RESULTS AND DISCUSSIONS

A. Two-frequency separation

Most previous researches focused on a single frequency signal for particle separation, because it’s a simple and straightforward method, and is quite effective for many applications. In this paper, we present a two-frequency separation technology. As discussed in [11], under certain situations especially when the crossover frequencies of the two particles are very close, the single frequency method doesn't work efficiently. Thus, multi-frequency excitation becomes an alternative choice for DEP separation. In addition, from a system control point-of-view, multi-frequency excitation provides more powerful and flexible control of the device. For example the frequency range for particle separation can be wider when two frequency signals are combined, which we demonstrate here.

The particles we used have a nominal diameter of 1.9 and 0.71 micrometers respectively. The conductivity of the medium is 12 \( \mu \text{S/cm} \).

Before using multiple frequencies, we ran the experiment with a single frequency. At 10V pk-pk and 100 kHz, both particle sizes undergo positive-DEP. They are attracted to the edges of the electrodes where the electrical field gradient is strongest. While at 10V pk-pk and 1MHz, both of the particles show negative-DEP phenomena. They are repelled away from the edges of the electrodes and levitate toward the cover. Therefore neither of the two above frequencies
can be used for the purpose of particle separation. This result fits the mathematical calculation of the real part of the Clausius-Mossotti factor under the given experimental parameters (see Fig.3).

In contrast, if two frequencies are added and applied to the system, we observe that large particles move away from the electrodes (N-DEP) and small particles accumulate on the electrodes (P-DEP). Figure 4a shows the channel filled with particles. Large particles appear brighter and the focal plane is on the top surface of the electrodes. There is no flow in the channel and the motion of the particles is only due to the Brownian agitation.

Figure 4b shows the particle distribution 0.5 seconds after the electric field was turned on. Large particles are moving away from the electrodes. They appear out of focus because they are moving in the vertical direction above the plane of the electrode. Positive DEP forces move small particles to the electrode edges, which correspond to the highest field gradient. Small and large particles are then separated. Note that some large particles appear on the plane shown in Fig.4 (b). These particles did not move because they were attached on the electrodes before the electric field was applied.

If one studies Fig.3 carefully, one can see that in fact for this specific case, in the range around 150 kHz to 500 kHz, a single frequency can also achieve separation, where large particles undergo positive DEP and small particles undergo negative DEP. However, the two-frequency method broadens the frequency range for particle separation. From Fig.3, one can see that for \( f_1 \) smaller than 100 kHz and \( f_2 \) between 300 kHz to 2 MHz, the large particles will have N-DEP while the small particles have P-DEP. Therefore large particles can be separated in this very broad frequency range. Special attention should be paid to the low frequency range.

Next we show an experimental result that low frequency perturbation can destroy DEP trapping zones.

Figure 4a: Top view of the channel filled with particles. The electric field is off. Electrodes width is 20 microns

Figure 4b: Top view of the channel filled with particles. The electric field is on.

B. Adding a low frequency perturbation to destroy the DEP trapping zones

Tuval et al. recently described theoretically that when a low frequency is added to the system, DEP trapping would break [14]. This phenomenon is similar to the fact that small time-dependent perturbations to steady flows can break up trapping barriers in conservative dynamic systems [15]. One explanation is that the large electrical field gradient on the chip interacts strongly with the suspended medium through several electrohydrodynamical effects such as AC electroosmosis, which in turn disturbs the particle trajectories via drag force. Here we experimentally verified the prediction with two sinusoidal signals at the potential of 10V pk-pk and frequencies of 10 kHz and 500 Hz respectively. The solution consisted of a 1-micrometer diameter latex beads in suspension.

Fig. 5 (a) shows stabilized flow without the influence of the electric field, where particles are uniformly suspended in the fluid, and only move under Brownian agitation. When the electrical signal is applied, particles begin to accumulate onto the electrodes and gradually form the pearl-chain at the edge of the electrode and also on the center of the electrodes, because of the electrokinetic convection as shown in Fig.5 (b).
V. CONCLUSIONS

The use of dielectrophoresis for particle manipulation has been studied extensively. Yet not much attention has been paid to dual or multi-frequency DEP. In this paper, we experimentally demonstrated that two frequencies can be used for particle separation. We also demonstrated that there is a good agreement between theory and experiment regarding the second frequency (if the frequency is lower than the diffusion time for the temperature front) signal for breaking the DEP trapping zone. This disturbance might be applied for DEP mixing and opens a door to advanced control of bioparticles using dielectrophoresis.

ACKNOWLEDGMENT

The authors would like to thank C. D. Meinhart for helpful discussions and lab assistance.

REFERENCES