Chapter

Applications of Electrokinetics and Dielectrophoresis on Designing Chip-Based Disease Diagnostic Platforms

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Abstract

This chapter discusses the concepts of electrokinetics, dielectrophoresis and how they intertwine with other forces in microsystems to aid microfluidic disease diagnostics. Methods of obtaining the intrinsic electrical properties of biological materials are first discussed alongside the mechanisms governing the variations in the intrinsic properties when biological entities become diseased/infected. The procedure and importance of modeling and simulation of disease detection platforms prior to their fabrication and testing is also discussed. Fabrication techniques for low- and high-resource settings are presented as well. The various applications of the synergy of dielectrophoresis and electrokinetics for disease detection are discussed. The chapter will end with some novel ideas about the believed future directions of the electrokinetic methods for early, intermediate, and late-stage disease detection either as adjuncts of various existing diagnostic methodologies or as a stand-alone diagnostic alternative.

Keywords: electrokinetics, dielectrophoresis, microsystems, disease diagnostics, dielectric properties

1. Introduction

Electrokinetics (EK), as the name implies, is a technique of using electric field to cause motion. The motion can be that of a liquid or colloidal particle (microscopic solid particles suspended in a fluid). This concept of electrokinetics has four main features: Electro-osmosis (EO), electrophoresis (EP), streaming potential (StP), and sedimentation potential (SeP). In the disease diagnostics arena—an integral part of the application of microfluidics in healthcare, electro-osmosis and electrophoresis are the main electrokinetic forces that particles experience. Electro-osmosis involves the bulk motion of a liquid through a solid surface under the influence of electric field. On the other hand, electrophoresis is the motion of a solid material through a liquid under an electric field effect. Both electrophoresis and electro-osmosis require that the surface of the solid be charged. As shown in **Figure 1**, when the surface of the particle, placed within a uniformly distributed electric field, is positively charged (A), the particle (suspended in a characterized liquid) moves to the left but to the right (against the field direction) when the charge on the surface

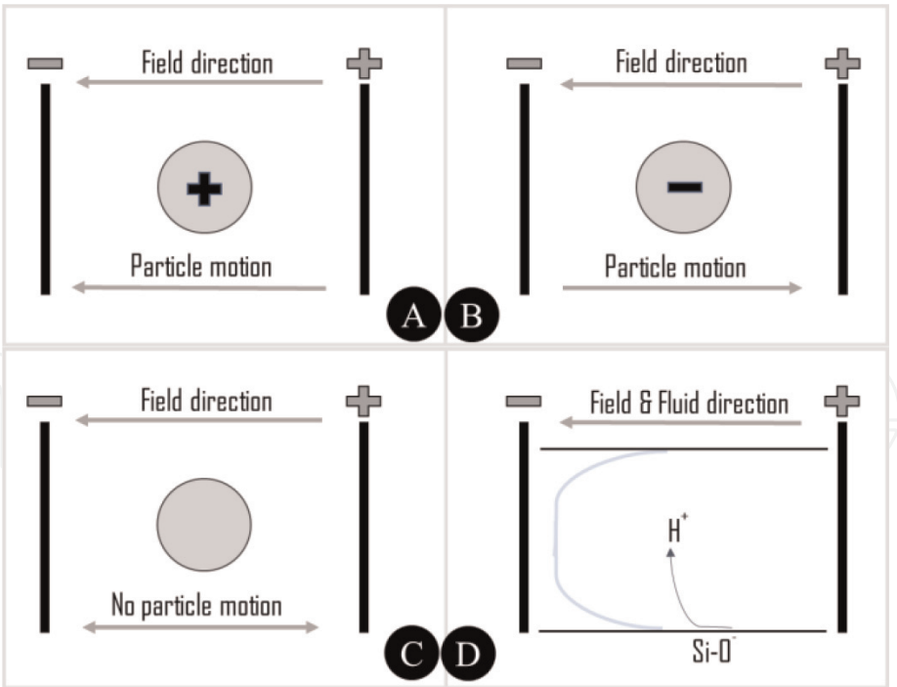

Figure 1.
The representation of electro-osmosis and electrophoresis as the two main electrokinetic phenomena in microfluidic channels: (A) positively charged particle under a uniform field region effect will move toward the negative electrode according to the fundamental attraction-repulsion laws. (B) Negatively charged particle placed under the same electric field region will migrate toward the positive electrode. (C) A no-charge (neutral) particle placed in the same field region will experience no electrophoretic motion because electrophoresis only applies to charged particles. (D) Representation of the deprotonation of the terminal (SiOH)/surface chemical group of the channel wall (treated poly(dimethyl siloxane) (PDMS) or uncoated glass), which births the electro-osmotic pumping within the channel.

is reversed (B). If the particle is a neutral body (C), no electrophoretic effect will be seen. **Figure 1D** is a case where liquid is flowing through a charged solid surface. The solid surface (usually glass or surface-treated polymer) become deprotonated when in contact with the liquid such that counter (positive) ions from the liquid goes into the channel surface to firmly replace the detached positive ions forming what is referred to as the Stern layer. Columbic force of attraction causes the Stern layer to, in turn, attract negative ions from the liquid forming the diffuse layer (together with some unattached positive charges) beyond which the region of electro-neutrality is initiated. A plane, called the slipping plane, formed around the loosely-bound diffuse layer is very crucial to how the bulk of the liquid would move when the electric field if applied across the static upstream and downstream flow region. The potential of this slipping plane is called the Zeta potential, ζ [1]. The same concept of Zeta potential applies to a charged solid moving through a liquid. For a charged solid moving through a liquid under an electric field effect, the rate of motion of the solid, r_{ep}, depends on the Zeta potential at its slipping plane, ζ_p, viscosity of the liquid in which it is moving, η, dielectric constant of the liquid, ε_m, as well as the strength of the applied electric field, E. Mathematically, this has been represented as

$$r_{ep} = f\left(\zeta_p, \varepsilon_m, \eta, E\right) = \left(\zeta_p \varepsilon_m E\right)/\eta \tag{1}$$

On the other hand, when a liquid is flowing through a charged wall of Zeta potential, ζ_w, the positional rate of flow of the liquid, r_{eo}, is given by

$$r_{eo} = f(\zeta_w, \varepsilon_m, \eta, E) = -\zeta_w \varepsilon_m E)/\eta \tag{2}$$

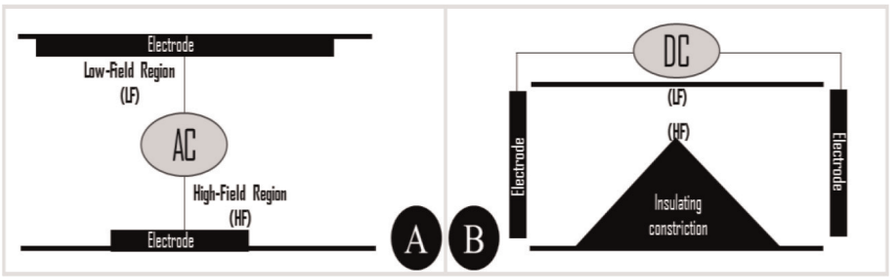

Figure 2.
Generation of non-uniform electric fields for dielectrophoretic applications; (A) simple unequal spatial electrode-pair arrangement connected to an AC source with the lower electrode acting as the high-field region while the upper electrode on the low-field region. The field low-high regions remain the same even when the electrode terminals are interchanged owing the frequency component of the AC source (B) insulating constriction (or obstacle) changed the uniform field between the electrodes to non-uniform field at its location.

In dielectrophoresis (DEP), the applied electric field must be non-uniform. The movement of the particles in non-uniform electric field does not depend primarily on the particle charge but on the ability of the particle to become polarized relative to that of the suspending medium [2].

Therefore, DEP force is always in effect when the particle is charged or not. This means, dielectrophoresis, therefore, safely be defined as a technique of using non-uniform electric field to induce the motion of a charged or an uncharged particle. Electric field can be rendered non-uniform in different ways. **Figure 2** shows some examples of how to generate non-uniform electric fields using AC or DC source. For the AC field, a simple unequal spatial electrode-pair arrangement (**Figure 2A**) would generate non-uniform field (AC DEP or classical DEP). A second method is to place an insulating constriction within a simple straight channel operating under DC condition that would make the field non-uniform (DC-iDEP or iDEP). There is never any hard and fast rule regarding how the electrodes should be arranged or the constrictions be distributed but simulation could assist in formulation of the device architecture. Every researcher has a purpose in mind and that purpose drives the architecture of the channel or device without denying the underlining physics.

2. Characterization of biological cells

Characterization of biological entities like cells involves the utilization of various methods including but not limited to electrical, magnetic, acoustic, and optical characterization to explore cell properties. In this section, electrical method will be discussed (with focus on dielectrophoresis) since dielectrophoretic force is related, in part, to the electrical properties of the biological cell. In the utilization of electrical method for bioparticle characterization, it is not uncommon to use impedance cytometry, dielectrophoresis, and electrorotation. Impedance cytometry works on the principle that when a particle suspended in a conductive fluid passes through a small orifice (comparable to the size of the particle) created by two electrodes, the passage of the particle through the (usually AC) electric field between the electrodes results in the generation of electric signal, which can be processed to provide valuable information about the electrical properties of the particle. In electrorotation, four electrodes are each charged with AC voltage of different phases to generate a rotating electric field, thus setting up an electrical torque. When a (spherical) particle is placed within this rotating field, it becomes polarized inducing a dipole. The dipole moment induced within this particle rotates with the electric field at certain velocity. However, the multiphase nature of the four electrodes causes the particle to lag behind the field by a factor that depends on the

frequency of the rotating field. Since the particle velocity is determined by the torque in the rotating electric field, electrical properties of the particle can be extracted measuring the dependence of the torque on the field frequency. With dielectrophoresis, the case is different. When a charged or uncharged spherical particle is placed between an unequally dimensioned AC electrode-pair which is generating non-uniform electric field, (**Figure 2A**) the particle becomes polarized just as the medium in which the particle is suspended [3]. The particle could then move toward the region of high field (HF), low field (LF) or remain unperturbed by the field depending on the properties of the applied electric field, suspending medium and the particle itself. When the particle moves toward the HF region, the phenomenon is termed positive dielectrophoresis (pDEP) while it is called negative dielectrophoresis (nDEP) if the particle's translational motion is toward the LF region [4]. Usually, when a particle is experiencing nDEP, for instance, it does so over a range of frequency. As the frequency changes further, the particle can translate to the pDEP regime. Before this happens, however, at a specific point of inflection where the particle comes to a halt before changing regime must have been reached. The frequency at such point of inflection is termed crossover frequency. At the crossover frequency i.e. after the application of the AC electric field parameters, the particle is only seen vibrating at a spot without any appreciable translational motion. At this point, the particle experiences no DEP force (F_{DEP} = 0).

$$F_{DEP} = 2\pi r^3 \varepsilon_0 \varepsilon_m Re\left[f_{CM}\right] \nabla E^2 = 0 \tag{3}$$

where $f_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}$ connecting both permittivity and conductivity (electrical properties) of both the particle $(\varepsilon_p, \sigma_p)$ and its suspending medium $(\varepsilon_m, \sigma_m)$ respectively.

This implies that the real part of the Clausius-Mossotti (CM) factor, $Re\left[f_{CM}\right] = 0$.

That is, $Re\left(\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right) = 0$ where $\varepsilon_i^* = \varepsilon_i - j\frac{\sigma_i}{\omega}$

Hence, $Re\left[\frac{\left(\varepsilon_p - j\frac{\sigma_p}{\omega}\right) - \left(\varepsilon_m - j\frac{m}{\omega}\right)}{\left(\varepsilon_p - j\frac{\sigma_p}{\omega}\right) + 2\left(\varepsilon_m - j\frac{\sigma_m}{\omega}\right)}\right] = 0$

$$\mathrm{Re}\left[\frac{\left(\varepsilon_\mathrm{p} - \mathrm{j}\frac{\sigma_\mathrm{p}}{\omega}\right) - \left(\varepsilon_\mathrm{m} - \mathrm{j}\frac{\mathrm{m}}{\omega}\right)}{\left(\varepsilon_\mathrm{p} - \mathrm{j}\frac{\sigma_\mathrm{p}}{\omega}\right) + 2\left(\varepsilon_\mathrm{m} - \mathrm{j}\frac{\sigma_\mathrm{m}}{\omega}\right)} \mathrm{x} \frac{\left(\varepsilon_\mathrm{p} - \mathrm{j}\frac{\sigma_\mathrm{p}}{\omega}\right) + 2\left(\varepsilon_\mathrm{m} - \mathrm{j}\frac{\sigma_\mathrm{m}}{\omega}\right)}{\left(\varepsilon_\mathrm{p} - \mathrm{j}\frac{\sigma_\mathrm{p}}{\omega}\right) + 2\left(\varepsilon_\mathrm{m} - \mathrm{j}\frac{\sigma_\mathrm{m}}{\omega}\right)}\right] = 0 \tag{4}$$

$$Re\left[\left(\varepsilon_p - j\frac{\sigma_p}{\omega}\right) - \left(\varepsilon_m - j\frac{m}{\omega}\right)\right]\left[\left(\varepsilon_p - j\frac{\sigma_p}{\omega}\right) + 2\left(\varepsilon_m - j\frac{\sigma_m}{\omega}\right)\right] = 0 \tag{5}$$

$$\begin{aligned}&\varepsilon_p^2 + \varepsilon_m\varepsilon_p - j\frac{\varepsilon_p\sigma_p}{\omega} - 2j\frac{\varepsilon_p\sigma_m}{\omega} - 2\varepsilon_m^2 + j\frac{\varepsilon_m\sigma_p}{\omega} + 2j\frac{\varepsilon_m\sigma_m}{\omega} + j\frac{\varepsilon_p\left(\sigma_m - \sigma_p\right)}{\omega}\\ &+ 2j\frac{\varepsilon_m\left(\sigma_m - \sigma_p\right)}{\omega} - j^2\frac{\sigma_p\left(\sigma_m - \sigma_p\right)}{\omega^2} - 2j^2\frac{\sigma_m\left(\sigma_m - \sigma_p\right)}{\omega^2} = 0\end{aligned} \tag{6}$$

Setting the real part to zero with $j^2 = -1$ gives $\varepsilon_p^2 + \varepsilon_m\varepsilon_p - 2\varepsilon_m^2 + \frac{\sigma_p\left(\sigma_m - \sigma_p\right)}{\omega^2} + 2\frac{\sigma_m\left(\sigma_m - \sigma_p\right)}{\omega^2} = 0$, $\exists\ \omega = 2\pi f_{co}$ with f_{co} as the crossover frequency, then using the identity; $a^2 + ab - 2b^2 = (a - b)(a + 2b)$, we can rearrange to obtain f_{co} as

$$f_{co} = \frac{1}{2\pi}\sqrt{\frac{\left(\sigma_p - \sigma_m\right)\left(\sigma_p + 2\sigma_m\right)}{\left(\varepsilon_p - \varepsilon_m\right)\left(\varepsilon_p + 2\varepsilon_m\right)}} \tag{7}$$

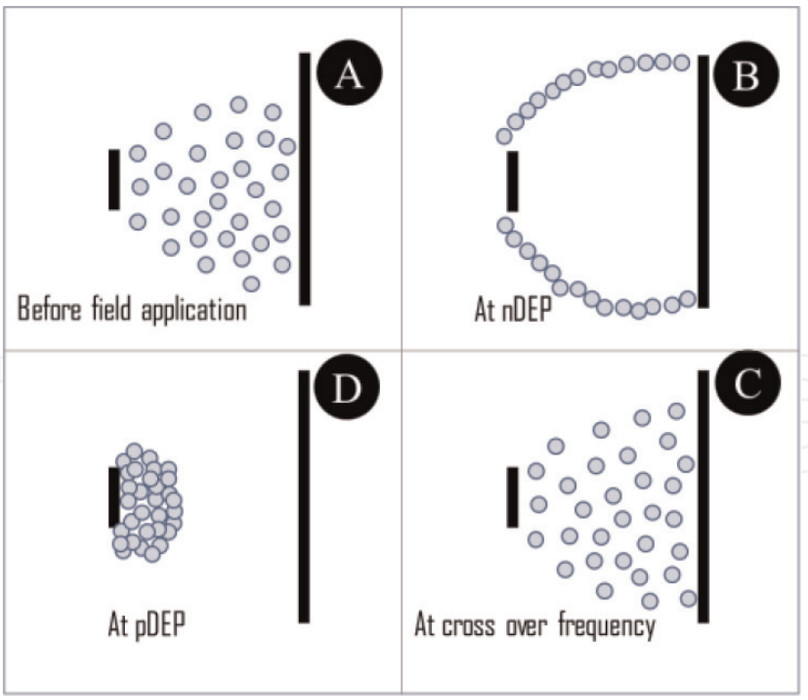

Figure 3.
Images of the particle behavior under different field frequencies. (A) Particles well dispersed before field application. (B) Particles undergoing nDEP forming chains with increased particle-particle interaction. (C) Particles at cross over frequency; no response to the dielectrophoretic force. (D) Particles clinging to the high-field region depicting pDEP.

Eq. (7) represents a simplified presentation of the first crossover frequency (f_{co}) of a particle in relation to the permittivity and conductivity (electrical properties) of both the particle (ε_p, σ_p) and its suspending medium (ε_m, σ_m) respectively. To extract the electrical properties of the particle, it is common to obtain a data set comprising of varied medium conductivity and hence, varied crossover frequency.

The conductivity-frequency data is then fitted with the appropriate model representing the biological materials of interest. Human red blood cells (RBCs), for instance, can be modeled as a bag of cytoplasm have an insulating plasma membrane. This type of model is popularly referred to as the single-shell model [5, 6]. **Figure 3A** shows the representative images of nDEP, pDEP, and crossover states for RBCs at a given AC amplitude and sweeping frequencies. Experiments resulting in these images are generally conducted by suspending the particles in an isotonic medium (5% dextrose and fed into a reservoir sealed onto a borosilicate glass with an interfacial 90°, low-separation electrode-pair connected to an arbitrary waveform generator. At a fixed amplitude output, the frequency of the AC field was varied until the crossover frequency was reached and surpassed. The conductivity of the suspending medium was then sequentially increased using phosphate-buffered saline (PBS) or other conductivity conditioners and at each increase, the corresponding crossover frequency was obtained. For other bioparticles such as the nucleated white blood cells and bacteria, the double-shell and three-shell models can be applied respectively. Detailed shell analysis for biological cells is available in diverse literatures [7–10].

3. Modeling and simulation of microdevices

The popularity of six sigma DMAIC (define, measure, analyze, improve, and control) approach has made more companies to embrace extensive modeling and

simulation in order to save cost and time. In the production of chip-based disease diagnostic platforms, numerical computation is seen as an important process step as it affords the flexibility of exploring how various parameters affect device performance without the need for extensive experimental research. In cases where experimental activities are needed to improve numerical computations, the utilization of the design of experiment principles is usually a wise choice. In the design of experimental principles, ability to obtained related factors, which can be confounded optimally is a key component of the cost and time-saving strategy. Microfluidic devices for disease diagnostics can be operated using AC or DC sources. Discussion in this section will be based on DC operations. Details of AC-operated designs have been given elsewhere [11–13].

In DC models for disease diagnostics, electro-osmosis and dielectrophoresis are usually the electrokinetic mechanism that governs the transport phenomena prior to the region of electric field non-uniformity within the channel [14]. At the region of non-uniform electric field, the dielectrophoretic force is combined with these electrokinetic forces to bring about the desired particle differentiation either through trapping (pDEP) or streaming (nDEP). A smart idea, then, is to locate the exit channels close to the region immediately following the field gradient so that sorted bioparticles can be collected appropriately. Failure to do this might result in the recombination of streamlines, which tend to restore the separated cells to their ab initio states.

Models of the envisaged diagnostic devices are usually drawn to scale using any suitable software. (AutoCAD, SolidWorks, *etc.*). These models are then interfaced with multi-physics simulation software such as COMSOL Multiphysics, FLUENT, to explore parameter dependence and their effects of targeted outcomes. It is not uncommon to draw the models using the functionalities available in the simulation software themselves. One requirement to emphasize here is the sound knowledge of the physics governing the operations of the diagnostic device. These inexhaustible physics are discussed in this section. For insulator-based (iDEP) diagnostic devices, the physics usually involve momentum transport (Newton's Law), mass transport (Fick's Law), energy transport (Fourier's Law) and charge transport (Maxwell's Laws/Ohm's law) [15].

Momentum transport involves the transfer of momentum from one particle to another. This transfer results in the continuous change in fluid's positional space leading to the concept of fluid dynamics (hydrodynamics for liquids). Depending of the focus of any project, momentum transport can be explored in 1D, 2D or 3D. One good approximation in iDEP devices is that the complexity of 3D consideration can be avoided by using 2D analysis provided the channel's width-to-depth ratio is about 5:1. The 3D to 2D approximation is also good on the basis that turbulence is not a common occurrence in micron-sized devices. The governing equations for momentum transport are generally the Navier-Stokes and mass continuity equations [16] (Eqs. 8 and 9). Navier-Stokes equation is usually reduced to Stokes equation when the continuity equation is applied at static conditions under the assumption that the Reynolds number is very low. This makes the computer solve, numerically, for the pressure and velocity distributions within the channel. Prior to obtaining the pressure and velocity profiles, appropriation boundary conditions are utilized to completely define the system. In iDEP operations, electroosmotic wall is specified in lieu of the 'no-slip' wall condition. This is because, in electro-osmotic flow, the bulk motion of the fluid is driven by the wall effects - a phenomenon termed electro-osmotic pumping. Electro-osmotic pumping makes the use of external pumping mechanism unnecessary. The boundary mainly utilizes the electric field solution obtained from the second physics (electric current node in, for instance, COMSOL Multiphysics) which is usually solved together with the fluid

flow equations in stationary mode. The electric current module requires that the electric potential be specified in addition to electrical insulating boundaries. The spatial distribution of the electric field strength with the channel reveals that when constrictions are placed within a uniform microchannel, the effect is non-uniformity in field strength. This non-uniformity is usually seen using, in COMSOL Multiphysics for instance, color pallet or legend. As theory suggests, dielectrophoretic force acts only at the region with field gradient (i.e. at the constriction(s)). Suffice it to say that the solved electric current equations in iDEP systems usually include Ohm's law, electric displacement and the charge conservation.

After solving the fluid and electric current equations in stationary mode to obtain the distributions of velocity, pressure and electric field within the diagnostic device, it is expedient to visually and quantitatively verify if the sorting or trapping process results in the desired output. Two approaches are possible; (1) using Fick's law of diffusion to classify the components of the mixture as unique tubes through the transport of diluted species module or (2) using a balance of viscous drag and dielectrophoretic force through particle tracking module. In the former, it is expected that the concentration gradient between the inlet and the outlet ports may cause diffusion and when the electro-osmotic flow is initiated for bulk fluid motion, convective flux also comes into play. This implies that the total particle flux can be expressed as the sum of diffusive, EP, DEP and convective flux:

$$N_i = -D_i \nabla c_i + \left(u + \mu_{EP,i} + \mu_{DEP,i} \nabla E^2\right) c_i \tag{8}$$

and

$$\frac{\partial_{ci}}{\partial t} + \nabla . N_i = R_i \tag{9}$$

where $\vec{u}$ is the hydrodynamic velocity vector, D_i is the diffusivity of the particle, $\vec{E}$ is the electric field applied, and μ_{EP} and μ_{EP} are the EP and DEP motilities respectively. The DEP mobility is a function of CM factor and for a spherical particle it is expressed as:

$$\mu_{DEP} = \frac{\pi d_p^2 \varepsilon_m}{12\eta} f_{CM} \tag{10}$$

where d_p is the particle diameter and η is the medium viscosity. In the latter, the drag force is given as; $F_{drag} = \frac{9\eta}{2\rho_p r_p^2} m_p (u - v)$ which is balanced with the DEP equation (Eq. 3).

4. Microdevice fabrication

Microdevice fabrication has traditionally been through the lithographic and etching process. In iDEP devices constructed with polymer, a combination of lithography, etching and rapid prototyping is usually utilized. While lithography prints the patterns on the substrates (glass or silicon wafers), etching creates the grooves on those patterns and rapid prototyping transfers the substrates patterns to polymer. The lithography process starts with the printing of the patterns made in the modeling and simulation stage. Usually, the patterns assisted by laser or electron-beam (and other process steps) are printed on a transparent-opaque pair plate which could further transfer the printed patterns to a resist-coated substrate in

the next stage of the microfabrication process. The plate is transparent where light is desired to pass but opaque where it is undesirable. This transparent-opaque pair plate is referred to as photomask. The architecture of the photomasks determines the final patterns (convex or concave, depending on the type of photoresist used) on the substrates after photoresist development, depending on the type of photoresist on the substrate. **Figure 4** shows the two forms of photomasks commonly used in iDEP device making process.

Details of the process steps involved in photomask printing have been given by Mack [17]. One important point to note is that mask making process is probably the most important step in microfabrication. Any error associated with dimensions during this mask-making process will propagate through the whole microfabrication process. In iDEP devices, all dimensions (especially at the constrictions regions) are critical. A change in the fillet angle, for instance, can affect the gradient of the electric field within the device and this could, in turn, change the efficiency of the device. Following the mask-making process is the cleaning of substrates onto which the mask patterns will be transferred. Cleaning substrate can be achieved through chemical (acetone, ethanol) or physicochemical (plasma cleaning) means. In some cases, cleansed substrates are treated with adhesion promoting agents such as silane, organotitanates, organozirconates and their derivatives. These coupling agents tend to act as binders between the substrates and the photoresist. Depending on whether concave or convex patterns are desired, positive or negative photoresists are spin-coated on the cleansed substrates at some pre-determined spin coating parameters to ensure uniform surface roughness and film thickness and prevent speckle or void formation. Following the spin coating process is the low-temperature baking step. This is the step that removes some of the solvent in the resist itself. Lowering the solvent content of the film is essential to preserve its integrity at room temperature since moisture content of the film could change the film properties, enhance contamination and thus damage the entire lithographic process. It is important to note that this baking step, which tend to lower the moisture content of the film, could reduce film thickness. It is therefore expedient to factor this effect into consideration when quantifying the resist volume and spin coating parameters prior to coating. A pre-baked resist-coated substrate is ready for UV exposure after the photomask has been correctly aligned with its positional space to circumvent any overlay. The dosing period of the UV depends, amongst other factors, on its intensity, the type, and percentage composition of the photoactive component of the resists. Usually, photoresists manufacturers provide the guidelines for UV exposure using certain substrate (usually silicon-based). Using a different substrate from the manufacturers' test substrate might have its own effect on the critical dimensions of the microstructure. If positive photoresist is used (together with mask A in **Figure 4**), it is expected that the exposed region will be less soluble owing to the change in the chemistry of the resist after exposure to ultraviolet (UV) light. Post-exposure baking and development of the resist will create the pattern vials which act as guides for the etchants during the etching

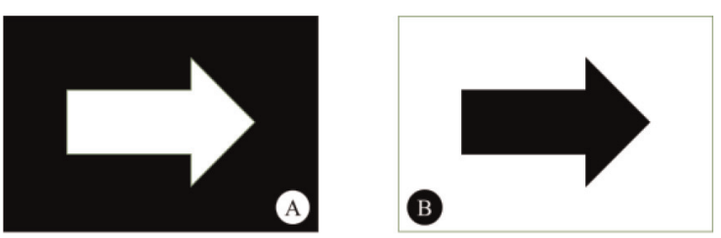

Figure 4.
Different forms of mask used in photolithography. (A) The mask intend for positive substrate photoresist and (B) the mask for negative photoresist.

process. After UV exposure, the substrate is usually baked to create a final structure that would reduce undercut and improve selectivity during the etching process. After this post UV-exposure baking, the resist is developed to remove the part of the resist that have been weakened by the UV. Another type of resist called chemically amplified resist has also become more common. This resist is not weakened or hardened by the photo exposure. Rather, it generates some form of cationic entity that becomes amplified during the post exposure baking and thus makes development meaningful. As mentioned earlier, the structural integrity of lithographic features is partly a function of the UV dosage. Over exposure and under exposure should be avoided. After the resist development stage, it is important to conduct some form of metrology to verify the conformance of critical dimensions and check if any overlay has occurred because of substrate relocation or displacement during mask alignment. Critical Dimension-SEM is probably the best metrological undertaking. As much as possible, the use of laser profiling system should be avoided as the collimated laser tend to form unwanted patterns on the resist. Once the dimensions of the features have been verified and defects such as edge effects have been substantially avoided/minimized, the substrate is then moved to the etching phase. Etching is the removal of some parts of the substrate as guided by the resist patterns. There are two main types of resist: Dry and Wet. Wet etching usually in HF or its mixture with HNO_3, i.e., HF/HNO_3 mixture has been traditionally used as etchant. However, with the Moore's law becoming obsolete and lithographic features turning smaller day by day, dry/plasma etching are becoming preferable. After etching, some metrological analysis could also be made to check the efficiency of the etching process. Once the etched substrate has been certified okay, the next stage is the transfer of the etched patterns to the polymer. In iDEP devices, several polymers can be used for the patterning, but silicone elastomer (PDMS) seems to be more widely accepted. By mixing silicone with its curing agent in the prescribed ratio (10:1), an air-ridden mixture whose air component can be removed through vacuum-degassing operation in any closed container is formed. Degassed mixture could then be poured onto the etched substrate (herein referred to as the master), cured thermally or at ambient conditions and systematically peeled off the master, diced, punched and completed with electrodes for dielectrophoretic experiments. Depending on the goal of the researcher, external devices such as syringe pump, microsensors, imaging line, *etc.* can be attached to the iDEP device. The completion of the device fabrication stage is the hydrophobic ceiling of the cured PDMS to glass or other PDMS material. PDMS is known to be hydrophobic owing to its terminal Si-O-CH_3 bond. This terminal bond will prevent wall-surface ionization which is necessary to initiate electrodynamic flow within the channel. There are several ways to treat this hydrophobic wall in order to render it hydrophilic by changing the Si—O—CH_3 bond to Si—O—H. One common way is plasma oxidation. The plasma oxidation process involves using the radio frequency (RF) generated by changing magnetic field under low-pressure and certain gas to produce plasma, which is a mixture of electrons, atoms, molecules and other components depending on the nature of the gas used. It is believed that certain component of the plasma is responsible for the surface reaction which replaced —OCH_3 with OH terminal. After surface treatment, high surface energy of the PDMS allows its bonding to glass/other treated PDMS and also prevents the suspending medium from beading up within the microfluidic channel thus preventing air bubbles from being trapped. To ensure that the sealing is perfect (i.e. the sealing is leak proof), the sealed reservoir can be filled with deionized water and anhydrous copper sulfate spread around the sealed interface. The whole set up is then placed in a controlled environment for confirmatory test. If the white color of the sulfate turns blue at the end of the test period, then sealing is not leak-free, and the device should be discarded.

It is important to do this check because leaking device would lead to an untold operation error since the leakage will induce pressure gradient which will belie the desired electro-osmotic flow within the channel.

5. Disease detection

Sections 1–3 have detailed how bioparticles can be characterized, iDEP devices modeled/simulated and how the numerically obtained device architecture can be fabricated using lithography and transferred to polymer using prototyping. This section will focus more on testing the fabricated devices and comparing its efficiency with the numerically obtained one. The experimental process of disease detection using iDEP devices starts with preparation of the bioparticles for DEP experiment. In some cases, the bioparticles of interests are cultured while in other cases, they are obtained and tested directly from donors, stored temporarily and worked on. Several other methods exist for obtaining samples. Irrespective of the method of obtaining the bioparticle samples, one important factor to consider before experimentation is the properties of the suspending medium. It is customary in iDEP experimentations to suspend bioparticles in a medium prior to feeding them into the inlet reservoir. During the operation of the iDEP device, it is necessary to prevent Joule heating or electrolysis in the iDEP devices by using low-conducting medium. Preventing Joule heating circumvents, amongst other things, any form of recirculation within the channel especially at the post-constriction regions [7]. Knowing that cells are cultured in relatively conducting medium at pH range that depends on the nature of the bioparticles, it is necessary to pre-treat the samples and make them fit for dielectrophoretic experimentation. The pH value of the suspending medium affects the Zeta potential of the bioparticle as well as that of the iDEP channel wall. These will in turn affect the electro-osmotic and electrophoretic contributions to the flux of particles within the channel. Apart from the medium pH, its tonicity (which is related to its osmolality) is also important. A hypertonic medium causes plasmolysis while a hypotonic result in cell turgidity. According to Eq. 3, the dielectrophoretic force experience by bioparticles depends, amongst other factors, on the size of the particle. If the suspending medium is hypertonic, it results in an increased particle radius whose effect on the DEP force is to the third power. Reduction in particle radius when the particles are suspended in a hypotonic solution has a reversed effect on the DEP force. For this reason, isotonic solution may be the more appropriate medium for preserving the cell size since there is no net movement of fluid when the cells are suspended. 5% dextrose and 0.9% NaCl are considered isotonic medium and are therefore good choices for iDEP experiments. Using direct current (DC) to generate electric field for iDEP experiment requires that one leverage the membrane characteristics of the bio-particles in context. This is because DC manipulation of cells cares less about the variation in field frequency which is the main factor in AC fields (higher AC frequency tends to put cytoplasmic contributions into account). According to the complex permittivity relation, $\tilde{\varepsilon}_i = \varepsilon_i - j\frac{\sigma_i}{\omega}$, the first term on the right represents, for a cell membrane, the polarization component of the dielectric (membrane) while the second component represent the dielectric loss component. Since DC and low-frequency AC fields seem to be comparable in terms of their effects on biological cells, it is expected that dielectric loss might be an issue. Primarily, a healthy cell has a membrane that separates its intercellular contents from its outer environment. Situated within the membrane are channels that allow certain ions to be exchanged between the internal and external environment of the cell depending on their concentrations on either side of the membrane. The disparity in the concentration-pair results in

membrane potential which is usually maintained as resting membrane potential through ion leakage and pumping. This potential difference places certain capacitance on the membrane relative to the charges associated with both sides of the membrane. At the onset of infection (intracellular infection, for instance), the damage to the membrane causes excessive ion change, which results in a change in the capacitance of the membrane. If the infection had engendered some proteins to be expressed on the cell membrane, that would have disrupted the charge distribution on the external environment contiguous to the membrane resulting in a different membrane capacitance. This difference is enough to make healthy and infected move into different paths when placed in a dielectrophoretic micro channel [18]. When infected and healthy cells move to diverse exit ports, they can be easily quantified and/or qualified. Common qualification technique involves post separation microscopic evaluation. This might involve cell staining and fluorescence characterization. In some cases, post separation qualification is achieved by integrating sensors i.e. measuring impedance or capacitance along the exit channels. These sensors work in a similar fashion to impedance cytometry. When separated a particle passes through the gap between the sensor, spectra are generated from where the nature of the particle is verified and compared with any baseline spectra. For identifying extracellular infection in human blood, for instance, there is a need to primarily isolate the blood plasma and then explore the possibility of singling out the pathogen. Lyme disease is best detected this way, but the process can be very challenging due to the complexity of the plasma component.

6. The future of dielectrophoresis

Having walked through the essential steps involved in the design, fabrication and testing of iDEP disease diagnostic device, a very pertinent question remains: where is this dielectrophoretic analysis heading to? iDEP is currently the choicest dielectrophoretic methodology for bioparticle separation/disease diagnostic purposes. This method could still be classified as being in the teething stage going by the number of research work that are published, especially since 1992. Therefore, iDEP is poised do more than separation and detection of bioparticle. For instance, bioparticle are currently being characterized using electrode-based (AC) dielectrophoresis. iDEP can be used to replace this age-long method through directional etching of glass substrate. This will boost the expansion of AC-DC DEP for simultaneous particle characterization, separation and detection. On glass, groove can be etched systematically and conducting metals, such as copper, can be electrochemically deposited.

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Conflict of interest

The authors declare no conflict of interest.

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