

# Review of bio-particle manipulation using dielectrophoresis

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**Abstract** — During the last decade, large and costly instruments are being replaced by system based on microfluidic devices. Microfluidic devices hold the promise of combining a small analytical laboratory onto a chip-sized substrate to identify, immobilize, separate, and purify cells, bio-molecules, toxins, and other chemical and biological materials. Compared to conventional instruments, microfluidic devices would perform these tasks faster with higher sensitivity and efficiency, and greater affordability. Dielectrophoresis is one of the enabling technologies for these devices. It exploits the differences in particle dielectric properties to allow manipulation and characterization of particles suspended in a fluidic medium. Particles can be trapped or moved between regions of high or low electric fields due to the polarization effects in non-uniform electric fields. By varying the applied electric field frequency, the magnitude and direction of the dielectrophoretic force on the particle can be controlled. Dielectrophoresis has been successfully demonstrated in the separation, transportation, trapping, and sorting of various biological particles.

**Index Terms** — AC electrokinetics, Dielectrophoresis, Particle manipulation, Particle separation

## I. INTRODUCTION

There is currently a high level of interest in developing means to manipulate biological particles. Expensive and time-consuming conventional analytical technique to separate and identify cells, proteins, viruses and DNA, is being replaced by low cost microfluidic devices. Such

devices are commonly referred as lab-on-a-chip or micro-total analysis systems ( $\mu$ TAS). To manipulate these bio-particles, whose diameters range from 10 nm to 100  $\mu$ m, researchers have exploited electrostatic force as it becomes dominant at micrometer scale [1]. The methods include electrophoresis, electroosmosis, electrofusion, electrowetting, and dielectrophoresis. Among these methods, electrophoresis has been used to separate and detect proteins and DNA, and devices based on this technique are commercially available [2]. Dielectrophoresis, the center of discussion in this paper, is traditionally recognized as a cell separation technique.

The term dielectrophoresis was first used by Pohl [3], which he described as the translational motion of neutral matter caused by polarization effects in a non-uniform electric field [4]. Originally, this term was strictly referred to the phenomena of induced dipole on particles due to a non-uniform field. However, the term has now been broaden to include other electrokinetic phenomena arises from non-uniform electric fields, in particular, traveling-wave dielectrophoresis [5] and electrorotation [6].

In the early stage, dielectrophoresis was performed using pin-wire electrodes [3]. Manipulation is limited to large particles like cells. Using this electrode, Pohl and Hawk [3] have demonstrated the separation of viable and non-viable yeast cells in 1966. Pohl [4] has then extended the experiments to separate other biological cells, including canine thrombocytes, red blood cells, chloroplasts, mitochondria, and bacteria.

In recent years, microelectrodes with dimension as small as 0.5  $\mu$ m have been fabricated using photolithography technique [7]. Electrodes are now small enough to generate high electrical field gradients to manipulate sub-micrometer particles. Dielectrophoresis can now be used to separate viruses [7-13], proteins [14], and DNA [15-17].

This paper reviews some of these particle manipulation methods, including separation, transportation, trapping and sorting.

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## II. THEORY

### A. Particle polarization

When an external electric field  $\vec{E}$  is applied across a particle suspended in a fluid medium, both the particle and the suspending medium are being polarized. The result is net unpaired surface charges  $\sigma_s$  cumulated at the interface between the particle and the fluid medium. These surface charges generate another electric field and distort the original electric field. A typical resulting electric field is shown in fig. 1. The amount of charges at the interface depends on the field strength and the electrical properties of the particle and the suspending medium. The key electrical properties involved are conductivity and permittivity, where conductivity is a measure of the ease with which charges can move through a material, while permittivity is a measure of the energy storage or charge accumulation in a system [18].

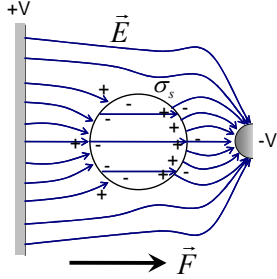


Fig. 1 Interfacial polarization and dielectrophoresis.

### B. Dielectrophoretic force

The surface charges interact with the electric field to produce Coulomb forces. Since the electric field distribution is not uniform in fig. 1, the electric field density is higher on the right than on the left, resulting in a net force  $\vec{F}$  in the direction as shown in fig. 1.

Few methods have been developed to find the total electrical force on the particle, including effective moment method [19] and Maxwell Stress Tensor method [20]. Effective moment method is more commonly used since it provides a simple analytical solution while maintaining a good physical insight of the behavior of the system. The basis of the effective moment method is the hypothesis that the force and torque upon a particle can be expressed in terms of the effective moments identified from the solution for the induced electrostatic field due to the particle [19]. On the other hand, the Maxwell Stress Tensor method requires a rigorous surface integration of stress tensor over the particle. The analytical solution has been limited to the case of a homogeneous spherical particle [20]. However, the Maxwell Stress Tensor method is preferred for numerical calculation, and has been implemented in some commercial finite-element software.

Assuming that the observation point is far enough relative to the size of the particle, the surface charges on the particle in fig. 1 can be approximated as a dipole, which is oriented with the direction of the electric field.

With this approximation, the total electrical force on the particle is found as [19]

$$\vec{F} = (\vec{p} \cdot \nabla) \vec{E} \quad (1)$$

where  $\vec{p}$  is the effective dipole moment specific to the particle-fluid system, and  $\nabla$  is a del operator. This electrical force is termed as dielectrophoretic force.

When the dipole approximation is not accurate, higher order multipoles have to be considered. The general solution has been solved by Jones and Washizu [21-24].

### C. Frequency response

For an isotropic homogenous spherical particle with radius  $a$ , the time-averaged dielectrophoretic force in equation (1) can be generalized as [25]

$$\langle \vec{F}(t) \rangle = 2\pi\epsilon_m a^3 \left\{ \text{Re}[K] \nabla E_{rms}^2 + \text{Im}[K] (E_{x0}^2 \nabla \varphi_x + E_{y0}^2 \nabla \varphi_y + E_{z0}^2 \nabla \varphi_z) \right\} \quad (2)$$

where  $E_{rms}$  is the root-mean-square of a sinusoidal electric field having magnitude  $(E_{x0}, E_{y0}, E_{z0})$  and phases  $(\varphi_x, \varphi_y, \varphi_z)$ ,  $\text{Re}[\ ]$  and  $\text{Im}[\ ]$  respectively denote real part and imaginary part, and  $K$  is the Clausius-Mossotti factor defined as

$$K = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad (3)$$

$$\text{and } \epsilon^* = \epsilon - i \frac{\sigma}{\omega} \quad (4)$$

$\epsilon_p^*$  and  $\epsilon_m^*$  are the complex permittivity of the particle and the suspending medium, respectively. The Clausius-Mossotti factor is a frequency dependent variable. A normalized plot for the Clausius-Mossotti factor is shown in fig. 2. When  $K > 0$ , the particle is said to be experiencing a positive dielectrophoresis, where the particle moves towards the high electric field gradient regions. Likewise, when  $K < 0$ , the particle experiences a negative dielectrophoresis and moves away from the high field gradient regions [19]. By changing the electric field frequency, the Clausius-Mossotti factor can experience a transition from a positive value to a negative value, which causes the dielectrophoretic force on the particle to change its direction accordingly. The Clausius-Mossotti factor is a unique property of the particle under the specified suspending medium, and it is this property that is being utilized in the dielectrophoresis for particle manipulation.

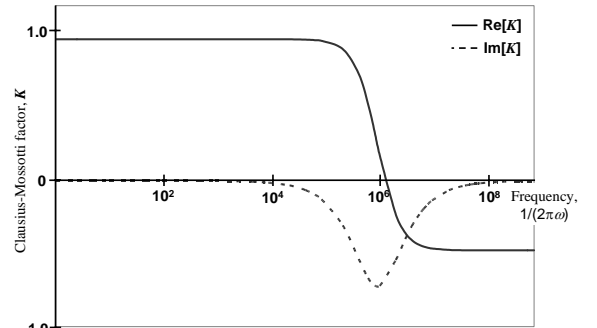


Fig. 2 Normalized Clausius-Mossotti factor vs frequency.

### III. PARTICLE SEPARATION

One of the most important applications of dielectrophoresis is particle separation. It relies on the fact that one particular sub-population of particles has unique frequency-dependent dielectric properties, which is different from any other population. The relative magnitude and direction of the dielectrophoretic force exerted on a given population of particles depends on the conductivity and permittivity of the suspending medium, together with the frequency and magnitude of the applied field. Therefore, differences in the dielectric properties of particles manifest themselves as variations in the dielectrophoretic force magnitude or direction, resulting in separation of particles.

The transition of particles from negative dielectrophoresis into positive dielectrophoresis on castellated electrodes was demonstrated by Pethig and co-workers [26]. Spatial separation of blood cells [27] and separation of blood cells from bacteria was also performed on such an electrode array [6]. The spatial separation of sub-micrometer particles on a castellated electrode array has also been demonstrated [11]. The spatial separation of a heterogeneous population of sub-micrometer particles of identical size can also be accomplished using electrode arrays [28].

It is also being demonstrated that small biological particles such as viruses, DNA and macromolecules can be separated using dielectrophoresis. For example, the spatial separation of two different viruses, Tobacco Mosaic Virus and Herpes Simplex Virus, using a polynomial electrode is shown in fig. 3 [11]. The Herpes Simplex Virus is trapped under negative dielectrophoretic forces at the field minimum in the center of the electrode array, while simultaneously Tobacco Mosaic Virus experiences positive dielectrophoresis and collects at the high-field regions at the electrode edges, resulting in the physical separation of the two particle types. This is illustrated schematically in fig. 3(a) and the photograph of the observation is shown in fig. 3(b).

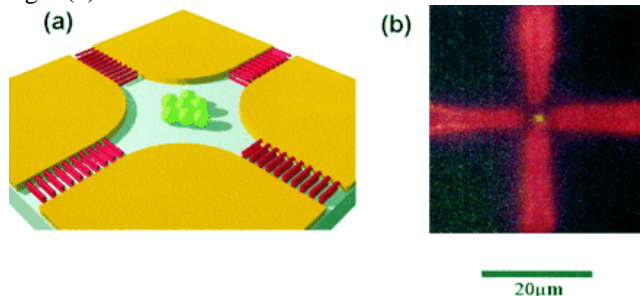


Fig. 3 Separation of Tobacco Mosaic Virus and Herpes Simplex Virus [11].

Physical separation of a mixture of particles into two populations is achieved by subjecting the electrode array to a flow of liquid of sufficient pressure to remove particles trapped at field minima leaving the other particles trapped at the electrode tips. The remaining particles can then be removed by switching off the field and flushing fresh

liquid across the electrodes [29]. This physical separation technique is based on the knowledge that the particles trapped at field gradient maxima by positive dielectrophoresis are held by a stronger force than those experiencing negative dielectrophoresis [26].

These are the basic separation techniques using dielectrophoresis. It only relies on the real part of the Clausius-Mossotti factor, since the applied electric field only changes in magnitude but not phase. The major disadvantage is that the particles are localized at the electrodes after separation, and flushing needs to be performed to collect the separated particles. A better technique is to separate and transport the particles at the same time. This is achieved using traveling-wave dielectrophoresis or dielectrophoretic – field flow fractionation technique.

### IV. TRAVELING-WAVE DIELECTROPHORESIS

In the traveling-wave dielectrophoresis, the applied electric field has varying magnitude and phase, which induces both the real and imaginary part of the Clausius-Mossotti factor on the particles [25]. For an interdigitated electrode [30,31], the real part of the Clausius-Mossotti factor determines the levitation of the particles from the electrode plane, whereas the imaginary part of the Clausius-Mossotti factor controls the translational movement of the particles along the electrode plane. Particle separation is achieved by applying a frequency where the first sub-population is levitated and translated, while the second sub-population is immobilized on the electrodes. This technique has been demonstrated to separate viable and non-viable yeast cells [31,32].

Particle separation is still possible even if both sub-populations are levitated and travel in the same direction, due to the fact that particles with different sizes travel at different velocities. This technique was demonstrated by Morgan and co-workers [33] to separate erythrocytes and leukocytes cells.

### V. DIELECTROPHORETIC – FIELD FLOW FRACTIONATION (DEP-FFF)

Dielectrophoretic forces can be combined with hydrodynamic forces in a separation method known as field flow fractionation (FFF), which is a general chromatographic separation technique in chemistry and biology [34]. In DEP-FFF, particles are separated according to a combination of their effective polarisability and density [35,36]. Particles are repelled from the electrodes under a dielectrophoretic levitation force, which acts on a suspension of particles. This force is combined with fluid flow with a parabolic velocity profile, where particles levitated at different height are transported in different speeds. In contrast to other DEP separation methods, where particles remain on the same plane and are either eluted or remain trapped, DEP-FFF exploits the

velocity gradient in the flow profile to achieve highly selective separation. Recent examples of applications include the separation of latex particles [36,37] and blood cells [38,39].

However, due to randomness, the particles travel at a Gaussian-shaped distribution, where two subpopulations often overlap. Thus, the separated subpopulation often contains residue of other subpopulations [40]. This is an area which needs improvement.

## VI. PARTICLE TRANSPORTATION

Dielectrophoresis can be used to transport particles as well, other than conventional pump and electrohydrodynamic methods. This is achieved through the use of interdigitated electrodes generating traveling-wave dielectrophoresis, which is essentially the same equipment discussed in section IV. Particles are moved in a traveling electric field energized with a four phase signal [41,42]. There is no need to pump liquid along the device in order to produce horizontal motion.

However, the traveling-wave dielectrophoresis can only allow one-dimensional transportation of the particle. An improved design is a grid electrode system, which allows two-dimensional movement of a particle [43]. It is constructed of two glass plates, where there are vertical electrode strips on the top glass, and horizontal electrodes strips on the bottom glass, as shown in fig. 4. A high field region is developed at the intersection of two strip electrodes to which AC signals are applied. The particles are attracted or repelled from the intersection depending on whether the particles are experiencing positive or negative dielectrophoresis.

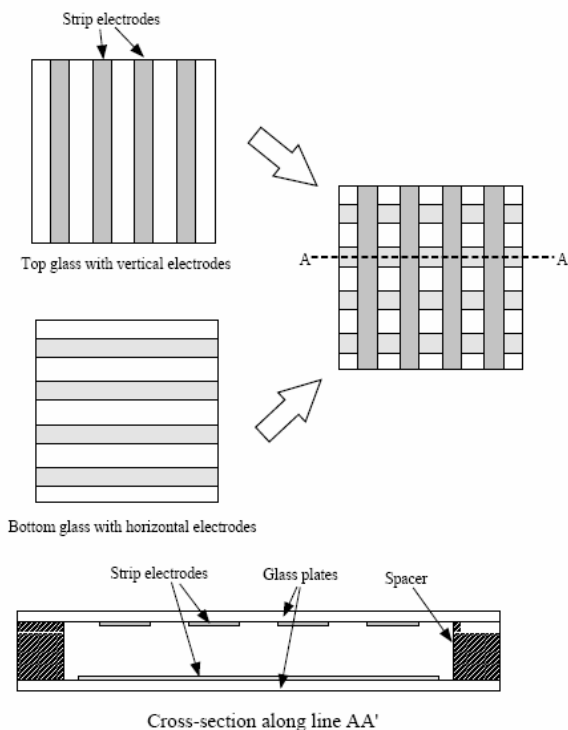


Fig. 4 Grid electrode system [43].

## VII. PARTICLE TRAPPING

Another important application of dielectrophoresis is the non-contact trapping of single particles. A polynomial electrodes system is used to generate a potential energy well at the center of the electrodes. Particles are trapped at the center of the electrodes under negative dielectrophoresis. The trapping of single sub-micrometer particles in quadrupole microelectrode structures has been demonstrated experimentally by Hughes and Morgan [44]. Fig. 5 shows the trapping of a 92 nm diameter latex sphere. Trapping in this manner is of particular interest, since it allows single particles to be isolated without resorting to invasive physical or chemical methods.

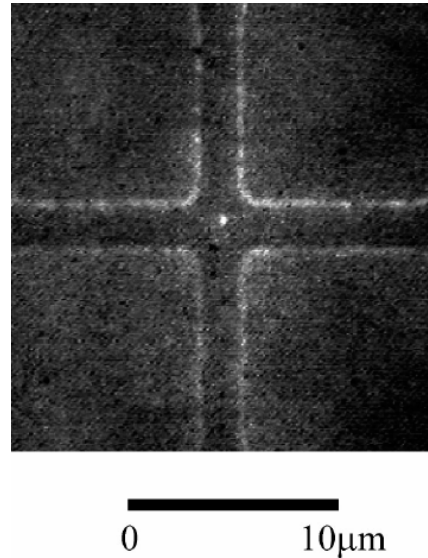


Fig. 5 Trapping of a 93 nm diameter latex sphere [44].

Similar trapping has been shown by other researchers [9,45,46]. Müller and co-workers [45] designed a quadrupole electrode array to expect to trap 650 nm latex beads, but to their surprise, they were able to trap particle as small as 14 nm. This prompted them to question the minimum particle size that can be stably trapped and the role of electrohydrodynamics. It was later proved by other researchers that the minimum radius is proportional to 1/3 of the trap width and the gradient of the electrical field [44]. It was later presented that electrohydrodynamic is accounted for to the discrepancy above, where electrothermal dominates at high frequencies, and AC electroosmosis dominates at low frequencies [46].

However, such quadrupole microelectrode structures are not a closed trap. It has an open top and gravity is responsible for the downward force holding the particle on the surface. Particles with near neutral buoyancy are less likely to be held in the trap by gravity. A closed trap can be made using two polynomial electrodes placed one above the other, to produce an octopole [47]. Surface of constant dielectrophoretic force potential for octopole electrodes is shown in fig. 6.

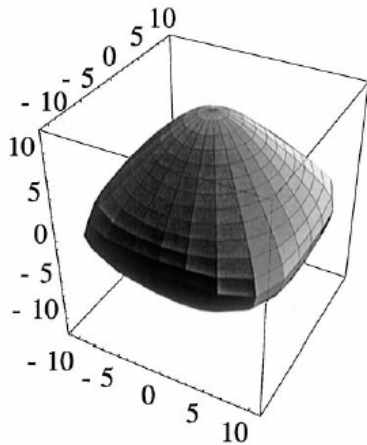


Fig. 6 The calculated surface of constant force potential [47].

An improved design of such particle trapping system is demonstrated by Voldman, who created extruded-quadrupole electrode array [48,49]. It consists of a set of four metallic gold posts, as shown in fig. 7. Their measurements show that it can confine particles over 100 times more strongly than a planar counterpart, yet it allows the flow-chamber height and trap geometry to scale independently.

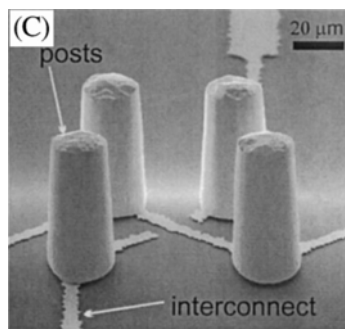


Fig. 7 Extruded-quadrupole electrode [49].

Although particle trapping has been demonstrated, it is still not an easy task to trap only one single particle. For instance, Hughes has observed more than one cell trapped in the quadrupole electrodes [44]. Similarly, Voldman has noted that several of the traps in the extruded-quadrupole electrode array contained two cells, instead of one [48]. Voldman argued that this can be improved by choosing a more stringent operating condition, and can be addressed with a closed-loop electrical sensing scheme.

It is also important to note that particle trapping is not suitable when the particles are experiencing positive dielectrophoresis. The dielectrophoretic force will pull the particles away from the center and immobilized it at the electrodes. Some researchers have tried to overcome this problem by using feedback control system [50].

### VIII. PARTICLE SORTING

A major development in particle handling has occurred through the integration of several types of electrokinetic particle trapping and manipulation devices into one

microchip [51,52]. Such device [52] consists of two layers of electrode structures separated by a 40  $\mu\text{m}$  thick polymer spacer forming a flow channel. The electrode elements are formed by funnel, aligner, cage and switch; which are designed to focus, trap and separate eukaryotic cells or latex particles with a diameter of 10–30  $\mu\text{m}$ , see fig. 8. Each set of electrodes can be separately addressed with suitable AC fields and frequencies. Particles are suspended in an electrolyte of high conductivity such that the system operates under negative dielectrophoresis. In the experiment, efficient handling of particles could be achieved with flow rates up to 3500  $\mu\text{m}/\text{s}$ , with electrodes operated at 5–11 V and 5–15 MHz.

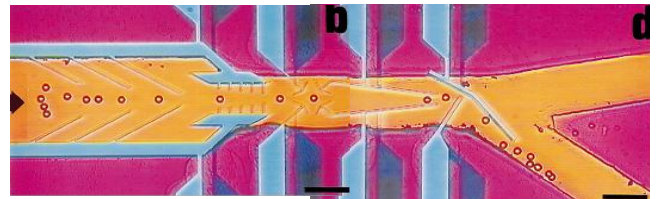


Fig. 8 A particle sorting and analysis system [52].

The field cage is the most critical design in this type of integrated system. Müller and co-workers [52] showed a dependency of critical voltage required to hold a latex particle in the cage subject to a laminar flow. A decrease of the amplitude resulted in displacement of the particle from the field minimum (along the  $x$ -axis) up to a point where the particle left the cage due to the applied flow. This poses a critical problem to such design, where the dimensions of the cage must be optimized for the size of the particles which are to be handled by the system, and greatly limits the type of particles that can be processed.

However, such particle sorting system is restricted by its processing time, since it treats one particle at a time. A different type of particle sorting system is proposed by Voldman [48]. It consists of an array of extruded-quadrupole electrodes as shown in fig. 7. The system can simultaneously load, interrogate, and sort an ensemble of single cells.

### IX. CONCLUSION

Dielectrophoresis is a promising technology as a building block for lab-on-a-chip devices. It can be used to separate, transport, trap, and sort particles. The device can be easily fabricated using the existing microelectrode photolithography techniques. Particle manipulation is achieved by controlling the applied frequency, to change the direction of the movement of the particles. The design of the electrodes, the choice of the suspending medium, and the applied peak voltage can be pre-determined to optimize the operation of the device.

The next phase of the research in dielectrophoresis would most likely be focused on the integration of these individual manipulation techniques to form a complete lab-on-a-chip, where dielectrophoresis can be used to transport



and separate particles.

Hitherto, most of the techniques were demonstrated using particles having identical physical sizes. There is still no study to prove their capability in treating particles having different order of sizes. This is important considering that the sample to be analyzed, e.g. blood sample, might consist of particles with huge difference in sizes.

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