Simple, Cost-Effective and Continuous 3D Dielectrophoretic Microchip for Concentration and Separation of Bio-Particles†

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ABSTRACT: Dielectrophoresis is a robust approach for manipulating bio-particles in microfluidic devices. In recent years, many groups have developed dielectrophoresis-based microfluidic systems for separation and concentration of various types of bio-particles, where the gradient of the electric field causes dielectrophoresis force acting on the suspended particles. Enhancing the gradient of the electric field with three-dimensional (3D) electrodes can significantly improve the efficiency of the system. Implementing planar electrodes in a 3D arrangement is a simple option to form a 3D-electrode configuration. This paper reports the development of a novel dielectrophoretic microfluidic system for continuously manipulating microparticles such as polystyrene microbeads and *Saccharomyces cerevisiae* cells. The fabrication process was relatively simple, cost-effective and precise. Moreover, the device was tested to find the impact of various parameters on the concentration of polystyrene microbeads and separation of live and dead cells. The optimum working conditions, including flow rate, applied voltage amplitude, and frequency, were obtained accordingly. Furthermore, the experimentally observed trajectories of the particles agreed well with simulated counterparts. The device was able to efficiently and continuously perform with high throughput. Under an optimum condition, an efficiency of approximately 100 % was obtained, confirming the capability of the proposed design with four triangular electrodes for continuous focusing and separation of live and dead cells as well as polystyrene particles.

1. Introduction

Separation and concentration of cells and particles are of great importance in engineering and biotechnology. With the advent of lab-on-a-chip devices and owing to their small length scale, these platforms are widely used for selective manipulation of microparticles in various

applications. Many methods have been employed to manipulate bio-particles suspended in a solution. Dielectrophoresis (DEP) is one of the most common techniques used by different research groups for this purpose.¹ DEP offers label-free, rapid, high purity and controllable manipulation of microparticles. The interaction between the particle and the non-uniform electric field leads to a DEP force that depends on the dielectric properties of the particle.² Thus, DEP response depends on particle characteristics such as dimensions, structure, viability, morphology and chemical properties.³ For this reason, DEP is both highly sensitive and selective.

Both AC and DC electric fields can be used for DEP.⁴ Thus, time-averaged DEP force is calculated in AC applications. Several geometries have been proposed for both the microchannel and the electrode, which are used to form a non-uniform electric field within the fluid.⁴ The main purpose of designing DEP-based systems is deforming the electric field lines relative to the microchannel. This can be achieved by a specific geometry of the microchannel, or shape and position of the electrodes. Insulator-based DEP (iDEP) systems with no electrodes within the microchannel have become popular in recent years. Many successful separation applications with iDEP have been reported.⁵ In iDEP, the non-uniform electric field is formed by employing obstacles and curvatures within the microchannel and applying the field from the inlet to the outlet. Because of the relatively large channel length, a high voltage is needed to achieve the required DEP force. High-frequency AC voltage is not practical to implement, limiting the possible applications of a DEP-based system. On the other hand, DC or low-frequency field may generate undesirable electrokinetic phenomena within the microfluidic network.

In contrast to iDEP, DEP systems based on a microelectrode array use electrode arrangement to generate the desired non-uniform electric field. Three-dimensional (3D) electrodes enhance the gradient of the electric field, which in turn improves the capability of the system, but usually raises

its complexity and consequently its cost.⁶ This paper focuses on the use of 3D-electrodes in a DEP system and elaborate on their design and optimization.

A range of concepts have been proposed for 3D-electrode systems in microfluidic devices. The device containing microchannels were made of a variety of materials such as polydimethylsiloxane (PDMS) using soft lithography,^{3, 7, 8} PDMS membrane,⁹⁻¹¹ photoresist,¹²⁻¹⁵ adhesives,¹⁶⁻¹⁸ and etched glass or Pyrex.^{17, 19} Three-dimensional electrodes within the microchannel have been implemented through techniques such as lift-off,^{11, 20} etching of thin films,^{16, 21} carbon-based 3D electrodes,²²⁻²⁴ conductive PDMS,²⁵⁻²⁷ liquid electrodes,²⁸ contactless 3D electrodes,²⁹ 3D silicon electrodes,³⁰ and metal sided electrodes.^{3, 31}

Besides the above-mentioned methods to create a 3D-electrode system, there is also the option of making parallel thin film electrodes. Many research groups have taken the advantage of this particular method and demonstrated experimentally excellent device performance in manipulating bio-particles. Three-dimensional thin film electrodes have been utilized for capturing,²⁰ manipulating,^{11, 18, 32} accumulating,³² sorting,¹³ focusing¹⁶ and separating^{11, 12} the bio-particles. Some methods are developed to bond and to seal microfluidic devices. Martinez-Duarte et al.²³ assembled a dielectrophoretic chip, where a pressure sensitive adhesive (PSA) layer formed the fluidic network. Carbon pillars were utilized as 3D electrodes. By applying positive DEP (pDEP) force, the team managed to filter and focus *Saccharomyces cerevisiae* and *Drosophila melanogaster* cells. Cheng et al.¹⁶ introduced a high-throughput and continuous bio-particle sorter based on traveling wave DEP with embedded 3D paired electrode design and sorted different cell types successfully. A thin layer of UV-curable glue was spun and laminated to seal the top and bottom surfaces, which is a complex choice for bonding. Chen et al.³² presented a 3D paired microelectrode system for manipulation of Latex particles in different sizes. The authors used

epoxy-based adhesive to bond two glass substrates. Yasukawa et al.²⁰ used a thin polymeric film to bond the glass slides with planar electrodes to make a 3D symmetric electrode-system. Dür and colleagues¹⁸ studied DEP forces acting on micro and nanoparticles under the electric field of 3D face-to-face electrodes on the top and bottom surfaces of the channel. The authors employed a 3D dielectrophoretic deflector for size-based separation, where a standard microfabrication procedure (thin film lithography for electrodes and SU8 photolithography for microchannel) was used. Finally, the chip was packaged using ultrathin UV-curable adhesive layer by means of specific apertures. Using curable adhesive is a relatively expensive choice that only provides an average bonding strength.¹⁸ Furthermore, imperfect glue coverage and remained residuals are possible. The authors analyzed the performance of the system with latex particles under the negative DEP (nDEP) and applied the same approach to a heterogeneous immunoassay device.

Muratore et al.¹³ developed a chip with 3D quadruple funnel electrode arrays, which were aligned and bonded using SU8 photoresist. The team demonstrated a DEP sorting system to distinguish between myoblast and fibroblast cells. The same fabrication method was utilized by Haandbaek et al.¹⁵ for single-cell impedance cytometry and optical imaging. The team used 3D quadrupole triangular electrodes to focus *Saccharomyces cerevisiae* cells and polystyrene beads. The device was fabricated by bonding a SU8 structure to glass. It is worthwhile to mention that using SU8 structures increases the need for specialized material and clean-room equipment. Hsu et al.³³ utilized face-to-face asymmetric planar electrodes to apply nDEP force to particles. The top electrode was a glass substrate with indium tin oxide (ITO) coating. The bottom electrode was a patterned thin film electrode. A cover glass was placed between the top and bottom plates as a spacer. Glass spacer is not a good choice because it is **challenging to create microchannels**, and leakage is possible. Li and co-workers¹¹ presented a DEP-based device with 3D electrode pairs.

The team achieved continuous separation and concentration of polystyrene particles and yeast cells. In this setup, a PDMS membrane between the electrodes was patterned by laser ablation. The PDMS membrane has its limitations because of the need for specific equipment. Laser ablation of the PDMS membrane as the fabrication technique limits the dimension and geometry of the microfluidic network. Park and colleagues¹⁷ used semi-circular microchannel and 3D asymmetric electrodes. Because of the particular design, the magnitude of the electric field varied along the lateral direction of the channel. The shape of the microchannel was obtained by wet etching of a Pyrex wafer. This fabrication process is time-consuming and expensive as compared to other methods.

In this paper, we proposed a straightforward technique to fabricate 3D microelectrodes for the manipulation of bio-particles. Our method for integrating 3D electrodes has significant advantages over traditional methods due to its simplicity, speed and low cost of fabrication. The 3D distribution of the electric field is achieved by placing four symmetric 2D thin film electrodes in a quadrupole arrangement, obviating the need for common microfabrication processes such as photolithography, lift-off, and thermo-pressure bonding. The shadow mask method was employed to fabricate the electrodes. This fabrication method is rapid, of low cost and can be repeated for several times as compared to the conventional lift-off method. The sputtering process is needed for fabricating thin film electrodes. However, the amount of the consumed gold target is negligible due to the thin electrode layer. The PSA layers eliminates the need for photolithography, which requires specific clean-room equipment and materials. Soft lithography with PDMS is relatively time-consuming and expensive. With our method, the device can be fabricated in less than 3 hours.

2. Theoretical Background

2.1. Governing Equations

When an uncharged dielectric particle is subjected to a non-uniform electric field, the interaction of the created dipole and the spatial gradient of the electric field generates an external DEP force. This force depends on several parameters, including the particle characteristics, properties of the suspending medium and the test conditions. More importantly, frequency and distribution of the electric field, which are the results of the geometry and electric boundary conditions, directly affect the DEP force. The time-averaged vector of the DEP force exerted on a homogenous spherical particle is given as³⁴:

$$\vec{F}_{DEP} = 2\pi r^3 \varepsilon_m RE[f_{CM}(\omega)] \nabla E_{rms}^2, \quad (1)$$

where r is the radius of the particle, ε_m is the permittivity of the medium, $RE[f_{CM}(\omega)]$ is the real part of the Clausius–Mossotti (CM) factor, and ∇E_{rms}^2 is the root-mean-square (RMS) value of the time-averaged electric field. As we used a harmonic electric field, a fluctuating DEP force component exists. Thus, we used the time-averaged equation to evaluate the DEP force vector.³⁵ Contrary to electrophoresis, force direction in DEP does not change by changing polarity of the alternating electric field. In fact, the force direction only depends on the condition of negative or positive DEP. The use of time-averaged equation is not limited to dielectrophoresis. Researchers have been using time-averaged expressions in the presence of AC electric fields in other nonlinear electrokinetic phenomena such as induced-charge electrokinetics,³⁶⁻⁴¹ electroosmosis^{42, 43} and electroconvection.⁴⁴

Note that Equation (1) is only valid when the particle size is smaller than the characteristic length scale of the electric field,¹⁹ which is the case of the present work. In addition, Equation (1) is derived for ideal spherical particles. Although yeast cells are not perfectly spherical, they are generally approximated as spheres in the literature.^{34, 45} The term r^3 indicates that DEP force is

strongly dependent on the size of the particles. That is why DEP is a useful method for size-based separation of particles in microfluidic devices.^{3, 16}

CM factor, a complex number describing how the particle and the suspending medium are polarized, is given as³⁴:

$$f_{CM}(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}, \quad (2)$$

where subscripts p and m refer to particle and medium, respectively. For an alternating electric field, ε^* is complex permittivity defined as³⁴:

$$\varepsilon^* = \varepsilon - \frac{i\sigma}{\omega}, \quad (3)$$

where $i = \sqrt{-1}$, σ and ε are the real conductivity and permittivity values, respectively, and ω is the angular frequency of the applied electric field. The real part of the CM factor ($RE[f_{CM}(\omega)]$) varies between -0.5 and +1 depending on the values of permittivity, conductivity and the frequency. In regard to the value of the aforementioned parameters, when $RE[f_{CM}(\omega)]$ is positive, particles are forced to regions with higher electric field gradient. The effect is called positive dielectrophoresis (pDEP). In contrast, negative values of $RE[f_{CM}(\omega)]$ lead to the repulsion of the particles from these regions. The effect is called negative dielectrophoresis (nDEP). The frequency of the applied field is a crucial factor in determining the sign of $RE[f_{CM}(\omega)]$ and consequently nDEP and pDEP.

Note that the definition introduced in Equation (3) is only valid for homogeneous spherical particles such as latex microparticles. Otherwise, complex permittivity of the biological particles is complicated, because of their structure heterogeneity.^{34, 46} In-depth details on how to model the dielectric behavior of various types of cell are provided by Pethig.⁴⁷

The real part of the CM factor plays a significant role in the particular case of dielectrophoretic separation of two different types of microparticles with approximately identical dimensions such

as live and dead cells. According to Equation (1), the direction and magnitude of the DEP force experienced by different particles would be similar unless the values of $RE[f_{CM}(\omega)]$ differ among them. In the above-mentioned case, separation is controlled by the frequency of the electric field; due to the fact that the CM factor precisely depends on the frequency of the applied electric field, Equation (3). As such, changing the frequency can accordingly tune the DEP response of the particles.

There are two other active forces in DEP-based systems: electrokinetic (EK) and hydrodynamic forces. The EK effect is a combination of fluid electro-osmosis and particle electrophoresis. In our current work, AC electric field is applied on the microparticles, which have a relatively large size and a small net charge. The EK effect is more dominant in DEP systems with a low-frequency electric field with small charged particles.¹⁹ As the EK phenomena are linearly proportional to the electric field, they vanish in high-frequency applied AC electric fields. Thus, EK force is neglected in this work.

The applied DEP force pushes in the direction of the force, but the particles are opposed by the hydrodynamic friction force.⁴⁸ For a small spherical particle moving through a viscous fluid, Stokes drag force is given as:

$$\vec{F}_f = 6\pi r\mu(\vec{V} - \vec{U}), \quad (4)$$

where μ is the fluid viscosity, r is the radius of the particle, $\vec{U}$ is the velocity of the particle, $\vec{V}$ is the velocity of the fluid and $(\vec{V} - \vec{U})$ is the relative velocity.

3.1. Working Principle

The basis of the presented microfluidic system is to force the diluted particles suspended in the fluid stream and separate them actively using DEP force. This concept is achieved by using the unique design of 3D paired symmetric electrodes located within the main channel. The device

consists of the main channel, four identical separation regions, and three outlet branches. The fluid containing a mixture of particles is introduced to the main channel (1000 μm wide), and ultimately separated particles are collected through the branches (350 μm wide) into different outlets. As shown in Figure 1, the injected fluid flows through the main channel and is subjected to DEP force in the separation region, where different particles experience pDEP or nDEP depending on the sign of their CM factor close to the edge of the electrodes. Each separation region includes four triangular electrodes, which are similar and form a 3D-asymmetric microelectrode configuration. Each electrode is a triangle angled at 18 degrees relative to the fluid flow, and its corner is rounded. On each side of the channel, two identical pairs of electrodes are located in a parallel arrangement. A 250- μm gap exists between the pairs of face-to-face electrodes to allow the deflected particles to pass to the centre of the channel. Four separation regions are located inside the main channel. The distance between each separation region is long enough to avoid the interference of their electric field.

Figure 1. Schematic representation of the dielectrophoretic device. The image illustrates the inlet and outlet reservoirs, the main channel and the four separation regions, each consisting of four triangular electrodes in a quadruple geometry. The magnified view depicts the dimension of the main channel as well as the process of separation of particles depending on the sign of their CM factor. The particles affected by nDEP move towards the center of the channel, whereas, particles affected by pDEP are forced in the opposite direction. Consequently, two types of particles are separated and collected at different outlets.

Figure 2a illustrates the vectors of ∇E^2 in an arbitrary 2D cross-section of a rectangular channel with the cross arrangement of electric boundary conditions ($V = 0$ and $V = V_0$). When there is a difference in the electric potential of two facing electrodes, the distribution of the electric field

causes a repulsive nDEP force near the edge of the electrodes towards the center line of the channel. This force reaches its maximum value near the electrode edges because of the high gradient of the electric field.

Figure 2. The working principle of face-to-face electrodes in DEP: (a) The image shows the normalized electric potential distribution (V/V_0), the vector of $-\vec{\nabla}|E^2|$ (proportional to nDEP force) **along the electrodes edge-to-edge area**, contour of electric field magnitude and boundary conditions in a rectangular geometry (height of $100\ \mu\text{m}$, width of $350\ \mu\text{m}$, electrode width of $90\ \mu\text{m}$). Electrodes are located on the top and bottom of the channel and are grounded ($V = 0$) and connected to the electric potential ($V = V_0$) crosswise. The normalized electric potential distribution was calculated using numerical simulation, which is independent of material properties and the frequency of applied electric potential; (b) Illustration of the nDEP forces acting on a single particle near the edges of the electrodes. The nDEP force repels the particle. In case of a strong nDEP force, the nDEP force and the fluid friction force are balanced so that the path-line of a particle would be similar to the illustrated schematic path-line. Due to the special design of the microfluidic system, the particles affected by nDEP force would be concentrated in the middle of the channel, and an enriched fluid would be collected at the middle outlet of the device.

The described nDEP force acts as an invisible barrier against the particles that have a negative CM factor at such frequencies. Under the conditions that nDEP force is adequately stronger than the friction force, the forces become balanced at a certain distance from the electrode edge. In this equilibrium condition, the total force exerted on the particle is parallel to the electrodes edge. Thus, the particles follow a path-line as depicted in Figure 2b. Under these circumstances, the particles are deflected towards the centerline of the channel, and after passing the separation region, are collected in the center outlet. Since laminar flow conditions prevail, streamlines within the main channel are straight and parallel so that particles hold their lateral position after crossing the separation region. The main channel is divided into three outlet channels to collect the separated particles. The outlet branches are designed to avoid any further remixing of the concentrated particles.

To separate the particles efficiently, DEP force must overcome the friction force continuously on the path of the separated particles:

$$F_{DEP} > F_f \sin(\theta). \quad (5)$$

In the current setup, friction force depends on the local flow velocity, determined by the unique parabolic profile of laminar flow.

In contrast to nDEP, particles experience an opposite force vector under pDEP. In the case that the pDEP force is significantly stronger than the friction force, the affected particles move towards the planar electrode edges and remain attached there. Otherwise, if the friction force is dominant, particles experience a small lateral movement (with respect to the direction of the flow) towards the channel sidewall and move with the flow stream after crossing the space between the face-to-face electrodes (within this space the electric field is uniform, and no DEP force is present). Our proposed 3D paired electrode arrangement provides a mechanism to form an active gate in the microfluidic device that is sensitive to the real part of CM factor of passing particles, and its

function can be adjusted by altering the frequency. In this paper, latex microparticles have been used for concentration tests. Particles injected into the device were concentrated and collected from the middle outlet (nDEP). By sampling the volumes of the outlet fluids, the impact of the test parameters was studied. Next, live (pDEP) and dead (nDEP) yeast cells were separated using the device. In this experiment, live cells and dead cells were separated continuously due to their response and collected at different outlets. Impact of system throughput and applied voltage on the separation efficiency was analyzed using experimental data.

3. Materials and Methods

3.1. Numerical Simulations

Comprehensive numerical modeling was carried out previously by our group to optimize DEP-based particle separation.^{49, 50} The numerical simulation indicated highly efficient separation of dead and live cells. In order to model the function of the proposed dielectrophoretic device, flow field and electric field were first solved. Next, the exerted forces (hydrodynamic and DEP forces) on the suspended particles were obtained to predict their trajectory. Numerical simulations were conducted in three dimensions to investigate the efficiency. Appropriate boundary conditions, including the inlet velocity, applied electric potential, as well as the medium and particle properties, were applied.

To model the laminar flow, inlet velocity was set at the inlet of the main channel, atmospheric pressure was assumed for the outlet boundary condition, and the no-slip boundary condition was set for channel walls. The fluid field was solved in the steady-state mode. The fluid flow distribution was obtained in the whole domain by solving the simplified Navier-Stokes equation for an incompressible, steady and laminar flow with no external source:

$$\nabla p = \mu \nabla^2 \vec{V}, \quad (6)$$

where $\vec{V}$ is the flow velocity, p is the pressure and μ is the dynamic viscosity of the fluid.

Hydrodynamic properties (density and dynamic viscosity) of the medium were set to be similar to those of water.

The electric study was done in the frequency domain mode for the whole geometry. There is no concentrated net charge inside the fluid for homogeneous ion concentration distribution. Moreover, the metal electrodes are fixed considering the Maxwell stress originating from the induced surface charge at the electrode surface. The simplified Laplace equation for electric potential was solved:

$$\nabla^2 \varphi = 0, \quad (7)$$

where φ is the electric potential. Subsequently, the electric field was obtained from:

$$\vec{E} = -\vec{\nabla} \varphi, \quad (8)$$

Electric potentials were assigned to electrodes as shown in Figure 2a. Note that face-to-face electrodes must have opposite potentials to form an electric field, but for the cross arrangement, it is not necessary. For example, both bottom electrodes may be grounded while top electrodes have electric potential or vice versa. Other surfaces were assumed to be insulated.

After computing these fields, DEP force and friction force vectors were obtained for a given particle position. After defining the initial positions, the particle position was updated using the following differential equation:

$$\vec{F}_t = \frac{d(m\vec{U})}{dt}, \quad (9)$$

where $\vec{F}_t$ is the total force, m is the mass of a single particle, $\vec{U}$ is the 3D velocity vector of the particle and t is the time. Particle concentration in the medium was so low that the particle-particle interaction was neglected.

3.2. Fabrication of the Microfluidic Device

The device consisted of three parts: upper and lower electric plates and the fluidic layer. The electrodes were deposited on the glass substrates and precisely aligned together with the middle PSA layer to form the quadruple geometry of the electrodes, Figure 3a. Four sets of electrodes were fabricated within the main channel, but only one set was activated for each test. Glass slides and PSA layer were chosen because they are optically transparent and of relatively low cost. Upper and lower plates with a thickness of 1 mm were cut from glass slides. Next, inlet and outlet holes were drilled in their predicted position into the upper plate using computer numerically controlled milling by a diamond drill. Glass plates were previously washed with piranha solution followed by a deionized (DI) water rinse for 30 seconds. Then, the plates were rinsed with acetone (Sigma-Aldrich, USA) for 10 seconds followed by Isopropyl alcohol (Sigma-Aldrich, USA) for 10 seconds, and finally washed again with DI water and dried with pressurized nitrogen gas.

The electrodes were fabricated using stainless steel shadow masks. In a microelectrode-array based DEP system, thin-film electrodes is mostly fabricated with microfabrication techniques such as photolithography and lift-off etching. We utilized shadow-mask technique to pattern the electrodes and achieved the minimum feature size of the 0.25 mm. Nevertheless, the resolution of the fabrication is mainly limited by the mask and may vary case by case. First, stainless steel sheet with a thickness of 0.1 mm was cut into rectangles with the same dimension as the glass plates. Then, the geometry was patterned by a 30 W customized fiber laser, Figure 3c,d. Finally, the parts were washed carefully with soapy water, rinsed with DI water and dried to remove any residues. Deposition of thin film gold-titanium electrodes (100 nm of titanium and 100 nm of gold) was done by magnetron sputtering. In order to get sharp edges, the shadow masks must completely

stick to the glass plates. This was done by placing a planar magnet sheet on the backside of the glass plate during the sputtering process, which held the mask in position.

Figure 3. Fabrication procedure of the microfluidic device: (a) Schematic representation of the chip components and the alignment process in water bath under a microscope; (b) Fabricated chip with connected wires and tube, and the chip was filled with red food colorant to depict the fluidic channels; (c) and (d) show the fabrication method employed in this work due to the relatively large feature sizes of electrode patterns; (c) Lower glass substrate with sputtered Ti/Au electrodes on its surface; (d) Fabricated shadow mask for the substrate.

The CAD design of the channels and reservoirs was transferred to a cutter plotter machine (GS24, Roland, Japan) to fabricate the fluidic layer. Channel height has a significant impact on the magnitude of DEP force since it varies with the power of three⁴² and must be selected according to the desired function of the device. The channel was cut from an 80- μm thick PSA sheet (FLEXcon, Spencer, MA, USA). After the preparation of the chip components, the parts were aligned precisely together. The issue arises during the alignment is that PSA layers are highly sticky and adhere to thin film electrodes and may accidentally damage them. This issue was resolved by immersing the alignment system in DI water under the microscope such that water could act as

a lubricant. After alignment, the device was sandwiched using a lab-made adjustable screw clamp for two hours to package the 3D paired electrode dielectrophoretic device. Excellent adhesion was observed, and no leakage was detected during the tests. The fluid was injected into the device through the tube using a cylindrical PDMS part, which was bonded to the upper plate using oxygen plasma bonding. Interconnect pads were placed around the chip and outside of the PSA layer to be accessible for conducting wires. A total number of 16 wires were connected to the pads (four pads for each separation region) using an electrically conductive adhesive (MCH-DJ002, China). Due to the manual fabrication process we were not able to determine the minimum accessible feature size of this fabrication method.

3.3. Sample Preparation

Two types of particles were used in the experiments: (i) latex microparticles (polystyrene, BS-Partikel, Germany) and (ii) yeast (*Saccharomyces cerevisiae*) cells. Latex microparticles with diameters of 15 μm and 5 μm were diluted by phosphate buffered saline (PBS). The electric conductance of the medium was controlled prior to the experiment by adjusting the PBS concentration and was measured using a conductivity meter (Metrohm, Switzerland). To prepare the cell suspension, instant-dried yeast powder was utilized. In brief, yeast cells were first dissolved in 10 cc of DI water (40°C) at a cell concentration of 1% w/v for 30 min. In addition, 1% bovine serum albumin (BSA) was added to the suspension to avoid cell adhesion problems. Afterward, the mixture was centrifuged and pipetted at 1000 g centrifugal acceleration for 10 min twice to eliminate any chemical residuals. Nonviable (dead) yeast cells were produced by raising the temperature of the cell suspension to 80 °C for 20 min. After centrifuging and pipetting, dead cells were stained by trypan blue, using the standard protocol, to be optically recognizable.

3.4. Experiment procedure

The device (Figure 3) was placed under an inverted microscope (Labomed TCM400, CA, USA) to observe the experiments. For cell counting using Neubauer hemocytometer under the microscope, a CCD camera (MD-30, Mshot co., China) was utilized. However, due to the low frame rate of this camera, an iPhone 5s smartphone (Apple, CA, USA) was mounted on the microscope eyepiece by means of a 3D printed mount to monitor the performance of the DEP-based system through the particles' motion. The device records videos at 120 frames per second (via the slow-motion feature), which suited this application. Sinusoidal voltages were generated by a function generator (JFG-2MD, Afzar Azma Co., Iran) and measured simultaneously with an oscilloscope (GDS-1102-U, GW Instek, Taiwan). The fluid was injected into the microfluidic device using a precise syringe pump (ET-SP-V1, SATEECO Co., Iran), and the fluid was extracted at the output reservoirs using micropipettes. For each experiment, 10 μL of sample was collected from the outlets and analyzed using a hemocytometer to determine the particle concentration. After each experiment, the device was flushed with DI water at the flow rate of 1 mL/min for 5 min.

To achieve the desired outcome, adjusting the CM factor is vital, as it determines the direction of the DEP force as described in section 3.1. CM factor was tuned by two parameters: (i) electric properties of suspending medium and (ii) working frequency. As electric properties of the latex particles and yeast cells (dead and live) remain unchanged, only the real conductivity of the medium (σ_m) and its permittivity (ϵ_m) can be altered to control the value of the real part of the CM factor, according to Equation (2).

Due to the low concentration of the introduced ions in the solution, its electric permittivity does not vary significantly ($\epsilon_m = 80$). The frequency of the applied field is another choice for altering the CM factor. Working frequency and σ_m were chosen with respect to the test conditions. The

relative permittivity of the latex particles was assumed to be 2.56.⁵² Inherent conductivity of polystyrene is very low compared to that of the medium. However, surrounding a polystyrene particle with the medium results in induced surface conductance.² Thus, the size of the particle plays a major role in its dielectric behavior. As shown in Figure 4, a medium with high conductivity was chosen, because increasing the ionic strength leads to the dominance of the nDEP condition and set the real part of the CM factor near the -0.5 (green lines) across the wide range of frequency spectra.⁵³ Thus, PBS 0.1X ($\sigma_m = 1.7$ mS/cm) was used as the buffer solution for the focusing experiments with latex particles. The values were calculated for 5- μ m and 15- μ m diameter latex particles at different solution conductivities by the method described by Ermolina and Morgan.⁵⁴ For live/dead yeast cells, the properties and method explained by Huang et al.³⁴ were used.

Figure 4. Plots of the real part of the CM factor ($RE[f_{CM}(\omega)]$) as a function of electric field frequency for latex particles and yeast cells (black lines). The separation region is highlighted, in which live and dead cells experience opposite DEP force due to the sign of the CM factor.

For live and heat-treated dead yeast cells in low conductivity buffer (0.055 S/m), the CM factor was calculated by the double-shell method. Mernier et al.^{55, 56} used the same frequency range and the buffer conductivity to separate the live and the dead *Saccharomyces cerevisiae* cells based on the sign of their CM factor. The experimental results of the research conducted by Doh et al.⁵⁷ confirm the data presented in Figure 4.

4. Results and Discussion

This section presents the experimental results of the two main applications of the fabricated microfluidic device viz., (i) focusing of latex particles under nDEP and (ii) separation of viable (pDEP) and nonviable (nDEP) yeast cells. Particle trajectories were simulated numerically to obtain the particle behavior. More detailed discussion regarding the numerical simulation of the same scheme was reported in our previous studies.^{49, 50} Finally, experimental results are presented for each category, and the effects of the experimentally controllable parameters (applied voltage amplitude and inlet flow rate) and particle size on performance are studied.

4.1. Focusing of Latex Particles

4.1.1. Numerical Validation

In this section, numerical results of the proposed DEP-based system are presented. Latex particles in two different sizes were tested under the nDEP force as illustrated in Figure 2. Test conditions are listed in Table 1.

Table 1. The parameters used for focusing the latex particles

Parameter	Value	Description
Medium Conductivity	1.7 mS/cm	0.1X PBS
Particle Concentration	2000 particle per milliliter	
Electric Field Frequency	100 kHz	$RE[f_{CM}(\omega)] = -0.5$
Applied Voltage Amplitude (V_p)	2,4,8 and 12	Peak voltage
Inlet Flow Rate	0.25, 0.5, 1, 2 and 3 μ L/min	

Figure 5 shows the numerical results of the trajectories of latex particles. The numerical model was made with regard to the parameters listed in Table 1. For a better understanding of the particle behavior, the device performance was modeled for 15- μ m latex particles, Figure 5a. Particles were released simultaneously from a grid at the inlet of the channel. Evidently, the velocity of the

particles depends on their initial position. However, the system focused all particles successfully regardless of their initial position. Before getting close to the electrodes, the friction force is dominant, and particles have a direct path-line, but as the distance between the electrodes and the particles decreases, the DEP force repels the particles to the channel center. Finally, the focused particles pass the separation region and retake a straight path.

Figure 5. Numerical validation of the presented design: (a) 3D view of focusing 15- μm latex particles released from a grid: Line colors represent the instantaneous velocity of a particle within its trajectories (Flow rate = $1 \mu\text{L/min}$, $V_p = 8 \text{ V}$); (b) Simultaneous focusing of 5- μm (blue lines) and 15- μm (red lines) latex particles with the similar initial position and test condition (Flow rate = $0.5 \mu\text{L/min}$, $V_p = 8 \text{ V}$); (c) As evident from the simulated streamlines, the trifurcation geometry is designed carefully so that the particles moved to the middle gap of the electrodes would be directed to the middle outlet (red lines).

Figure 5b depicts the calculated trajectories of 15- μm and 5- μm latex particles released into the channel from an approximately similar initial position. The simulations were performed for the worst case. In such case, the particles are located exactly between the top and bottom of the channel, where velocity is in its maximum, and larger DEP force is needed to act against the friction force. Although both particle types have been concentrated in the middle, there is a

difference in their trajectory that depends on the size. The particles follow a certain line parallel to the electrodes because forces are in equilibrium over this line until they reach the proximity of the electrodes. The equilibrium depends on the forces and the particle size. As larger particles experience the greater force, they move away from the edges of the electrodes.

The geometry of branches was designed with the intention of separating the focused particles from the main fluid stream. The streamlines of the fluid are plotted in Figure 5c for each outlet. It is impractical to fabricate sharp edges using a cutter plotter machine. Thus, all edges were rounded.

4.1.2. Experimental Results

This section presents the experimental results on the performance of the fabricated device. **Figure 6a and video S1 show** the focusing effect of 15- μm latex particles. The images were taken from the bottom of the device. The particles have different velocities due to their spatial position within the flow. As shown, latex particles were repelled to the middle of the stream as a result of the nDEP force exerted on them by the electric field.

Figure 6. Illustration of the device performance by optical imaging: (a) The trajectories of 15-μm latex particles (flow rate = 2 μL/min, V_p = 12 V, particle concentration = 100 particles/mL). The concentration of the particles was selected low enough to determine the pathlines of the particles. The figure is obtained by overlaying video frames (4 frames per second) using ImageJ software (NIH, USA); (b) Microscope image of 5-μm latex particles focused towards the middle of the channel after passing through the 3D face-to-face electrodes (flow rate of 0.2 μL/min, voltage V_p of 12 V, particle concentration of 1000 particles/mL).

Proficiency of the suggested continuous and high-throughput scheme is demonstrated in Figure 6b and Video S2, where all of the 5-μm latex beads are concentrated in the center of the main channel. In the upstream flow, the particles are distributed all over the channel width, whereas, after reaching the electrode pairs, they are forced to the center of the channel.

Next, the experimental data obtained from the tests are presented and interpreted by studying the role of the effective parameters. A practical method is needed to interpret the performance of

the device. In the focusing experiments, the performance of the system was measured by the focusing efficiency (FE) number:

$$FE = \frac{C_m - C_s}{C_m} \times 100 \quad (10)$$

where C_m is the particle concentration of the middle outlet sample, and C_s is the average concentration in the side outlet samples. The purpose of focusing is driving particles to the center of the main channel, Figure 2b. In the ideal case of focusing, all particles move to the center of the channel and the focusing efficiency equals 100 %. Conversely, in the condition that no focusing happens, $FE=0$ because C_m and C_s are the same as the inlet concentration. To examine the efficiency, samples were collected for each experiment three times and the average FE is reported.

DEP force is affected by several items. In this work, three different parameters were examined through the experiments: (i) amplitude of the applied voltage, (ii) fluid flow rate and (iii) particle size. According to Equation (1), DEP force is proportional to the cube of particle radius and the square of the amplitude of the applied voltage. The electric field is proportional to voltage according to Equation (8). Moreover, the friction force is proportional to the fluid velocity according to Equation (4), which changes linearly with the fluid inlet flow rate.

The electric potential was applied to the electrodes as shown in Figure 2a. Two electrodes were connected to the ground potential. The two remaining electrodes were connected to the AC function generator with a fixed frequency of 100 kHz. As depicted in Figure 3 (green lines), altering the frequency does not affect the CM factor due to the high conductance of the buffer fluid. We observed that the latex particles experienced negative DEP force at frequencies in the range of 100 kHz to 1 MHz. The frequency was not set lower than 100 kHz to avoid any possible damage to the thin film electrodes. Moreover, electrode polarization problem was not observed

during the experiments. This problem is likely to occur in the case of low fluid conductivity and field frequency below approximately 15 kHz,⁵⁸ which is not the case of our current work.

Figure 7a shows the impact of the voltage amplitude on the FE numbers of 5- μm and 15- μm latex particles. To demonstrate the role of the voltage amplitude, the amplitude was adjusted to the values presented in Table 1, while the flow rate and the applied voltage frequency were kept at 1 $\mu\text{L}/\text{min}$ and 100 kHz, respectively. Obviously, by increasing the voltage amplitude, the DEP force magnitude and consequently the FE number increases. At lower voltages ($V_p = 2$ V) weak focusing was observed. Otherwise, both particle sizes were focused successfully at a high voltage ($V_p = 12$ V). At voltages higher than 12 V, the DEP force was large enough to focus particles with diameters larger than 5 μm . Under this condition, even particles with the maximum velocity were affected by the DEP force and pushed towards the center of the channel.

Figure 7. The impact of the controllable test parameters on the device performance: (a) The impact of electric potential magnitude on device performance: Increasing the applied voltage amplitude increases the focusing efficiency of the dielectrophoretic device for two particle diameters (the flow rate was fixed at 1 $\mu\text{L}/\text{min}$); (b) The impact of flow rate on focusing efficiency of the DEP-based device for two particle diameters ($V_p = 8$ V): Decreasing the flow rate of the injected fluid led to higher focusing efficiency values. Larger particles experienced stronger DEP force and greater FE values.

As depicted in Figure 7b, both particle sizes were placed under test with a constant electric potential ($V_p = 8$ V) applied on the chip. At a low flow rate of 0.25 $\mu\text{L}/\text{min}$, most of the passing

particles were subjected to nDEP force and therefore were driven to the center of the channel. Focusing efficiency was enhanced to approximately 100 %. In contrast, at a high flow rate of 3 $\mu\text{L}/\text{min}$, the nDEP force was not strong enough to oppose the frictional force acting on particles. Thus, only some of the particles with relatively low velocity were affected, and other particles passed the separation region with a slight lateral movement. Although efficiency was improved by decreasing the flow rate, this reduces the throughput of the device.

Regarding the data depicted in Figures 7a and b, FE values of small particles were always seen to be less than those of large particles under the same test condition. This confirms the fact that particle size directly influences the DEP force magnitude.

4. 2. Separation of Live and Dead Cells

As explained earlier, live cells experience pDEP and dead cells experienced nDEP due to the **controllable** operation conditions. The diameter of yeast cells was approximately between 5 to 6 μm . The experiments were conducted based on the parameters listed in Table 2.

Table 2. The values of the parameters used for the separation of live and dead yeast cells

Parameter	Value	Description
Medium Conductivity	0.55 mS/cm	Adjusted by adding PBS to DI water
Electric Field Frequency	1050 kHz	See Figure 3.
Cell Concentration	2000 particle per milliliter	
Live/Dead cell ratio	50% / 50%	
Applied Voltage Amplitude (V_p)	4,8 and 12	Peak voltage
Inlet Flow Rate	0.25, 0.5 and 1 $\mu\text{L}/\text{min}$	

Dead cells acted under nDEP in a manner similar to what is described for latex particles. However, live cells ($RE[f_{CM}(\omega)] > 0$) were forced in the opposite direction, Section 3.1. For strong pDEP forces (high voltage or low flow rate), live cells are likely to be attracted to the top

and the bottom of the channel close to the electrode edges (Figure 8a) and remain there during the experiment. Under this condition, the pDEP force was strong enough to trap live cells. Nevertheless, this did not disrupt the device performance in our experiments. However, most particles experienced a lateral movement towards the channel sides and pursue their path to the side outlets.

Figure 8. Separation of dead yeast cells from live yeast cells: (a) Live cells are accumulated close to the electrode edge due to pDEP force (flow rate of 0.5 $\mu\text{L}/\text{min}$, $V_p = 12$ V). Live and dead cells are detected according to the blue color caused by staining the dead cells; (b) FE values for various flow rates and applied voltage amplitudes; (c) Illustration of the live and dead cells using the trypan blue staining.

To evaluate the enrichment of dead cells in the middle outlet, we proposed the measurable separation efficiency (SE) number:

$$SE = \frac{C_{d,m} - C_{d,i}}{C_{d,m}} \times 100 \quad (11)$$

where C_d is the concentration of dead cells. Subscripts m and i refer to the sample collected from the solution leaving the middle outlet and the injected solution, respectively. As described earlier, staining caused the color difference within the live and dead cells, Figure 8c. Yeast cells are not monodispersed, thus smaller particles experience weaker DEP forces than the friction force.

Nine different test conditions were implemented, and each was repeated for three times. The averaged values of measured SE are plotted in Figure 8b. As predicted, the best performance was observed for the lowest flow rate (0.25 $\mu\text{L}/\text{min}$) and the highest voltage ($V_p = 12 \text{ V}$). Under this condition, most dead cells were moved to the middle outlet, and the collected sample was enriched with them.

5. Concluding Remarks

This paper presents a dielectrophoretic device based on the 3D-electrode system for two applications: concentration and separation of bioparticles. The quadrupole arrangement of four triangular planar electrodes was used to form a non-uniform AC electric field within the fluidic channel. Interaction between the electric field and the suspended particles resulted in a DEP force. By optimizing the operating conditions, the exerted DEP force was controlled to continuously concentrate and separate passing particles, which were then collected from the different outlets. Live and dead cells were used for separation experiments. Polystyrene microparticles in two different sizes were utilized for concentration experiments. By optimizing the medium electric conductivity, the electric field frequency, the flow rate, and the applied voltage amplitude, an efficiency of about 100% was obtained for both applications. We conclude that decreasing flow

rate and increasing amplitude of the applied voltage improve the device performance. Furthermore, the observed particle trajectories in concentration tests agree well with the numerical results. Moreover, the fabrication method employed in this research was relatively simple and cost-effective. A PSA layer was utilized as the fluidic channel, and steel shadow masks were used to fabricate thin film electrodes. The fabricated device performed well during the experimental tests. This study demonstrated the capability of the device to concentrate and separate particles and cells, which paves the way to have a reliable platform for future works employing any types of bio-particles with the same technique.

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