Improving the Design and Application of Insulator-Based Dielectrophoretic Devices for the Assessment of Complex Mixtures

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Improving the Design and Application of Insulator-based Dielectrophoretic Devices for the Assessment of Complex Mixtures

by

Mario A. Saucedo-Espinosa

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Microsystems Engineering

Rochester, New York June 2017
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Mario A. Saucedo-Espinosa

Committee Approval:

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Dielectrophoresis (DEP) is an electrokinetic (EK) transport mechanism that exploits polarization effects when particles are exposed to a non-uniform electric field. This dissertation focused on the development of high-performance insulator-based DEP (iDEP) devices. A detailed analysis of the spatial forces that contribute to particle movement in an iDEP device is provided. In particular, this analysis shows how particle size and shape affects the regions where particles are likely to be retained due to dielectrophoretic trapping. The performance of these trapping regions was optimized using a systematic approach that integrates the geometrical parameters of the array of insulating structures. Devices that decrease the required electrical potential by ~80% were found. The optimization strategy enabled the detection of structures that promote and discourage particle trapping. By combining the “best” and “worst” structures in a single asymmetric structure, a novel iDEP device was designed. This device selectively enriches the larger particles in a sample and drives the smaller particles away from the enrichment region. A quick enrichment and elution of large cells was achieved. This is important when dealing with samples containing eukaryotic cells, which can be harmed by the electrical treatment. Yeast cells were successfully separated from polystyrene particles in under 40 seconds using this device and a high cell viability of 85% was achieved. Finally, an enhancement of traditional iDEP devices is proposed, where some insulating posts are replaced by conducting structures. That is, insulating and conductive posts are intimately combined within the same array. The performance of this hybrid device is presented to show the advantage of using insulating structures with microelectrodes in the same array to dominate par-
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# Contents

Abstract iii

Acknowledgements v

1 Introduction 1

1.1 Particle separation in microfluidic devices 1

1.2 Electrokinetics 6

1.3 Dielectrophoresis 7

1.4 Objectives 11

2 Theoretical background 13

2.1 Electric field distribution 13

2.2 Electrokinetic force and velocity 14

2.3 Dielectrophoretic force and velocity 16

2.4 Thermal and electrical cell damage 17

2.5 The Clausius-Mossotti factor 19

2.6 The dielectrophoretic signature 21
3 Materials and Methods

3.1 Microdevices fabrication ........................................ 23
3.2 Polystyrene microparticles .................................... 25
3.3 Cells ............................................................. 26
3.4 Equipment and software ........................................ 27
3.5 Experimental procedure ........................................ 27
3.6 Cell viability assessment ....................................... 29

4 Fundamentals of the particle trapping mechanism ............... 31

4.1 Electric field and particle velocity at the constrictions ........ 34
4.2 Particle trapping condition and trapping regions .............. 37
4.3 The correction factor as a function of particle size and shape .. 42
4.4 Location and shape of the trapping regions ................. 43

5 Optimization of the insulating structures ....................... 47

5.1 Particle trapping and the channel design .................... 48
5.1.1 Insulator posts shape ..................................... 48
5.1.2 Insulator posts geometry ................................ 50
5.1.3 Insulator posts arrangement .............................. 52
5.1.4 Parameters considered for optimization .................. 53
5.2 Effect of the length of the insulator posts ................. 54
5.3 Achievable precision in the fabrication process .......... 57
5.4 Effect of the longitudinal spacing between posts .......... 58
5.5 Effect of the lateral spacing between posts ........................................ 59
5.6 Effect of the microchannel lengthscale .............................................. 61
5.7 Particle trapping performance of the optimal devices ......................... 62

6 Design of asymmetric posts to reverse the elution order ........................ 68
   6.1 Mechanism behind the asymmetric posts effect ................................. 70
   6.2 Separation of inert polystyrene particles ...................................... 74
   6.3 Separation of yeast cells and polystyrene particles ......................... 79

7 Design and fabrication of a hybrid DEP device .................................... 83
   7.1 Motivation for the hybrid device .................................................. 88
   7.2 Combination of insulating and conducting structures ....................... 90
   7.3 Electrical characterization of the composite material ....................... 93
   7.4 Fabrication of the insulating structures ....................................... 97

8 Conclusions ....................................................................................... 101
   8.1 Future work ................................................................................ 103

References ........................................................................................... 106
List of Publications


List of Figures

1.1 Schematic illustration of some current technologies for microscale particle separation. (a) Pinched flow fractionation. (b) Inertial microfluidics sorting. (c) Deterministic lateral displacement. (d) Membrane dead-end filtration. (e) Membrane cross-flow filtration. ....... 3

1.2 Schematic illustration of the microfluidic channel with an array of insulating posts employed in this work. ......................... 9

1.3 Schematic illustration of the relative polarization of a particle (in blue) when its conductivity, $\sigma_p$, is (a) much greater and (b) much lower than the conductivity of the suspending medium, $\sigma_m$. These limits represent the cases where the particle is (a) much more and (b) much less polarizable than the suspending medium, and experience either (a) positive or (b) negative DEP behavior. The red arrows show the direction of the electric field, while the blue arrows show the direction of the DEP force. .............. 10
2.1 In blue, the CM factor for a particle ($\sigma_p = 1 \times 10^{-2}$ S/m and $\varepsilon_p = 2.4$) which is much more conducting than the suspending medium (DI water, $\sigma_m = 1 \times 10^{-4}$ S/m and $\varepsilon_m = 78.4$). The arrow shows the location of the crossover frequency. In red, the CM factor for a particle ($\sigma_p = 1 \times 10^{-2}$ S/m and $\varepsilon_p = 2.4$) which is much less conducting than the suspending medium (DI water, $\sigma_m = 1$ S/m and $\varepsilon_m = 78.4$). The regions where positive and negative DEP behaviors are observed are separated by the magenta line.

3.1 Images of the high-resolution transparency mask employed to pattern the photoresist.

3.2 Schematic illustration of (a) the master mold used to create (b) the PDMS piece containing the channel walls and insulating structures. Images of (c) the master mold and (d) the PDMS piece containing the channel walls and insulating structures.

3.3 Images of (a) the manifold interfacing the iDEP device and (b) the large cylindrical liquid reservoirs adapted to the iDEP device.

3.4 Fluorescence of viable (green) and non-viable (red) cells after staining them with Syto 9 and propidium iodide.

4.1 Experimental flow regimes for 1-µm polystyrene particles (showing a negative DEP behavior) obtained by applying a DC electric potential of (a) 100, (b) 200 and (c) 300 V in the iDEP channel shown in Figure 1.2. The suspending medium is DI water with a pH of 8 and a conductivity of $2 \times 10^{-3}$ S/m.

4.2 Schematic illustration of the microfluidic channel with an array of insulating posts, along with their geometrical parameters and measurements.
4.3 Predictions obtained with numerical simulations in (a) a constriction for the (b) electric field, (c) EK velocity, (d) gradient of the square of the electric field, (e) DEP velocity and (f) net particle velocity for 1-µm particles at potentials of 200, 400 and 800 V. A negative value indicates that the particles move in the direction of the inlet. The centerline and the center of the constriction are shown as a red horizontal line and a brown vertical dotted line, respectively. ........................................... 35

4.4 (a) Illustration of the adjusted correction factor to a value of 43 that matches the experimental band of trapped 2-µm particles and the predicted dielectrophoretic barrier at 800 V. The trapping barrier is depicted as a white line that corresponds to a net particle velocity of zero. The inset shows the direction of the DEP and EK velocities along the centerline. Predicted dielectrophoretic barriers for correction factors of (b) 41, (c) 42, (d) 44 and (e) 45. .......................................................... 40

4.5 Predictions obtained with numerical simulations for (a, d and g) the trapping condition curves and (b, e and h) the dielectrophoretic barriers for the 1-µm and 2-µm particles and E. coli cells, respectively. The predictions are compared to (c, f and i) experimental trapping regions for the same species when potentials of 200, 400 and 800 V were applied to the microchannels shown in Figure 4.2, respectively. The suspending medium corresponds to DI water with a pH of 8.0 and a conductivity of $2 \times 10^{-3}$ S/m. .................................................. 41

4.6 (a) Experimental band of trapped 2-µm particles at 800 V. Surface plots of the magnitude of the (b) DEP and (c) EK velocities in a constriction at 800 V. (d) Surface plot of the magnitude of the net particle velocity at 800 V. The arrows indicate the direction of the net particle velocity. The suspending medium corresponds to DI water with a pH of 8.0 and a conductivity of $2 \times 10^{-3}$ S/m. ................................. 45
4.7  (a) Experimental band of trapped *E. coli* cells at 800 V. Surface plots of the magnitude of the (b) DEP and (c) EK velocities in a constriction at 800 V. The suspending medium corresponds to DI water with a pH of 8.0 and a conductivity of $2 \times 10^{-3}$ S/m. 46

5.1  Insulator post shapes considered: (a) star-shaped post, (b) diamond-shaped post, (c) circle-shaped post and (d) square-shaped post. Magnitude distributions of the (e-h) electric field and (i-l) gradient of the square of the electric field at the centerline caused by the presence of the insulator posts. 49

5.2  (a) Representation of the opening region located between four insulating posts. (b) Illustration of the magnitude and direction of the net particle velocity in the opening region when a DC potential of 1,400 V is applied. The upstream and downstream regions represent the zones where particle velocity increases (when the particle comes out of the constriction) and decreases (when the particle enters the next constriction), respectively, as a result of the negative DEP force. 51

5.3  Parameters considered in the optimization procedure. The post length, lateral and longitudinal spacings, and channel length-scale are optimized in independent, consecutive processes. 54

5.4  Distribution of the dielectrophoretic force magnitude in (a) in-line and (b) staggered designs under the same conditions. 55
5.5 (a) Normalized average trapping condition ($T_C$) for the star, diamond, circle and square insulator posts as a function of post length ($L$), where $L \in \{20, 40, 60, 80, 120, 160, 200, 300, 400, 600, 1000\}$ µm. The width ($W = 200$ µm), lateral ($W_S = 50$ µm) and longitudinal ($L_S = 50$ µm) spacing were the same for all post shapes. Results for each post shape were normalized with respect to the design with $W = 200$ µm, $L = 200$ µm, $W_S = 50$ µm and $L_S = 50$ µm, shown with an arrow. The left-hand side of all figures represents narrow posts, while the right-hand side represents broad posts. (b-e) Normalized average trapping condition ($T_C$) when the vertices of the (b) star, (c) diamond, (d) circle and (e) square insulator posts are rounded in 10, 20 and 30 µm, as shown with arrows. .................................................. 56

5.6 Normalized average force ratio ($F_R$) for the diamond, circle and square insulator posts as a function of the longitudinal spacing ($L_S$), where $L_S \in \{10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 400, 600, 800\}$ µm. The length ($L$) corresponded to the optimal length for each post shape from Figure 5.5a. The width ($W = 200$ µm) and lateral spacing ($W_S = 50$ µm) were the same for all post shapes. Results for each post shape were normalized with respect to the optimal designs from Figure 5.5a (where $L_S = 50$ µm), shown with an arrow. The left-hand side of the figure represents closely-spaced structures, while the left-hand side represents distantly-spaced structures. .................................................. 60
5.7 Normalized average force ratio ($F_R$) for the diamond, circle and square insulator posts as a function of the lateral spacing ($W_S$), where $W_S \in \{10, 20, 30, 40, 50, 60, 70, 80, 90, 100\}$ µm. The length ($L$) and longitudinal spacing ($L_S$) corresponded to the optimal values for each post shape from Figures 5.5a and 5.6, respectively. The width ($W = 200$ µm) was the same for all post shapes. Results for each post shape were normalized with respect to the optimal designs from Figure 5.6 (where $W_S = 50$ µm), shown with an arrow. The left-hand side of the figure represents closely-spaced structures, while the left-hand side represents distantly-spaced structures. 

5.8 Normalized average trapping condition ($T_C$) for the diamond, circle and square insulator posts as a function of the post shape ($W$), where $W \in \{40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 200, 300, 400, 600, 1000\}$ µm. The length ($L$), longitudinal ($L_S$) and lateral spacing ($W_S$) corresponded to the optimal values for each post shape from Figures 5.5a, 5.6 and 5.7, respectively. Results for each post shape were normalized with respect to the optimal designs from Figure 5.7 (where $W_S = 200$ µm), shown with an arrow. The left-hand side of the figure represents small devices, while the left-hand side represents large devices.
5.9 Experimental bands of trapped 2-µm particles for (a-c) the initial devices, (d-f) devices with an optimal length included, (g-i) devices with an optimal longitudinal spacing included, (j-l) devices with an optimal lateral spacing included and (m-o) devices with an optimal lengthscale (or post width) included. Each subfigure shows the electric potential at which the picture was taken, and corresponds with the lowest electrical potential ($V_{LEP}$) that was necessary to apply in order to achieve well-defined bands of enriched particles. Moreover, each subfigure shows the performance impedance ($P_I$). The suspending medium corresponds to DI water with a pH of 8 and a conductivity of $2 \times 10^{-3}$ S/m. 65

6.1 (a) Schematic illustration of the microfluidic channel with an array of asymmetric insulating posts, along with their geometrical parameters and measurements. The upstream and downstream directions are the directions towards the inlet and outlet, respectively. The figure shows the regions of interest where fluorescence measurements were made. 70

6.2 (a) Regions between two posts where the gradient of the square of the electric field ($\nabla E^2$) has local maximum values when 500 V are applied. (b-e) Schematic representation of the forces acting on particles upon the application of a square DC-biased AC signal ($+V_p = 500$ V, $-V_p = -700$ V and $f = 400$ mHz) to a binary mixture with red 500-nm and green 1-µm polystyrene particles. The arrows show the migration direction for the red 500-nm and green 1-µm polystyrene particles. (f-i) Sequential time response of the experimental system when the electric potential signal was applied for (f) 1.85, (g) 1.98, (h) 2.12 and (i) 3.57 seconds. The sequential images show the operational principle of the asymmetric posts device. The suspending medium corresponds to a 0.2 mM K$_2$HPO$_4$ solution with a pH of 7.2 and a conductivity of $6 \times 10^{-3}$ S/m. 72
6.3 (a) Corrected total fluorescence (CTF) of the red 500-nm and green 1-µm particles with respect to time when a square DC-biased AC signal (+Vp = 500 V, -Vp = -700 V and f = 400 mHz) was applied for 60 seconds. (b) CTF of the red 500-nm and green 1-µm particles resulting when fluorescence was sampled at the same time period during each cycle. (c-f) Sequential time response of the red 500-nm and green 1-µm polystyrene particles mixture when the electric potential signal shown in (a) was applied for (c) 18.65, (d) 28.97, (e) 38.75 and (f) 48.67 seconds. The sequential images show the increasing fluorescence concentration at a subset of the sampled times in (b). The suspending medium corresponds to a 0.2 mM K$_2$HPO$_4$ solution with a pH of 7.2 and a conductivity of 6×10$^{-3}$ S/m.  

6.4 (a) Characteristics of the electric potential signals applied during the four steps of the elution-release experiments for the green 1-µm and red 500-nm polystyrene particles mixture. (b-e) CTF of the red 500-nm and green 1-µm particles with respect to the overall time during the (b) 1-µm particles elution, (c) 1-µm particles release, (d) 500-nm particles elution and (e) 500-nm particles release. Fluorescence measurements for the elution and release steps were performed at the outlet of the post array, as depicted by the regions of interest drawn in Figure 6.1. The suspending medium corresponds to a 0.2 mM K$_2$HPO$_4$ solution with a pH of 7.2 and a conductivity of 6×10$^{-3}$ S/m.
6.5 (a) Characteristics of the electric potential signals applied during the elution and release of the green yeast cells from a mixture with red 2-µm polystyrene particles. (b-c) CTF of the green yeast cells and red 2-µm particles with respect to time during the (b) yeast elution and (c) yeast release. (d-e) Sequential time response of the binary mixture when the yeast elution signal was applied for (d) 4 and (e) 40 seconds. The sequential images show the increasing green yeast concentration and the small variation in the red 2-µm particles concentration. Fluorescence measurements for the elution and release steps were performed at the outlet of the post array, as depicted by the regions of interest drawn in Figure 6.1. The suspending medium corresponds to a 0.2 mM K$_2$HPO$_4$ solution with a pH of 7.2 and a conductivity of 6×10$^{-3}$ S/m.

7.1 (a-c) Distribution of the DEP force and (d-f) regions where particles are attracted or repelled according to their relative polarization in devices with cylindrical structures when (a,d) posts behave as electrodes, (b,e) posts are insulators and the electric potential is applied at the inlet/outlet reservoirs and (c,f) insulating and conducting posts coexist within the same post array. The DEP force magnitude was standardized with respect to (a). Particles showing positive and negative DEP behaviors are shown in magenta and green, respectively.

7.2 (a) Distribution of the DEP force when two triangular-shaped electrodes face each other. (b) Particles showing a positive DEP behavior are attracted to the electrodes edge. (c) Distribution of the DEP force when four insulator structures are placed between the electrodes shown in (a). (d) The same particles are attracted to the inner region of the insulating structures, where they are protected from the fluid flow. The DEP force magnitude was standardized with respect to (a). Particles showing a positive DEP behavior are shown in magenta.
7.3 (a) Schematic and (b) experimental image of the 4-point probe technique used to measure the electrical conductivity of the composite material samples. .............................................. 95

7.4 Electrical conductivity of the composite material as function of the MWCNTs content. ................................................................. 96

7.5 Experimental images of the connection lines (a) close to the terminal end and (b) below the conducting posts. The images show the selective casting of the conductive composite material. .............................................. 96

7.6 Schematic of the fabrication process for the hybrid device. The (a) top and (b) bottom master molds were fabricated using SU-8 on a silicon wafer. (c) Native PDMS was casted on the top mold to produce the channel walls and insulating structures. (d) The conductive composite material was casted on the bottom mold to produce the connection lines and conductive posts. (e) A thin layer of PDMS was applied to insulate the connection lines form the channel. (f) SEM image showing the conducting posts not being covered by PDMS. .............................................. 98

7.7 (a) Bottom piece of the device employed for cell lysis and fractionation. The channel is surrounded by the terminal ends that will connect the device with the voltage supply. (b) Copper sheets were glued on the terminal ends to facilitate the electrical connections. (c) Inlet/outlet ports were punched in the device, and the bottom and top PDMS pieces were sealed together. The inlet/outlet ports were connected to the syringe pump, and the copper sheets were connected to the voltage supply. .............................................. 100
List of Tables

4.1 Experimental minimum trapping voltage and correction factors for the 1-μm, 2-μm particles and *E. coli* cells. aThe diameter of the *E. coli* cells represents the equivalent diameter. . . . . . . . . . . . . . . . . . 39
1.1 PARTICLE SEPARATION IN MICROFLUIDIC DEVICES

Microfluidics is a rapidly growing field with potential applications in numerous areas, including the analysis of biological particles in clinical and environmental assessments, in food and water safety evaluations and in point-of-care devices [1, 2]. Scaling down fluidic processes offers attractive advantages, such as short processing times for analysis, the requirement of small quantities of samples and reagents, high resolution and sensitivity, fast diffusion mixing, and low cost devices [3, 4]. Further, microfluidics allows exploiting different transport mechanisms than those employed in macroscopic bench-scale techniques [5]. In consequence, there is a growing interest in the development of analytical techniques that can be successfully applied in miniaturized devices and lab-on-chip (LOC) platforms for the manipulation of microscale particles.

The most common functional modules of a LOC device are (i) sample transportation and preparation, (ii) separation, and (iii) detection and analysis. In the separation module, a sample is purified through the separation of both biological and non-biological components. Several technologies have been developed to enable particle separation at the microscale. These technologies can be classified in two main groups, as shown in Figure 1.1: those techniques that rely on immunoaffinity-based
methods, and those that exploit physical properties to differentiate particles and cells.

Some of the most powerful conventional cell separation strategies are derived from continuous flow cytometry techniques, which can provide information about cell population heterogeneity, and cell size and volume [6]. However, flow cytometry usually relies on external labels to distinguish between cell types. A label is defined as any foreign molecule that is chemically or temporarily attached to the cell of interest to detect cellular presence or activity [6]. The most commonly used separation technique in this category is fluorescent activated cell sorting (FACS). Using FACS, a cell mixture is first labeled with one or more cell surface marker-specific antibodies. These antibodies have been previously conjugated to fluorescent dyes, so the collected scatter and fluorescence data can be used to identify cell type [7]. This technique offers a high specificity, low chance of cell damage and short sorting time. However, FACS has a medium-range throughput (2000 particles per second), the preparation is labor-intensive, and the addition of labels to cells may affect their biological function [6,8]. An alternative to increase the sorting speed shown in FACS is using magnetic activated cell sorting (MACS), which uses antibody-coated magnetic nanoparticles to differentiate cells and an external magnetic field [8]. The downside of this technique is the low availability of markers conjugated with magnetic beads.

In general, FACS and MACS cell sorting technologies are expensive (around $250,000 USD [9]), so their application is limited. In contrast, technologies based on the use of physical properties to sort cells into distinct populations are inexpensive and constitute an important tool in healthcare applications. This is because cells that have been affected by some diseases experience a change on their physical properties. For example, cells infected by malaria parasites are 50 times more rigid than healthy cells, preventing them from passing through the capillaries and creating blood clots [10].

The group of techniques that exploit differences in physical properties are called “label-free” technologies. The biomarkers that are more employed for particle and cell discrimination are size, deformability, elasticity and electrical properties.

The easiest technique that allows a continuous sorting of particles is termed pinched
flow fractionation (PFF, Fig. 1.1a). In PFF, the fluid containing the particles to be sorted is focused by a particle-free fluid. The device includes a “pinched” segment, where particles align to the sidewall by the particle-free fluid. After the pinched segment, particles arrange themselves according to their size, the smaller particles being closer to the channel wall than the larger ones [11]. The main advantage of this technique is the simplicity of the device, while the main limitation is that vortex formation should be avoided. The device is then restricted to use slow flows, in the order of 1-16 µL/min [11, 12]. These flows result in a low throughput that ranges from 50 to 100 particles per second [11,12].

A similar approach for particle sorting is the one based on inertial fractionation (Fig. 1.1b), where the parabolic velocity profile developed inside straight microchannels is
exploited [13]. The resulting shear gradient experienced by particles induces a lift force that pushes particles towards the sidewall. As particles approach the sidewall, a secondary lift force arises. These two forces have opposite directions, so there is an equilibrium point that establishes the lateral position of particles, which is a function of particle size. When inertial fractionation is used, smaller particles are distributed closer to the sidewall than larger particles [14]. In the cases where the microchannel has a curved nature, the asymmetry of the fluid flow in the curved regions of the channel causes the development of a secondary flow. This flow, called “dean flow,” is the result of centrifugal forces, and affects the equilibrium position of particles [14]. In contrast to PFF, the sorting performance of these two techniques benefit from an increase in fluid velocity. However, fluid velocity is limited to around 1000 µL/min to avoid particles being irreversibly trapped by the generated dean vortices [14, 15]. Even so, these devices usually show a high throughput, ranging from $1 \times 10^4$ to $1 \times 10^6$ particles per second [14, 15].

The use of standing-free obstacles within a microchannel is another common approach for particle sorting. If the gap between obstacles is larger than particle size, then particles moving through the array of obstacles select their path in a deterministic manner (Fig. 1.1c), according to their size and deformability [16]. That is, particles with a similar size and deformability will follow equivalent migration paths as they move through the device. This technique, coined as deterministic lateral displacement, can handle flow rates up to 10 mL/min [17], which is higher than the flow rates used in most sorting methods. The throughput for these devices is in the order of $1 \times 10^6$ cells per second. A main disadvantage of this technique is that the displacement of cells perpendicular to the primary flow is determined by the pattern of the array. Hence, a device with a fixed design is only applicable to a given mixture.

A main area of particle separation is comprised by the development of selective barriers, also known as membranes. These filtering devices offer a sieving effect that separates particles based on their size [18]. The most common approach is the use of membranes that allow cells smaller than a given pore size to pass through, while
stopping larger cells [18]. If all the sample is forced to migrate through the membrane by an applied pressure (Fig. 1.1d), then the operation mode is called dead-end filtration. Current microfabrication technologies allow a high control over the distribution size of the membrane pores [18], enabling the manufacturing of membranes with high selectivities for the components to be separated. Membranes with different pore sizes can be placed sequentially inside a microchannel to enable the separation of multiple components from a sample [8]. One of the main issues associated with this filtration mode is the accumulation of particles on the filter [19], which reduces the separation efficiency of the membrane. To avoid fouling issues, the sample can be pumped across the membrane (i.e., parallel to its surface, Fig. 1.1e), and the membrane is said to work in a cross-flow mode [20]. This mode enables the use of periodic reverse flow as an alternative to further reduce fouling [20]. The main limitation of this approach is that a high velocity is usually needed to sweep off any retained solid at the membrane surface [8], which can damage shear sensitive materials. Moreover, cross-flow operation is usually less efficient than dead-end filtration [20]. In general, membrane-based separations offer high resolutions, but the size-based separation achievable requires an adequate pore size. Hence, a device with a fixed pore size is only applicable to the sorting of particles with a specific size.

Particle separation can also be achieved using optically-induced techniques. When a light beam is projected on a particle, light scatters and produces a change in the momentum of photons [21]. This momentum change results in the creation of a force that can be used to manipulate particles. This optical-based technique, termed as optical tweezers, allows adjusting the laser power and wavelength for achieving particle separation. The base for this separation is a difference in the refractive index between the particle or cell and the surrounding suspending medium. The applicability of this technique is limited because it requires a complicated experimental setup [21] and because it produces forces that are considerably smaller than other approaches. This is reflected in low throughput rates, which ranges from 20 to 100 particles per second [21, 22].
Finally, electric fields can be used in microfluidic devices to promote electrically controlled particle separation. The basis for this cell manipulation is called dielectrophoresis, and refers to the net force that cells experience when they are placed on a non-uniform electric field [23]. Using dielectrophoresis, particles and cells can be differentiated by their size and dielectric properties. The next section will cover in detail this separation technique.

1.2 Electrokinetics

Numerous materials employed in microfluidic devices acquire surface electric charges upon contact with an aqueous solution. These surface charges influence the distribution of nearby ions in the fluid and lead to the development of an electrical double layer (EDL) [24]. This interesting phenomena makes the application of electric field driven techniques an attractive option for the manipulation of particles. Indeed, electric field driven techniques have proved to be efficient and reliable methods when coupled with microfluidic devices for the manipulation of a wide range of particles [25], from colloidal particles to nematodes [26–29]. Successful applications for the analysis of biological particles, such as macromolecules and cells [28, 30–32], have also been reported using electric field driven techniques.

When a direct current (DC) electric field is applied in a microchannel with an EDL, the liquid within the double layer acquires momentum. This momentum is transmitted to adjacent layers of fluid through the action of viscosity, giving rise to an electroosmotic (EO) flow of the electrolyte fluid. The EO flow is particularly attractive as a transport mechanism, since it produces low sample dispersion due to its plug-like velocity profile and it can be easily controlled by manipulating the applied electric potential. The addition of EO flow suppressants is also widely employed to further control the EO flow velocity [33]. Hence, EO flow is commonly employed to
pump fluid and particles through microfluidic devices. If the particles to be transported are electrically charged, they will experience an attractive force towards the positive or negative pole, according to their charge. This force, coined electrophoresis (EP), results in a migration of the particles relative to the fluid [24]. Both mechanisms (EP and EO) are linear functions of the electric field, and their net contribution to particle movement can be considered in a single electrokinetic (EK) transport term, defined as the superposition of EO flow and EP [34,35].

1.3 DIELECTROPHORESIS

Particles with different charge magnitudes move at different EK velocities. Hence, particle separation can be achieved by simultaneously controlling EO and EP. If the particles are neutral or weakly charged, particle separation by these mechanisms is not feasible, since the EP forces are low and all particles move at the same velocity as the medium. If the neutral particles are exposed to a non-uniform electric field, however, they will experience an induced multipole [36], and the interaction of the non-uniform electric field with the multipole results in net particle movement [37]. This electrokinetic transport mechanism, known as dielectrophoresis (DEP), is caused by polarization effects when a dielectric particle is exposed to a non-uniform electric field [37, 38]. Particle manipulation by means of DEP does not require particles to have a net charge, as long as they are polarizable. This technique offers great potential for the manipulation of micro and nanoparticles, since particle motion is defined by physical and electrical properties of both the particles and the suspending medium. Thus, DEP-based techniques present an advantage over current methods in the ability to be highly specific with minimal sample preparation [39]. Particle properties such as size, shape and relative polarizability can be exploited through DEP to achieve a desired separation. Moreover, DEP is highly flexible, since it can be used with DC
and alternating current (AC) electric fields, providing means for simultaneous particle sorting and enrichment [32]. Due to these attractive characteristics, DEP has been increasingly investigated as a method for particle detection, sorting, enrichment and isolation [40]. Successful applications of DEP with bioparticles include the separation of proteins [41, 42], DNA [43, 44], viruses [44], bacteria [3, 45–48] and mammalian cells [49–52]. Non-biological particles, such as colloids [29, 53, 54], carbon nanotubes [55] and polystyrene particles [56–58], have also been manipulated by DEP. In all these studies, efficient particle manipulation and enrichment is a crucial issue for the development of integrated microfluidic systems.

The generation of non-uniform electric fields is a key aspect of DEP. Although these non-uniformities can be introduced in several ways, two main approaches comprise the development of DEP-based devices [2, 59]. The first one, electrode-based DEP (eDEP), uses asymmetric arrays of microelectrodes embedded within a device [2]. Relatively high electric field gradients can be generated by applying low electric potentials, due to the minute electrode dimensions, leading to high DEP forces. Many different electrode configurations have been successfully employed to date [2]. However, microelectrodes are prone to lose their functionality as a result of fouling [2], which is common when working with biological samples. Further, eDEP devices usually have complex and expensive fabrication procedures [2]. An alternative to the use of microelectrodes is the addition of arrays of electrically insulator structures within a channel (Fig. 1.2). The presence of the insulator structures decreases the cross-sectional area of the channel between two external electrodes, distorting the electric field distribution and creating regions with a non-uniform electric field [60, 61]. This technique, coined as insulator-based DEP (iDEP), produces simpler and inexpensive devices [4], specially when polymer casting is employed. Moreover, it enables EO flow to be used for pumping liquid and particles through channels, simplifying even more the device configuration [25, 62]. However, higher electric potentials are required to produce sufficiently strong electric fields, since electrodes are frequently located far apart from each other or are external. Despite these characteristics, iDEP has been
proved to be an effective method for particle manipulation [60,61].

![Figure 1.2: Schematic illustration of the microfluidic channel with an array of insulating posts employed in this work.](image)

Particles can either be attracted to or repelled from regions of high electric field gradient, depending on their relative polarizability with respect to that of the suspending medium. If the induced multipole moment of the particles is greater than that of the suspending medium, particles experience a force towards the regions of high electric field gradient (positive DEP, Fig. 1.3a). Particles that are less polarizable than the suspending medium, in contrary, experience a repulsive force and migrate away from these (negative DEP, Fig. 1.3b). An important parameter that greatly affects particle polarization is the frequency of the applied electric potential [63]. At low frequencies, conductivity effects dominate particle polarization, while permittivity effects dominate at high frequencies.

A significant portion of the studies where eDEP devices are employed focused on the manipulation of nanoparticles with high frequency electric fields, in the kHz to MHz range. When electric fields with frequencies in the kHz range are employed, inert polystyrene particles exhibit a positive DEP behavior. Green et al. [64] employed polynomial and castellated electrodes to manipulate 557-nm polystyrene particles. The authors observed a negative DEP behavior when 5 V were applied at 5 MHz using the polynomial electrodes and when 8 V were applied at 8 MHz using the castellated electrodes. Positive DEP behavior was observed, in contrast, when 8 V were applied at 500 and 700 kHz with the polynomial and castellated electrodes, respectively. Similar observations have been thoroughly reported in literature: inert dielectric particles, such as latex or polystyrene particles, exhibit positive behavior at
Figure 1.3: Schematic illustration of the relative polarization of a particle (in blue) when its conductivity, $\sigma_p$, is (a) much greater and (b) much lower than the conductivity of the suspending medium, $\sigma_m$. These limits represent the cases where the particle is (a) much more and (b) much less polarizable than the suspending medium, and experience either (a) positive or (b) negative DEP behavior. The red arrows show the direction of the electric field, while the blue arrows show the direction of the DEP force.

Most studies conducted using iDEP devices reported that inert and biological micron-sized particles exhibit negative DEP behavior under the application of DC and low frequency electric fields. The characterization of live and dead bacterial cells in a device with cylindrical insulating posts was performed by Lapizco-Encinas et al. [3,65] using DC electric fields. A negative DEP behavior was observed for both type of cells. Pysher and Hayes [34] developed the technique of gradient-iDEP by employing a converging microchannel with sawtooth walls. Upon the application of DC electric fields, live and dead bacterial cells were identified and separated by means of negative DEP. The same DEP behavior was reported by Kang et al. [66] in the sorting of polystyrene particles of different diameters (6-µm, 10-µm and 16-µm) in a channel with a single insulator structure. A similar study by Srivastava et al. [67] reported the negative DEP behavior of smaller polystyrene particles (3-µm, 6-µm and 10-µm diameters). Bhattacharya et al. [49] demonstrated the trapping of 10-µm polystyrene
beads by negative DEP, using DC electric fields and an elliptical-shaped insulating post. The study also reported a positive DEP behavior of MCF-7 cells under the same experimental conditions. Zhu and Xuan [68] employed curved and spiral channels to sort 5-µm and 10-µm polystyrene particles, observing only a negative DEP behavior. The same group reported the negative DEP behavior of 3-µm polystyrene particles under DC and low frequency fields [69]. When using the novel 3D-iDEP technique, Zellner et al. [70] reported positive and negative DEP behaviors in a mixture with E. coli cells and 1-µm polystyrene particles using DC and AC electric potentials below 600 kHz. Using the contactless-iDEP technique, Shafiee et al. [71] demonstrated the positive DEP behavior of the THP-1 leukemia and MCF-7 breast cancer cells using AC signals below 85 kHz.

1.4 Objectives

Particle motion in iDEP devices is defined by physical and electrical properties of both the particles and the suspending medium. The viscosity, permittivity and conductivity of the suspending medium are all parameters of interest. For a given suspending medium, however, three particle properties define the DEP force and behavior that particles will experience: size, shape and relative polarizability. In addition, there are parameters which are external to the system, namely the applied electric potential, frequency, and time that the potential is applied. All these parameters define the state of the system, the position of the particles and the trapping capacity of the device. This work has four main objectives towards the design of customizable, high-performance iDEP devices for the separation of complex mixtures.

Objective 1  Provide a deep analysis of the spatial forces acting on particles, along with the regions where particles are likely to be retained by DEP (also referred as
trapping regions). In particular, the analysis of how particle size and shape affect the trapping regions would be of benefit for the development of optimization strategies towards the design of high-performance iDEP devices.

Objective 2  Design a systematic approach that integrates the geometrical parameters and arrangement of the array of insulator structures to maximize the trapping performance of iDEP devices. This will, in turn, decrease the required electric potential to trap particles, enhancing the portability of these devices and enabling the incorporation of less-sophisticated voltage supplies.

Objective 3  Design of an iDEP device that inverts the order of particle elution by using arrays of asymmetric posts, so that larger particles leave the system before smaller particles, decreasing cell damage due to electrical exposition.

Objective 4  Design and fabrication of a novel dielectrophoretic device based on the polymer casting method, which preserves the easiness of fabrication observed in iDEP devices. The main distinction of the proposed approach with iDEP is that, while a subset of posts behaves as insulators, another subset behave as microelectrodes and have the capability of achieving high electric field intensities.
The array of insulator posts transverse the entire depth of a channel, creating a distortion of the electric field over the entire channel volume. Since previous reports demonstrated that changes in the electric field along the channel depth are negligible [72], the simulation domain can be considered as a 2-dimensional space. The AC/DC module of COMSOL Multiphysics 4.4 (COMSOL Inc., Newton, MA) was used in this work to estimate the distribution of the electric field and its gradient. Meshes with a minimum of 1,000,000 triangular finite elements distributed along the simulation domain were employed for the discretization of the channel.

The distribution of the electric field requires solving the Poisson equation for the electric potential, $\phi$:

$$\nabla^2 \phi = \frac{\rho_m}{\varepsilon_m},$$  \hspace{1cm} (2.1)

where $\rho_m$ is the space charge density and $\varepsilon_m$ is the real permittivity of the suspending medium. For simplicity, this work considers the applied electric potential to be DC and a charge free space. The first consideration can easily be extended to consider...
AC potentials when accounting for the time dependence or by calculating averages over time. The second consideration assumes that there are no charge sources inside the system, forcing the system to satisfy the Laplace equation in all points inside the channel:

\[ \nabla^2 \phi = 0. \quad (2.2) \]

Therefore, the Laplace equation (Eq. 2.2) was solved to describe the distribution of the electric potential in the microchannel. The electric field, \( \mathbf{E} \), can be determined from:

\[ \mathbf{E} = -\nabla \phi. \quad (2.3) \]

Dirichlet boundary conditions were employed to describe the electric potentials at the inlet (Eq. 2.4) and outlet (Eq. 2.5) of the microchannel, where \( V_i \) and \( V_o \) are the applied voltages at the inlet and outlet electrodes, respectively. The insulating structures and the walls of the microchannel were described using Neumann boundary conditions (Eq. 2.6).

\[ \phi = V_i, \quad (2.4) \]
\[ \phi = V_o, \quad (2.5) \]
\[ \hat{n} \nabla \phi = 0. \quad (2.6) \]

### 2.2 Electrokinetic Force and Velocity

Particle motion in iDEP devices depends on the behavior of particles under the influence of EP and DEP forces, as well as their transport by EO flow. The application of
an electric field acts on the ions within the EDL and exerts a drag force on the fluid. This produces an effective slip velocity outside the EDL, generating an EO flow. The EO flow velocity, \( \mathbf{v}_{eo} \), is given by the Helmholtz-Smoluchowski equation [24]:

\[
\mathbf{v}_{eo} = \mu_{eo} \mathbf{E} = -\frac{\varepsilon_m \zeta_w}{\eta} \mathbf{E},
\]

(2.7)

where \( \mu_{eo} \) denotes the EO mobility. The EO mobility can be defined in terms of the real permittivity and viscosity of the suspending medium, \( \varepsilon_m \) and \( \eta \), respectively, and the zeta potential of the microchannel wall, \( \zeta_w \). The channels employed in this work are made from PDMS, which has a negative surface charge and a negative zeta potential. Hence, the fluid motion is towards the negative electrode (outlet, Fig. 1.2).

The EP velocity, \( \mathbf{v}_{ep} \), which originates from the electrical charge of the particle, can also be described by the Helmholtz-Smoluchowski equation, in the limit of a thin EDL [24]:

\[
\mathbf{v}_{ep} = \mu_{ep} \mathbf{E} = \frac{\varepsilon_m \zeta_p}{\eta} \mathbf{E},
\]

(2.8)

where \( \mu_{ep} \) denotes the EP mobility, and \( \zeta_p \) refers to the zeta potential of the particle. The assumption of a thin EDL is true for high ionic strengths or when the particle size is large, generally in the micron size range. The particles employed in this work have a negative charge, and their EP motion is towards the positive electrode (inlet, Fig. 1.2).

According to Equations 2.7 and 2.8, the EO and EP velocities are both linear functions of the electric field. Hence, their contribution to particle velocity is usually considered in the single EK transport term, \( \mathbf{v}_{ek} \):

\[
\mathbf{v}_{ek} = \mu_{ek} \mathbf{E} = -\frac{\varepsilon_m (\zeta_w - \zeta_p)}{\eta} \mathbf{E},
\]

(2.9)

where \( \mu_{ek} \) is the EK mobility. The determination of the combined zeta potential \( (\zeta_w - \zeta_p) \) can be experimentally determined using particle image velocimetry [73,74].
Usually, the value for \((\zeta_w - \zeta_p)\) is lower than zero, indicating that \(\zeta_w\) is greater than \(\zeta_p\) (both are negatives). This observation also indicates that particles move from the positive to the negative electrode when a DC electric potential is applied (from the inlet to the outlet, Fig. 1.2). Further, the EK force \(\vec{F}_{ek}\) acting on a spherical particle of radius \(r_p\) that produces the EK velocity in Equation 2.9 is [24]:

\[
\vec{F}_{ek} = -6\pi r_p^2 \varepsilon_m (\zeta_w - \zeta_p) \vec{E}.
\] (2.10)

Equations 2.9 and 2.10 are directly applicable when the source of the electric field is a DC potential. If the applied electric field is generated from an AC electric potential, however, some considerations need to be assessed. Since the direction of the electric field changes periodically with time, EO produces back-and-forth fluid displacement. Similarly, the positive and negative electrodes exchange their positions periodically, producing a back-and-forth EP particle displacement. Hence, the time-averaged EK velocity and force tend to zero:

\[
\langle \vec{v}_{ek} \rangle = 0,
\] (2.11)

\[
\langle \vec{F}_{ek} \rangle = 0.
\] (2.12)

### 2.3 Dielectrophoretic Force and Velocity

When describing particle motion, the EK velocity can be defined as the superposition of the EO and EP velocities, since both phenomena are linear functions of the electric field. In contrary, DEP is a second order function of the non-uniformities in the electric field. The DEP velocity, \(\vec{v}_{dep}\), of a spherical particle upon the application of a DC electric field is defined as [24,36,37]:
\[ \vec{v}_{dep} = \mu_{dep} \nabla (\vec{E} \cdot \vec{E}) = \frac{r_p^2 \varepsilon_m}{3\eta} \text{Re}[f_{CM}] \nabla (\vec{E} \cdot \vec{E}), \] (2.13)

where $\mu_{dep}$ denotes the DEP mobility and $\text{Re}[f_{CM}]$ is the real part of the Clausius-Mossotti (CM) factor. If an AC electric potential is applied, the direction of the DEP force does not change along with the electric field, since DEP depends on the gradient of the electric field. The time-averaged DEP velocity in this situation is defined as [24, 36, 37]:

\[ \langle \vec{v}_{dep} \rangle = \mu_{dep} \nabla (\vec{E} \cdot \vec{E}) = \frac{r_p^2 \varepsilon_m}{6\eta} \text{Re}[f_{CM}] \nabla (\vec{E} \cdot \vec{E}). \] (2.14)

The DEP force acting on a spherical particle upon the application of DC and AC electric potentials are defined as [37]:

\[ \vec{F}_{dep} = 2\pi r_p^3 \varepsilon_m \text{Re}[f_{CM}] \nabla (\vec{E} \cdot \vec{E}), \] (2.15)

\[ \langle \vec{F}_{dep} \rangle = \pi r_p^3 \varepsilon_m \text{Re}[f_{CM}] \nabla (\vec{E} \cdot \vec{E}), \] (2.16)

respectively.

### 2.4 Thermal and Electrical Cell Damage

One of the main characteristics of iDEP devices is that the electrodes are located outside the microchannel, at the inlet and outlet reservoirs (Fig. 1.2). The use of external electrodes avoids a direct contact with the sample, preventing the accumulation of unwanted material on the electrodes surface and also avoiding the generation of electrolysis by-products inside the channel. However, in this non-contact mode, an electric field is created through the whole channel. Considering that high electric potentials are usually employed in iDEP devices for promoting DEP particle trapping,
a significant amount of Joule heating can be produced in the microchannel [75]. The generated Joule heat has the potential to increase the temperature of the suspending medium [76], impacting the magnitude of the EK and DEP forces. Moreover, a rise in temperature enacts a thermal stress on cells, which can result in cell damage by the denaturation of proteins triggered by the elevated temperatures [77]. This observation is especially true in the regions closer to the insulating structures, where the electric field intensity is largely amplified.

In addition to thermal stress, a high electric potential also develops an electrical stress on cells. This stress is mainly caused because the applied electric field is “amplified” in the form of a potential across the cell membrane [78]. If a spherical cell is considered, the frequency dependence of the field acting across a cell membrane ($E_m$) can be estimated as [79]:

$$E_m$$
where $R$ is the cell radius, $d$ is the membrane thickness, $E$ the applied electric field and $\theta$ is the polar angle with respect to the field direction. In Equation 2.17, $\tau$ is a time constant that depends on cell morphology and the conductivities of both the cell components and suspending medium. At low frequencies ($\omega \tau < 1$), as is the case when using iDEP devices, the field $E_m$ can exceed the applied electric field by a factor of 1000 or greater, depending on cell size [80]. Cell damage by the electrical treatment is then primarily caused by a field-induced breakdown of the physical integrity of the plasma membrane [80].

Both Joule heating and the potential across cell membrane have a strong dependence on the applied electric field. It has been reported that skin and muscle cells are electroporated with 1,200 V pulses with a duration of 20 ms [81]. A study of murine B lymphocytes showed that 5 µs pulses with electric fields of 3 kV/cm and 5 kV/cm resulted in 20% and 70% of the cells becoming nonviable, compared with 5-7% of non-viable cells for samples that were not electrically treated [82]. LaLonde et al. [83] shown that only 5% of yeast cells remained viable after a 4-minute exposure when a DC potential of 1,000 V was applied in an iDEP system. As observed, the design of high-performance iDEP devices should be able to promote cell trapping while decreasing cell damage, which can be achieved by decreasing the electric potential needed to polarize the device.

2.5 The Clausius-Mossotti factor

The CM factor, $f_{CM}$, arises when solving the Laplace equation and matching the boundary conditions for the electric field at the surface of the particle:
In Equation 2.18, \( \varepsilon_p^* \) and \( \varepsilon_m^* \) are the complex permittivities of the particle and suspending medium, respectively. The complex permittivity is comprised by the conductivity, \( \sigma \), and permittivity, \( \varepsilon \), of either the particle (\( p \)) or the suspending medium (\( m \)) [37]:

\[
\varepsilon^* = \varepsilon + \frac{\sigma}{\omega i},
\]

where \( i \) is the imaginary vector \( (i = \sqrt{-1}) \) and \( \omega \) is the angular frequency of the applied potential \( (\omega = 2\pi f, \text{ where } f \text{ is the ordinary frequency}) \). According to Equation 2.18, the CM factor accounts for the relative polarization of the particle and the suspending medium, and it can only take values in the range \( f_{CM} \in [-0.5, 1.0] \). The lower (or upper) bound indicates the case where the particle is much less (or much more) polarizable than the suspending medium, and the particle exhibit negative (or positive) DEP with a \( f_{CM} = -0.5 \) (or \( f_{CM} = 1.0 \)). An important observation derived from Equation 2.18 is that, beyond controlling the direction of the DEP force, the CM factor also has a contribution on the magnitude of the DEP force experienced by the particle. As noted by the limiting values, the negative DEP force can only be half as strong as the positive DEP force. This observation remarks the importance of particle polarization for the design of iDEP devices. Further, the mechanisms that dominate particle polarization at low and high frequencies can be derived by applying the appropriate limits to Equation 2.18:

\[
\lim_{\omega \to 0} f_{CM} = \frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m}, \quad (2.20)
\]

\[
\lim_{\omega \to \infty} f_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}. \quad (2.21)
\]

Conductivity effects dominate particle polarization at low frequencies (Equation 2.20, for approximately \( f < 50 \text{ kHz} \) [28]) and permittivity effects dominate at high frequencies (Equation 2.21, for approximately \( f > 50 \text{ MHz} \) [28]).
2.6 The dielectrophoretic signature

The limits presented in the forms of Equations 2.20 and 2.21 show the competition between different charging processes for intermediate frequencies. The total current in the particle has two main components, one associated with the conduction of free charges and one associated with the dielectric displacement, where the particle acts as a capacitor [28]. Figure 2.1 shows the CM factor (Equation 2.18) as a function of the frequency of the applied electric potential in two situations: (a) where a particle is much more conducting than the suspending medium ($\sigma_p \gg \sigma_m$), and (b) where a particle is much less conducting than the suspending medium ($\sigma_p \ll \sigma_m$). The DEP response as a function of the frequency of the applied potential, represented in Figure 2.1 by the CM factor, is known as the dielectrophoretic signature of the particle, whose determination has many practical applications. The first one resides in the selection of the frequency range where a mixture separation is feasible. Imagine a binary mixture with particles having a similar size and shape, but exhibiting different DEP responses (or behaviors) with respect to the applied frequency. A successful separation is more likely to happen when the difference of the dielectrophoretic signature of the particles is a maximum. For instance, particles (a) and (b) in Figure 2.1 can be easily separated if $f < 1\times10^4$ Hz, since they exhibit a positive and negative DEP behavior, respectively. The separation becomes difficult when $f > 1\times10^6$ Hz, since both particles exhibit a negative DEP behavior. Second, the crossover frequency, corresponding to the frequency where the DEP force is null, can be experimentally determined (as shown in Fig. 2.1). Several reports have used the characterization of the crossover frequency to compare and identify cells with different dielectric properties, like distinguishing between different mutant bacterial strains [84]. Third, the dielectrophoretic signature can be fitted to mathematical models (e.g., a two shell model to describe cells with a cytoplasm and membrane) to determine the electro-
physiological parameters of the cells. For instance, the cytoplasm conductivity (a measure of the ionic strength of the cytoplasm) and the specific membrane capacitance (related to the morphology of the membrane) can be determined from the dielectrophoretic signature of the cell [85].

![Diagram](image)

**Figure 2.1:** In blue, the CM factor for a particle ($\sigma_p = 1 \times 10^{-2}$ S/m and $\varepsilon_p = 2.4$) which is much more conducting than the suspending medium (DI water, $\sigma_m = 1 \times 10^{-4}$ S/m and $\varepsilon_m = 78.4$). The arrow shows the location of the crossover frequency. In red, the CM factor for a particle ($\sigma_p = 1 \times 10^{-2}$ S/m and $\varepsilon_p = 2.4$) which is much less conducting than the suspending medium (DI water, $\sigma_m = 1$ S/m and $\varepsilon_m = 78.4$). The regions where positive and negative DEP behaviors are observed are separated by the magenta line.
Chapter 3

Materials and Methods

3.1 Microdevices fabrication

The fabrication of an iDEP device started with a clean silicon wafer with a diameter of 10 cm. SU-8 3050 photoresist (MicroChem, Newton, MA) was spin-coated on the silicon wafer to form a 40-µm thick resist layer. The thickness was verified using a contact profilometer (Model P2; KLA Tencor, Milpitas, CA). A transparency mask (Output City, Bandon, OR) with a resolution of 25,000 dpi (Fig. 3.1) was used to pattern the photoresist after exposure to ultraviolet radiation.

![Figure 3.1: Images of the high-resolution transparency mask employed to pattern the photoresist.](image)

The resulting master mold contained structures that formed the channels walls and cavities that formed the insulating posts of the iDEP device (Fig. 3.2a). A 3-mm thick
layer of PDMS (Dow Corning, Midland, MI) was cast onto the mold to produce the microchannels and insulating structures (Fig. 3.2b). Multiple microchannels can be manufactured using a single master mold, as shown in Figure 3.2b, where 14 devices were fabricated. The PDMS layer containing the microchannels and insulator posts was then sealed to a glass substrate previously coated with PDMS, by employing a plasma corona wand (Electro Technic Products, Chicago, IL). Therefore, all the interior surfaces of the microchannels were made of PDMS and have the same zeta potential.

Figure 3.2: Schematic illustration of (a) the master mold used to create (b) the PDMS piece containing the channel walls and insulating structures. Images of (c) the master mold and (d) the PDMS piece containing the channel walls and insulating structures.

All microchannels employed in this dissertation were 10.16 mm long and 40 µm deep, and contained an inlet and outlet liquid reservoir. The cylindrical insulating structures in the microchannel design employed in Chapter 4: Fundamentals of the particle trapping mechanism were patterned in arrays of 16 columns with four posts each, and
were located at the center of the microchannel, separated from the microchannel wall by 25 µm. The cylindrical structures had a diameter of 200 µm and were spaced by 250 µm from center-to-center. The devices used in Chapter 5: Optimization of the insulating structures featured distinct post shapes, as clearly described in that section. Independently of the post shape, the array of insulator posts was located at the channel center, 4 mm away from the inlet reservoir and patterned in arrays of 16 columns of four posts each. In Chapter 6: Design of asymmetric posts to reverse the elution order, devices with asymmetric structures are fabricated. The sharp side of each asymmetric post is a half oval 40 µm long and 200 µm wide; while the long side of each asymmetric post is a half oval 150 µm long and 200 µm wide. The asymmetric insulating posts in the microchannels were patterned in arrays of 16 columns with four posts each, located at the center of the microchannel, separated from the microchannel wall by 10 µm.

The development of the hybrid device needed a personalized fabrication procedure that deviated from the standard procedure described in this chapter. Hence, the specific fabrication steps followed to manufacture the hybrid device will be described in Chapter 7: Design and fabrication of a hybrid DEP device.

### 3.2 Polystyrene Microparticles

Yellow-green (ex/em 505/515 nm) and red (ex/em 580/605 nm) fluorescent polystyrene microparticles with diameters of 500-nm, 1-µm and 1-µm (Invitrogen, Eugene, OR) were employed for experimentation. The stock suspensions of the microspheres were sonicated for 10 minutes to break aggregates and diluted in a low conductivity media, either DI water with KOH (pH = 8, conductivity = 20 µS/cm) or a 0.2 mM K₂HPO₄ (pH = 7, conductivity = 60 µS/cm), as described in each section. Each suspension had a concentration of \(7.0 \times 10^8\) particles/mL.
3.3 Cells

*Escherichia coli* (ATCC 25922) cells were cultured in a Lysogeny broth liquid medium at 37 °C in a shaker incubator for 12-14 hours, until they reached an optical density of 0.65 measured at 600 nm. This optical density corresponds to a cell concentration of $1.79 \times 10^8$ cells/mL. For labeling, 2 mL of culture were centrifuged at 5,000 rpm for 10 minutes and the supernatant was discarded. The pellet was washed twice in DI water and resuspended in 0.5 mL of DI water. Next, 3 µL of Syto 11 (Invitrogen, Carlsbad, CA), a green fluorescent bacterial stain (ex/em 508/527 nm), were added. Cells were later incubated with the dye for 20 minutes and centrifuged at 5,000 rpm for 10 minutes. The pellet was washed three times in DI water to remove the excess of Syto 11. Finally, cells were resuspended in 0.5 mL of low conductivity media. The final concentration was $7.16 \times 10^8$ cells/mL. Cells had a prolate shape and were $2.38 \pm 0.32$ µm long and $1.20 \pm 0.21$ µm wide.

*Saccharomyces cerevisiae* (ATCC 9763) cells were cultured in a yeast mannitol broth liquid medium at 30 °C in a shaking incubator for 12-14 hours, until they reached an optical density of 1.3 measured at 600 nm. This optical density corresponds to a cell concentration of $1.26 \times 10^7$ cells/mL. For labeling, 2 mL of cell culture were centrifuged at 5,000 rpm for 5 minutes and the supernatant was discarded. The cell pellet was washed twice with DI water and resuspended in 0.5 mL of DI water. Next, 3 µL of Syto 11 were added. Cells were then incubated with the dye for 40 minutes and centrifuged at 5,000 rpm for 5 minutes. The pellet was washed three times with DI water to remove the excess of Syto 11. Finally, labeled cells were resuspended in 0.252 mL of the same low conductivity media. The final concentration was $1.0 \times 10^8$ cells/mL. *S. cerevisiae* cells (or simply yeast cells) had a semi-spherical shape with a diameter of $6.3 \pm 0.4$ µm.
3.4 Equipment and Software

A high voltage sequencer (Model HVS6000D; LabSmith, Livermore, CA) was used to apply DC electric potentials by means of platinum wire electrodes (0.584-mm diameter; LabSmith, Livermore, CA). The voltage sequencer was manipulated with the software Sequence provided by the manufacturer. A waveform generator (33522A, Agilent Technologies, Santa Clara, CA) and a high voltage amplifier (PZD700A2, Trek Inc., Lockport, NY) were used to apply AC electric potentials. Images and videos of the experiments were captured with an Axiovert 40 CFL microscope (Carl Zeiss Microscopy, Thornwood, NY) equipped with an Infinity 2 camera (Luminera, Ottawa, Canada).

3.5 Experimental Procedure

The DEP trapping experiments started with a clean microchannel with insulating structures that was filled with low conductivity media. The channels were then connected to a vacuum chuck manifold (LabSmith, Livermore, CA) using a vacuum pump (Model 400-3910; Barnant Company, Barrington, IL). The manifold (Fig. 3.3(a)) provides larger inlet and outlet liquid reservoirs to reduce the effect of back pressure build up, and interfaces with slip tip syringes, which allowed a simple filling of the microchannels with the suspending medium using pressure. If the channel did not fit the manifold, large cylindrical liquid reservoirs with an height of 40.3 mm and a diameter of 8.2 mm (total volume = 1.5 mL) were embedded at the inlet and outlet port of the channels (Fig. 3.3(b)). Water electrolysis reactions take place on electrode surfaces in
contact with aqueous solutions when DC potentials are applied. The products from these reaction can significantly alter pH [86]. The height of these reservoirs ensures a separation distance of \(~3.5\) cm between the electrode tip and the channel inlet. This large separation between the electrode and the channel inlet, in combination with a large liquid reservoir volume, hinders electrolysis products from entering the channel, preventing the formation of large pH gradients inside the channel. Next, 15 \(\mu\)L of the sample suspension were introduced at the inlet reservoir of the microchannel. Platinum wire electrodes were then placed at the channel reservoirs and an electric potential was applied across the length of the microchannel by employing either the high voltage supply or the waveform generator and high voltage amplifier. Particles and cells response was observed and recorded for each experiment in the form of pictures and videos. Between uses, it was necessary to re-condition the PDMS microchannel to ensure a negative surface charge and a stable EO [87,88]; to do this, each channel was soaked in 0.1 N KOH for two hours and then soaked in DI water for one hour.

Figure 3.3: Images of (a) the manifold interfacing the iDEP device and (b) the large cylindrical liquid reservoirs adapted to the iDEP device.
3.6 CELL VIABILITY ASSESSMENT

The Live/Dead BacLight Bacterial Viability Kit (Invitrogen, Eugene, OR) was used for the assessment of cell viability. This kit consists of two stains, propidium iodide and Syto 9, both staining nucleic acids. Syto 9 is a green fluorescent dye (ex/em 505/515 nm) which is able to enter all cells, and was used for assessing total cell counts. In contrast, propidium iodide is a red fluorescent dye (ex/em 580/605 nm) that can only penetrate cells with damaged cytoplasmic membranes [89]. Cell viability was defined as a function of the membrane integrity, assessed by considering the cell capacity to uptake propidium iodide [90].

The viability of yeast cells was assessed by initially adding a 50 µL sample containing cells to the inlet reservoir. The sample was then exposed to the electric field under normal operation conditions, and extracted from the outlet reservoir. DI water was used to dilute the sample to a final volume of 100 µL. About 1 µL of each fluorescent stain was added to the diluted sample, followed by the incubation of cells for 40 minutes. Finally, the sample was placed in a hemocytometer and fluorescence microscopy was used to identify the viable (green cells in Fig. 3.4) and non-viable (red cells in Fig. 3.4).
Figure 3.4: Fluorescence of viable (green) and non-viable (red) cells after staining them with Syto 9 and propidium iodide.
Chapter 4

Fundamentals of the particle trapping mechanism

Particle polarizability depicts the DEP behavior that particles exhibit: it dictates if particles are attracted or repelled from the regions of higher electric field gradient. In other words, particle polarizability determines the direction of the DEP force experienced by the particles. Particle flow can be classified in three regimes [60], as a result of the interplay between DEP and EK forces to dominate particle movement in iDEP devices. The first one, EK flow, is produced at low electric field intensities, where particle transport is dominated by EK, along with a small contribution of DEP (Fig. 4.1a). Particles in EK flow mostly migrate along with the fluid in isoelectric field lines across the post array. If the electric field magnitude is increased, such that DEP is comparable to EK, the system undergoes a regime change into streaming DEP (Fig. 4.1b). Particles in this regime move in particular streamlines, which are observed as streams of fluid having low and high particle concentrations. If the electric field magnitude is further increased, DEP overcomes EK (trapping DEP regime) and the strong DEP force prevents particles from migrating along with the fluid, capturing them (Fig. 4.1c).

Although particle polarizability has a contribution on the magnitude of the DEP force, other physical properties have larger contributions. Of special interest is the size of the particle, since the DEP force scales with the cube of particle radius. Several reports depict the importance of particle size in the trapping capabilities of iDEP.
devices. Kang et al. [66,91] demonstrated that binary mixtures of particles and cells of different sizes can be separated into two different collecting wells using a single insulating block. When studying the effect of particle concentration in the trapping DEP regime, Lewpiriyawong et al. [92] shown that a larger particle size enhances the enrichment of particles. The same result was reported by Li et al. [93,94] for a similar device. The magnitude of the DEP force, and hence the capacity of a DEP device to promote particle trapping, is also affected by particle shape. Riahifar et al. [95] separated rod and cubic ZnO particles using low-frequency electric fields based on their shape. Valero et al. [96] balanced opposite DEP forces acting on yeast cells at multiple frequencies to sort them. The authors reported an interplay between the internal electric properties of the cells and their shape. Nili and Green [97] studied the effect of higher-order moments for spherical, ellipsoidal and cylindrical particles, showing the importance of higher-order DEP forces for non-spherical particles.

Moreover, the influence of particle size and shape in the generation of regions where particles are likely to be trapped in iDEP devices has been studied to some extent. Baylon-Cardiel et al. [73] studied the regions where particle trapping occurs in a glass iDEP device with cylindrical insulating structures. They predicted the intensity and location of the particle trapping regions for 1-µm polystyrene particles over a wide range of suspending medium properties. Kwon et al. [98] studied the effect of

Figure 4.1: Experimental flow regimes for 1-µm polystyrene particles (showing a negative DEP behavior) obtained by applying a DC electric potential of (a) 100, (b) 200 and (c) 300 V in the iDEP channel shown in Figure 1.2. The suspending medium is DI water with a pH of 8 and a conductivity of $2 \times 10^{-3}$ S/m.
the array of insulating structures on particle trapping and determined the optimal spacing between cylindrical posts that results in the strongest lateral-to-longitudinal force ratio to enhance particle trapping. Although these studies have made significant contributions, the assessment of the particle trapping capacity in most DEP systems has been qualitative in nature. More sophisticated mathematical models that are able to represent DEP devices to a greater detail still need to be developed [99]. In addition, numerous devices can achieve some particle trapping despite of design flaws.

The interplay between DEP and EK forces in iDEP devices can hinder the regions where particles are trapped. Therefore, a detailed study of the regions where particles are likely to be retained due to DEP was performed in a device with cylindrical insulating structures (Fig. 4.2). In particular, the effect that particle size (analyzed with polystyrene particles with diameters of 1-µm and 2-µm) and shape (analyzed with spherical polystyrene particles and prolate-shaped E. coli cells) pose on the dielectrophoretic trapping was studied. The magnitude of the electric field, the electric field gradient and the net particle velocity was studied at each constriction between consecutive insulating posts to predict the regions where particles trap over different flow regimes.

Figure 4.2: Schematic illustration of the microfluidic channel with an array of insulating posts, along with their geometrical parameters and measurements.

The experimentation was performed by applying DC electric potentials across PDMS channels with arrays of cylindrical insulating structures and observing the response of particles and cells. The mechanism behind particle trapping in iDEP devices
was depicted by both experimental and modeling results. The shape and trapping capacity of these enrichment regions are strongly affected by particle size and shape. It was found that the shape of the clusters formed by the trapped particles can be described by EK and DEP iso-velocity lines for the particles trapped far away and close to the constrictions, respectively.

4.1 Electric field and particle velocity at the constrictions

The electric field and the gradient of the square of the electric field ($\nabla (E \cdot E) = \nabla E^2$) in a constriction are shown in Figure 4.3. The figure also shows the resulting EK, DEP and net particle ($\vec{v}_p$) velocities for 1-μm particles. The applied electric potentials in Figure 4.3 are 200, 400 and 800 V. All calculations were estimated at the centerline (red horizontal line in Fig. 4.3a), considering a negative DEP behavior. The center of the constriction between the posts is illustrated as a brown dotted line. As observed, the electric field magnitude rises towards the center of the constriction and then decreases in a symmetrical pattern (Fig. 4.3b). The circular shape of the insulating structures results in a local maximum of the electric field intensity at the center of the constriction. The EK velocity, being a linear function of the electric field, also has its maximum magnitude at the center of the constriction (Fig. 4.3c). This effect influences the regions of the channel where particles are to be enriched.

The non-uniformities in the electric field, induced by the insulating structures, are relatively small for the lowest applied potential (200 V), as demonstrated by the semi-flat curve of $\nabla E^2$ (200 V; Fig. 4.3d). Therefore, the DEP force is not strong enough to prevent particle movement (200V; Fig. 4.3e). It is important to note that, in order to facilitate the analysis of the DEP velocity, the next convention was followed: the magnitude of the velocity vectors whose direction is towards the
Figure 4.3: Predictions obtained with numerical simulations in (a) a constriction for the (b) electric field, (c) EK velocity, (d) gradient of the square of the electric field, (e) DEP velocity and (f) net particle velocity for 1-µm particles at potentials of 200, 400 and 800 V. A negative value indicates that the particles move in the direction of the inlet. The centerline and the center of the constriction are shown as a red horizontal line and a brown vertical dotted line, respectively.
outlet (in the direction of the EK velocity) were considered positive. In contrast, the magnitude of the velocity vectors whose direction is towards the inlet (opposing the EK direction) were considered negative. Thus, a negative magnitude of the DEP velocity describes how particles are repelled by the DEP force. This convention was also followed when $\nabla E^2$ was plotted in Figure 4.3d, where a negative value indicates a decreasing gradient.

Away from the constrictions, the electric field is virtually uniform and particles move at the EK velocity. As particles approach the constriction, they experience an acceleration caused by the electric field and the EK velocity increases (200 V; Figs. 4.3b and 4.3c). Particles also experience the DEP force, a repulsive force opposing the direction of the EK velocity, and their net particle velocity decreases (200 V; Figs. 4.3e and 4.3f). Prior to the center of the constriction, the DEP force reaches a maximum and particles show their minimum net velocity (200 V; Fig. 4.3f). After this point, the DEP force decreases (as non-uniformities in the electric field vanish) and particles move again at the EK velocity as they approach the center of the constriction (200 V; Fig. 4.3f). After the center of the constriction, however, the non-uniformities in the electric field increase, and the increasing DEP force accelerates particles towards the outlet, away from the increasing electric field (200 V; Fig. 4.3f). The minimum and maximum magnitudes of the net particle velocity correspond with the regions where the DEP velocity has minimum and maximum values, respectively.

The non-uniformities in the electric field increase when a potential of 400 V is applied, as observed in the curve of the magnitude of $\nabla E^2$ (400 V; Fig. 4.3d). The maximum electric field has an intensity of over $0.8 \times 10^5$ V/m, while the maximum $\nabla E^2$ is in the order of $0.2 \times 10^{15}$ V^2/m^3. The EK velocity, being a linear function of the electric field, is doubled as a result of doubling the applied potential (400 V; Fig. 4.3c). Since DEP has a quadratic dependence on the electric field, the DEP velocity increases four times (400 V; Fig. 4.3e). Particles approaching the constriction experience the same initial impulse to accelerate with the increasing electric field. However, the larger DEP force causes particles to slow down until their net velocity is zero (400 V; Fig.
4.3f). At this point, the EK and DEP velocities are in equilibrium and most particles are trapped at a fixed position. If the equilibrium is disturbed (e.g., by collision with other particles entering the constriction) DEP prevents particles to move towards the center of the constriction and drives particle movement in the opposite direction (i.e., there is a trapping barrier that particles cannot penetrate). Here, EK prevents a further displacement away from the constriction and particles return to the trapping region.

If the applied potential is increased to 800 V, the electric field is greatly affected by the presence of the insulating structures, as observed by the large enhancement on the $\nabla E^2$ curve (800 V; Fig. 4.3d). Consequently, the DEP velocity matches the EK velocity earlier in the constriction and the trapping region is displaced towards the channel inlet (upstream in Fig. 4.2). Notice that, when the electric potential is increased, the shape of the net particle velocity curve (800 V; Fig. 4.3f) tends to the shape of the DEP velocity (800 V; Fig. 4.3e).

### 4.2 Particle trapping condition and trapping regions

Particles are considered to be trapped when the DEP force exerted on them is such that it prevents particle migration downstream along with the fluid. A particle has a net zero velocity when the EK and DEP velocities are in equilibrium. That is, when both velocities have the same magnitude but opposite directions, as described below in terms of the velocities and mobilities:

$$c\frac{\vec{v}_{dep}\vec{E}}{\vec{v}_{ek}\vec{E}} = c\frac{\mu_{dep}\nabla E^2 \cdot \vec{E}}{\mu_{ek} \vec{E} \cdot \vec{E}} = -1,$$

where $c$ is a correction factor. This factor, which scales the DEP mobilities to fit the
experimental trapping observations, is a rough estimation of particle mutual interactions, distortions in the electric field distribution caused by the presence of particles and the effect of higher order moments in particles. Hence, it depends on other parameters/conditions (e.g., the local concentration of particles). Similar correction factors have also been used in related studies [66, 92–94, 100]. The velocities or mobilities ratio in the left-hand side of Equation 4.1 is defined in this work as the trapping condition. In the regions where DEP dominates particle motion, the following condition must be satisfied:

\[ c \frac{\mu_{\text{dep}} \nabla E^2 \cdot \vec{E}}{\mu_{\text{ek}} \vec{E} \cdot \vec{E}} < -1. \]  

(4.2)

The trapping condition (Eq. 4.1) has been derived here from an equilibrium in the DEP and EK velocities (or forces). The minus sign in the right-hand side of Equations 4.1 and 4.2 is intuitive: it represents that the velocity vectors are equal in magnitude but have opposite directions. While other authors [3, 49, 98, 101] have an algebraic derivation of this trapping condition, we found this representation to be simpler to understand. For instance, in the case where particles exhibit negative DEP, the DEP mobility is lower than zero (\( \mu_{\text{dep}} < 0 \)). Thus, the mobilities ratio in Equations 4.1 and 4.2 is lower than zero and the trapping condition becomes the same as others reported in literature [3, 49, 98, 101]:

\[ c \frac{\mu_{\text{dep}} \nabla E^2 \cdot \vec{E}}{\mu_{\text{ek}} \vec{E} \cdot \vec{E}} \geq -1. \]  

(4.3)

Figure 4.4a illustrates the experimental trapping region and the predicted barrier region on a constriction for the 2-\( \mu \)m particles at 800 V. The barrier region corresponds to the zone in the constriction where the influence of DEP results in a net particle movement in a direction opposite to EK. The trapping barrier represents the boundary of the barrier region, as depicted by the white line in Fig. 4.4a. This means that particles will start being captured on the trapping barrier, where the net particle velocity is zero. In this sense, the trapping barrier must match the first line of trapped particles (inset in Fig. 4.4a), providing a simple method to estimate the correction
factor. The adjustment of the correction was performed as follows. First, a correction factor that resulted in a “complete” barrier region at the experimental minimum trapping voltage was estimated. Figures 4.4(b-e) illustrate how the correction factor affects the barrier region, and the concept of a “complete” barrier region. When the correction factor has a value of 41 or 42, the barrier region is “incomplete” (i.e., there is a region in the centerline where DEP does not overcome EK). In contrast, when the correction factor value is 44 or 45, the barrier region is “complete.” A larger correction factor results in a broader barrier region. Once the initial correction factor was estimated, it was adjusted to match the trapping barrier with the experimental trapped particles at 200, 400 and 800 V. A preference was given to match the centerlines of the experimental trapped particles and the trapping barrier, since the drag and DEP forces acting on particles along the centerline are parallel to each other (inset in Fig. 4.4a). Table 4.1 shows the estimated correction factors for the 1-μm and 2-μm particles and for the E. coli cells. The table also shows the minimum trapping voltage, determined by slowly ramping up the applied electric potential until particles were observed to be trapped.

<table>
<thead>
<tr>
<th>Particles/cells diameter</th>
<th>Minimum trapping voltage (V)</th>
<th>Correction factor, c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 μm (Polystyrene)</td>
<td>376.67 ± 5.77</td>
<td>64</td>
</tr>
<tr>
<td>2.0 μm (Polystyrene)</td>
<td>173.33 ± 5.77</td>
<td>43</td>
</tr>
<tr>
<td>1.3 μm (E. coli cells)</td>
<td>357.77 ± 15.28</td>
<td>69</td>
</tr>
</tbody>
</table>

Table 4.1: Experimental minimum trapping voltage and correction factors for the 1-μm, 2-μm particles and E. coli cells. aThe diameter of the E. coli cells represents the equivalent diameter.

The predicted trapping condition (Eq. 4.3) as obtained from the mathematical model for the 1-μm and 2-μm particles and the E. coli cells is shown in Figure 4.5 for applied potentials of 200, 400 and 800 V. The trapping condition is presented using two different approaches for each particle and cell: line plots (Figs. 4.5a, 4.5d and 4.5g) to show the dependence of the trapping condition curve on the magnitude of the DEP velocity, and as surface plots to illustrate the regions that serve as dielectrophoretic
Chapter 4. Fundamentals of the particle trapping mechanism

Figure 4.4: (a) Illustration of the adjusted correction factor to a value of 43 that matches the experimental band of trapped 2-µm particles and the predicted dielectrophoretic barrier at 800 V. The trapping barrier is depicted as a white line that corresponds to a net particle velocity of zero. The inset shows the direction of the DEP and EK velocities along the centerline. Predicted dielectrophoretic barriers for correction factors of (b) 41, (c) 42, (d) 44 and (e) 45.

barriers (Figs. 4.5b, 4.5e and 4.5h). These regions, shown in yellow in Figures 4.5b, 4.5e and 4.5h, represent the regions where the trapping condition value is lower than -1 and correspond to the zones where DEP repulses particles (i.e., the particles will not be able to enter these regions). Further, Figures 4.5c, 4.5f and 4.5i include images of experimental particle trapping to allow a comparison between the predicted trapping barriers and their experimental counterpart.

The behavior of the trapping condition curve (blue line in Figs. 4.5a, 4.5d and 4.5g) is mainly determined by the shape of the DEP velocity curve (Fig. 4.3e). The trapping condition is satisfied when it crosses the value of -1 (Eq. 4.1), depicted by the green line in Figure 4.5. The first captured particles will be located in the region where the DEP velocity has its minimum magnitude, since this is the location that
Figure 4.5: Predictions obtained with numerical simulations for (a, d and g) the trapping condition curves and (b, e and h) the dielectrophoretic barriers for the 1-µm and 2-µm particles and *E. coli* cells, respectively. The predictions are compared to (c, f and i) experimental trapping regions for the same species when potentials of 200, 400 and 800 V were applied to the microchannels shown in Figure 4.2, respectively. The suspending medium corresponds to DI water with a pH of 8.0 and a conductivity of $2 \times 10^{-3}$ S/m.
first crosses the trapping condition value of -1. For example, only the 2-µm particles are captured at 200 V, as depicted by the plot of the trapping condition crossing the value of -1 (200V; Fig. 4.5d), and also demonstrated by the yellow dielectrophoretic barrier (200V; Fig. 4.5e) and the experimental image of trapped particles (200V; Fig. 4.5f). An increase in the applied potential results in the displacement to the left of the trapping region (see the results for 400 V and 800 V in Figs. 4.5a, 4.5d and 4.5g). For example, for the 1-µm particles, a clear displacement to the left of the band of trapped particles is observed by comparing the pictures at 400 and 800 V in Figure 4.5c. At 400 V, the band is very close to the center of the constriction, while at 800 V the band is located further upstream. For a given suspending medium and a set of particles, the shape and location of the band of trapped particles depends mainly on the shape and spacing of the insulating structures.

4.3 The correction factor as a function of particle size and shape

The larger correction factor (Table 4.1) for the 1-µm particles, in comparison with the 2-µm particles, indicates that the experimental DEP velocity has a larger deviation from the theoretical one (Eq. 2.13). There are several parameters that affect the correction factor to different extents. For instance, using a cubic cell unit calculation and considering the most efficient particle arrangement in the experimentally trapped bands, we have estimated the void fractions for the 1-µm and 2-µm spherical particles to be 0.35 and 0.48, respectively. The lower void fraction for the 1-µm particles implies a lower permeability in the suspending medium in the regions where particles are trapped [102]; reducing the bulk conductivity at the trapping region and posing a larger deformation of the local electric field. Further, it is known that the dielectrophoretic dipole approximation used to derive the DEP velocity equation
(Eq. 2.13) leads to significant deviations between closely spaced particles. This is caused by mutual particle interactions, where the non-uniformities of the electric field induced by the particles themselves are comparable to particle size [37].

Contradictorily, the correction factor for the *E. coli* cells (which have an equivalent diameter of 1.3 μm) is larger than that of the 1-μm particles. We have estimated the void fraction of the *E. coli* cells to be 0.346, close to the void fraction of the 1-μm particles (0.35). However, this calculation considered that all cells have the same dimensions. The void fraction is expected to vary as a function of the size distribution of the cell population. Furthermore, while polystyrene particles have a more homogeneous composition, the *E. coli* cells are composed of a membrane, a wall and a cytoplasm, all with different dielectric properties. The three-shell model employed to describe cells is useful to estimate the cell dielectric properties, but it involves a considerable uncertainty. Moreover, higher-order DEP forces become important when non-spherical particles are studied [97]. Overall, the adjustment of the correction factor for cells is more challenging and less accurate than that for the polystyrene particles.

### 4.4 Location and shape of the trapping regions

Larger particles experience stronger DEP forces, which enlarge the barrier region and displace the trapping region towards the inlet. For example, by comparing the 1-μm and 2-μm particles at 400 V (Fig. 4.5b versus Fig. 4.5e), it is possible to observe a broader barrier region for the 2-μm particles that will push the band of trapped particles upstream. Comparing the experimental results at 400 V (Fig. 4.5c versus Fig. 4.5f), it is clearly seen than the band for the 1-μm particles is located much closer to the center of the constriction than the band for the 2-μm particles. A similar trend is observed at higher applied voltages. Further, the band of trapped particles seems
to be more compact at higher voltages, where higher magnitudes of the DEP and EK forces keep the bands more tightly packed and more defined (200 V and 400 V, Fig. 4.5f).

The shape of the trapping region (i.e., the experimental bands of trapped particles) is defined by two different mechanisms. The front-end of the band of trapped particles has a “flat” profile defined by DEP, while the back-end has a “curved” profile defined by EK (Fig. 4.6a). The front-end of the trapping region, which is close to the center of the constriction, is dominated by DEP forces, which are plotted in Figure 4.6b as “iso-velocity” lines. This image shows almost flat lines with a slight curvature at the center of each line (along the channel width). The behavior of the EK velocity is depicted in Figure 4.6c, where the iso-velocity lines show a strong curvature. As mentioned earlier, particles are trapped due to negative DEP to the left of the barrier regions. The first trapped particles are dominated by the DEP velocity, which follows almost flat lines (Fig. 4.6d). As more particles arrive to the trapping region, they accumulate to the left/behind the trapping barrier, in upstream locations away from the center of the constriction. In these locations, DEP becomes weaker and linear EK dominates particle behavior. The back-end of the band of trapped particles is then defined by the curved EK iso-velocity lines.

A similar behavior is expected for the bands of trapped *E. coli* cells, where the front-end should be flatter than the back-end. However, this is not the case; the band of trapped *E. coli* cells has a strong curvature in both ends (Fig. 4.7a). Since both the DEP (Fig. 4.7b) and EK (Fig. 4.7c) velocities follow similar profiles to the ones for the spherical particles, this observation suggests that the DEP velocity profile is not adequately described by the dipole approximation. In addition, the broad experimental band of trapped cells suggests that *E. coli* cells have more freedom to move in the region where the velocities are in equilibrium. It is important to note that heterogeneity in the cells population has an effect on both the DEP force experienced by cells and on the curvature of the trapping region.
Figure 4.6: (a) Experimental band of trapped 2-µm particles at 800 V. Surface plots of the magnitude of the (b) DEP and (c) EK velocities in a constriction at 800 V. (d) Surface plot of the magnitude of the net particle velocity at 800 V. The arrows indicate the direction of the net particle velocity. The suspending medium corresponds to DI water with a pH of 8.0 and a conductivity of $2 \times 10^{-3}$ S/m.
Figure 4.7: (a) Experimental band of trapped *E. coli* cells at 800 V. Surface plots of the magnitude of the (b) DEP and (c) EK velocities in a constriction at 800 V. The suspending medium corresponds to DI water with a pH of 8.0 and a conductivity of $2 \times 10^{-3}$ S/m.
Chapter 5

Optimization of the Insulating Structures

As noted earlier, the DEP forces in iDEP devices are produced with insulator posts, which distort an otherwise uniform electric field. Given the interplay of EK and DEP forces to dominate particle movement, the DEP trapping regime involves a careful balance between EK and DEP in the posts array, where the design of the insulator posts is crucial for the trapping performance. This performance has been extensively studied in channels with a single insulator structure [38], where rectangular [66,91,103] and triangular [91] shaped structures rank among the most studied structures. Since different sorting performances have been achieved with these devices, the design of the structure plays a main role in the manipulation of particles. Moreover, as observed by Lewpiriyawong et al. [104] when studying devices with rectangular-shaped structures, shifting from a single structure to multiple structures enhances the manipulation of particles. This observation suggested that each insulator structure influences the trapping capacity of neighboring structures. Since then, most studies employ arrays of insulating posts rather than single structures when particle trapping by DEP is desired [38]. Although circle-shaped structures are preferred for the array design [42,60,65,74,105–108], diamond [60,65,106] and rectangular shaped-structures [60,65] have also been thoroughly studied. The importance of selecting an adequate design for the posts array was first depicted by Kwon et al. [98], who determined the optimal longitudinal spacing between consecutive posts in an array of circle-shaped structures.
Chapter 5. Optimization of the insulating structures

The optimal spacing was selected so that the force ratio to induce particle trapping was the highest. The converging sawtooth channel designed by Pysher and Hayes [34] produced progressively higher electric field gradient regions along the channel length. This allowed a more precise control of the variations of the electric field gradient to efficiently manipulate particle movement [109]. These studies have made significant contributions to the performance improvement of iDEP devices. There is still, however, a need for a systematic approach that integrates the geometrical parameters and arrangement of the insulator posts with the aim of decreasing the necessary electric potential to induce particle enrichment.

The analysis of the particle trapping mechanism and the formation of enrichment regions in iDEP devices allowed for the development of functions that describe particle behavior within the post array. In particular, the influence that the geometrical parameters of the posts (i.e., length and width) and their spatial arrangement (i.e., longitudinal and lateral spacing between consecutive posts) have on DEP trapping was explored. The core of the study was composed by several numerical simulations executed in a systematic order, so the designs of the posts array that enhance particle enrichment can be determined. The fabrication feasibility by standard soft-lithographic techniques, which is a major concern on the selection of feasible designs, was also explored. Moreover, each optimization strategy of the parametric variation study was experimentally validated using 2-µm polystyrene particles.

5.1 Particle trapping and the channel design

5.1.1 Insulator posts shape

The distribution of the electric field along the channel is perturbed by the presence of the insulator posts, which reduce the cross-sectional area of the channel, creating non-
uniformities in the field. The electric field undergoes a transition from a relaxed state when no posts are present to a perturbed state between the posts; where the cross-sectional area reduction reaches its maximum. This area transition, which depends on the shape of the post, characterizes the trapping capacity of the device. In this contribution, four insulator shapes, which produce four distinct transitions, were considered (Figs. 5.1(a-d)). These area transitions are: (i) a modest initial decrease in the cross-sectional area followed by a sharp decrease (star-shaped post, Fig. 5.1a), (ii) a constant decrease in the cross-sectional area (diamond-shaped post, Fig. 5.1b), (iii) a sharp initial decrease in the cross-sectional area followed by a modest decrease (circle-shaped post, Fig. 5.1c) and (iv) a step decrease in the cross-sectional area (square-shaped post, Fig. 5.1d).

Figures 5.1(e-h) show the electric field magnitude distribution at the centerline for the considered post shapes. The modest initial decrease in the cross-sectional area obtained with the star shaped posts results in a relatively low increase of the electric

![Figure 5.1](image_url)
field (Fig. 5.1e). A higher local electric field magnitude is achieved by a sharper initial decrease in the cross-sectional area, as evinced by the gradual increase for the diamond (Fig. 5.1f) and circle (Fig. 5.1g) posts. The reduction in the cross-sectional area offered by the circle post produces a local electric field maximum that is \(\sim 1.5\) times the maximum achieved with the star post. The square post provides the sharpest cross-sectional area reduction. Even so, the maximum electric field magnitude does not significantly differ from that of the circle post (Fig. 5.1h). The maximum electric field, however, covers a larger region, whereas for the rest of the post shapes the maximum is reached at a single point. Notice that, with a sharper decrease in the cross-sectional area, the electric field magnitude is less able to completely return to its relaxed state.

Figures 5.1(i-l) present the magnitude distribution of the gradient of electric field square \((\nabla E^2)\), the function directly related to DEP. A significant increase in \(\nabla E^2\) is observed with a sharper decrease in the cross-sectional area between the posts. It is important to note that both the magnitude and size of the region affected by \(\nabla E^2\) are important. A sharp increase in the magnitude of \(\nabla E^2\) over a small region may not produce effective particle trapping. Moreover, a sharper decrease in the cross-sectional area directly affects the location of the maxima points for \(\nabla E^2\) associated with each shape. The location and spacing of these maxima points characterize the zones where trapping iDEP and streaming iDEP dominate particle movement.

### 5.1.2 Insulator Posts Geometry

To determine the particle trapping performance of a channel with a given post shape, the forces acting on particles located in the openings within consecutive posts (Fig. 5.2a) were studied. The geometrical parameters of the insulator posts that determine their shape are length \((L)\) and width \((W)\). Particles are trapped when the negative DEP force exerted on them is such that it prevents particle migration downstream along with the fluid. Hence, particles have a net zero velocity when the EK and DEP forces are in equilibrium. That is, particle trapping occurs when both velocities (or
forces) have the same magnitude but opposite directions, as described by Equation 4.1. The regions where the magnitude of the DEP velocity exceeds the magnitude of the EK velocity are regions that particles are not able to penetrate, as described by Equation 4.2.

The DEP and EK mobilities are a function of the particles and the suspending medium properties [24, 36, 37]. For a given particle in a suspending medium, the mobilities are independent of the electric field distribution and are held constant. If a negative dielectrophoretic behavior is considered, the DEP mobility is lower than zero ($\mu_{\text{dep}} < 0$) and the mobilities ratio in Equation 4.3 can be expressed as:

$$\frac{\nabla E^2}{E^2} \geq -\frac{\mu_{\text{ek}}}{\mu_{\text{dep}}}. \quad (5.1)$$

According to Equation 5.1, an increase in particle manipulation due to DEP can be achieved by simply modifying the geometrical parameters of a device to enhance the non-uniformities of the electric field. The left-hand side of Equation 5.1 is now considered as the trapping condition, and the average trapping condition ($T_C$) in an
opening can be defined as:

\[ T_C = \frac{1}{A} \int \left| \nabla (\vec{E} \cdot \vec{E}) \right| dA, \quad (5.2) \]

where \( A \) is the area of the opening between the insulator posts. Considering a set of insulator posts with different geometrical parameters, the post with the largest trapping capacity is the one with a maximum value for \( T_C \). An important observation in Equation 5.2 is the absolute value operator added to the integrand, which accounts for the posts symmetry. The numerator in Equation 5.1 is lower than zero between the first set of posts (upstream in Fig. 5.2b) and greater than zero between the second ones (downstream in Fig. 5.2b). Given that the electric field is symmetric on both regions (4.3b), \( T_C \) would tend to zero if the absolute value operator is not used.

### 5.1.3 Insulator Posts Arrangement

The average trapping condition \( (T_C) \) is useful to optimize the trapping capacity when only the geometrical parameters of the insulator posts are considered (i.e., length, width and shape). However, \( T_C \) does not appropriately represent the variation in the trapping capacity caused by closely spaced posts. Neighboring posts are longitudinally and laterally spaced by \( L_S \) and \( W_S \), respectively (Fig. 5.2a). This is related to the DEP force nature around the insulator posts. The spacing between consecutive posts optimization needs to consider that DEP effects decrease the net particle velocity in the right-hand side of the opening (downstream in Fig. 5.2c) while increasing particle velocity in the other side (upstream in Fig. 5.2c). The resulting particle velocity through the constriction is thus asymmetric (Figs. 4.3 and 5.2b). In order to analyze the effect of the spacing between posts, the EK and DEP forces acting on particles were divided into their longitudinal and lateral components, and the average lateral-to-longitudinal force ratio in the opening \( (F_R) \) was computed:

\[ F_R = \frac{1}{A} \int \left| \frac{\vec{F}_{ek,y} - \vec{F}_{dep,y}}{\vec{F}_{ek,x} + \vec{F}_{dep,x}} \right| dA, \quad (5.3) \]
where $\vec{F}_{ek,y}$ and $\vec{F}_{dep,y}$ represent the lateral components of the EK and DEP forces, respectively, and $\vec{F}_{ek,x}$ and $\vec{F}_{dep,x}$ are their longitudinal components. This methodology is consistent with the study performed by Kwon et al. [98], where the influence of the longitudinal spacing between cylindrical insulator posts was optimized to achieve the highest particle trapping considering positive DEP. When positive DEP forces are present, a higher value of $F_R$ indicates that a particle near the centerline between two posts would be trapped more rapidly as it approaches either of the posts [98]. For negative DEP, however, particle trapping only occurs in the zones where the EK and DEP forces are opposite to each other (downstream in Fig. 5.2c). In the cases where both forces have the same direction, streaming iDEP prevents particle trapping (upstream in Fig. 5.2c). Hence, a negative sign was introduced before $\vec{F}_{dep,y}$ to account for this effect. The sign penalizes or rewards the cases where the lateral components of the EK and DEP forces have the same or opposite directions, respectively.

5.1.4 Parameters considered for optimization

In order to analyze the effect of the posts geometrical parameters and spacing for improving the particle trapping performance of iDEP devices, we started an analysis with the four post shapes (star, diamond, circle and square posts) having a length and width of 200 $\mu$m. The lateral and longitudinal spacing between posts were set to 50 $\mu$m. The initial circle design (Fig. 4.2) is consistent with the microchannel device that our group has been successfully using during recent years to manipulate polystyrene beads, *E. coli*, *Bacillus subtilis* and *Saccharomyces cerevisiae* cells, among other microparticles [74, 83, 106]. The optimization strategy considers variations in the post length, lateral spacing, longitudinal spacing and channel length-scale (Fig. 5.3). The trapping performance of each device after each of these optimization steps is analyzed and described in the rest of this chapter.

In this dissertation, only in-line arrays of insulating structures (Fig. 5.4a) were considered. Since the electric field gradients that give rise to the DEP force depend only on the cross-sectional area decay, the employment of staggered arrays (Fig. 5.4b) do
5.2 Effect of the length of the insulator posts

The post length \((L)\) modifies the cross-sectional area reduction rate: a shorter post results in a sharper reduction rate. Figure 5.5a shows the average trapping condition \((T_C)\) as a function of \(L\) for each one of the four post shapes, keeping the post width
constant \((W = 200 \, \mu m)\), as well as the lateral \((W_S = 50 \, \mu m)\) and longitudinal \((L_S = 50 \, \mu m)\) spacings. The values of \(T_C\) are shown as percentages obtained by normalizing \(T_C\) with respect to the initial configuration of each post shape (where \(L = 200 \, \mu m\)). Overall, narrower posts \((L < 200 \, \mu m)\) increase \(T_C\) for all devices. This observation agrees with the results drawn from Figure 5.1: a sharper initial decrease in the cross-sectional area benefits the trapping performance. The enhancement in \(T_C\) is, however, bounded by a critical length; after which the post loses its ability to adequately distort the electric field and \(T_C\) starts to decay. The square post shows the highest benefit in \(T_C\) when the post length is reduced to the critical (or optimal) length, with an average trapping condition of 162\% when \(L = 40 \, \mu m\). That is, decreasing the post length to 40 \(\mu m\) results in a 62\% increase in \(T_C\) with respect to the initial configuration. The square post performance is followed by the star (optimal \(T_C = 154\%\) when \(L = 40 \, \mu m\)), circle (optimal \(T_C = 117\%\) when \(L = 80 \, \mu m\)) and diamond (optimal \(T_C = 110\%\) when \(L = 120 \, \mu m\)) posts. The decay after the optimal length is of special importance in the design of the square post. If the optimal length is decreased by 20 \(\mu m\), \(T_C\) decreases from 162\% to 139\%. If the optimal length is increased by 20 \(\mu m\), however, \(T_C\) only decreases to 159\%. The circle, diamond and star posts, in contrary, show a high design tolerance at their optimal lengths. A variation of \(\pm 20 \, \mu m\) with respect to their optimal length, results in a maximum decay of 3\%, 4\% and 6\%, respectively.
Figure 5.5: (a) Normalized average trapping condition ($T_C$) for the star, diamond, circle and square insulator posts as a function of post length ($L$), where $L \in \{20, 40, 60, 80, 120, 160, 200, 300, 400, 600, 1000\}$ µm. The width ($W = 200$ µm), lateral ($W_S = 50$ µm) and longitudinal ($L_S = 50$ µm) spacing were the same for all post shapes. Results for each post shape were normalized with respect to the design with $W = 200$ µm, $L = 200$ µm, $W_S = 50$ µm and $L_S = 50$ µm, shown with an arrow. The left-hand side of all figures represents narrow posts, while the right-hand side represents broad posts. (b-e) Normalized average trapping condition ($T_C$) when the vertices of the (b) star, (c) diamond, (d) circle and (e) square insulator posts are rounded in 10, 20 and 30 µm, as shown with arrows.
5.3 Achievable precision in the fabrication process

Figure 5.5a shows an important conclusion: narrower posts perform better. The exact shape of the insulator posts in the PDMS device, however, depends on all fabrication details. Soft-lithographic techniques can be controlled with high precision nowadays, but the post shape patterned on the photoresist is usually distorted from the original design. This distortion poses a great challenge for reproducing exact posts in the polymeric substrate, resulting in corner rounding for sharp corner features. The fabrication feasibility by soft-lithographic techniques is therefore an important consideration in the trapping capacity for PDMS devices. In practice, transparency-film masks used in soft-lithography with a minimum feature size of 7 \( \mu \text{m} \) can be easily found. Considering this, Figures 5.5(b-e) introduces the average trapping capacity \( (T_C) \) when the vertices of the star (Fig. 5.5b), diamond (Fig. 5.5c), circle (Fig. 5.5d) and square (Fig. 5.5e) posts are rounded by 10, 20 and 30 \( \mu \text{m} \), each corresponding to different achievable precision levels in the fabrication process. There is a limit where the approaching curves in the sharp region become indistinguishable from each other and cannot be fabricated with standard methods.

As expected, the star and diamond posts are more significantly affected by corner rounding effects. A rounding of even 10 \( \mu \text{m} \) applied to the star post (Fig. 5.5b) results in \( T_C \) drastically tending to zero. This is caused by the loss of their sharp edges, which cannot be replicated with standard soft-lithography processes [59]. Since the star post cannot be fabricated with standard equipment, it is not considered in the remaining sections of this work. The average trapping capacity for the diamond decreases progressively with corner rounding (Fig. 5.5c), where the maximum \( T_C \) roughly decreases from 110% to 92%, 84%, and 79% when its vertices are rounded by 10, 20 and 30 \( \mu \text{m} \), respectively. In addition to a decrease in \( T_C \), rounding the
corners of the diamond also increases its optimal post length (160, 160 and 200 µm, in the same order). This observation is expected: if corner rounding hinders the accurate fabrication of narrow diamond posts, an increase in post length is required to fabricate a structure that can adequately distort the electric field.

The circle post (Fig. 5.5d), contrary to the rest of post shapes, is not significantly affected by rounding effects (as explained, rounding affects oval geometries, i.e., narrow circles). For a rounding of 30 µm, the maximum $T_C$ only decreases 8% and its optimal length is unaltered. Ultimately, this translates to a high tolerance level on its design for the fabrication process. Interestingly, corner rounding effects seems to have a slightly beneficial effect on the square post (Fig. 5.5e). The maximum average trapping condition decreases from 162% to 145% for a rounding of 10 µm, but $T_C$ increases to 149% for a rounding of 20 µm and to 147% for a rounding of 30 µm. This observation may be explained as follows: when the corners of the square post are rounded, its shape slowly tends to that of the circle post. As a result, the square post still provides a sharp cross-sectional area change, but producing a larger region where DEP exceeds EK.

5.4 Effect of the Longitudinal Spacing between Posts

Once the optimal length for every post shape was determined, the ideal longitudinal spacing ($L_S$) that results in a complete relaxation of the electric field was determined. Figure 5.6 introduces the average force ratio ($F_R$) as a function of $L_S$ for the diamond, circle and square posts at their optimal lengths; the width ($W = 200$ µm) and lateral spacing ($W_S = 50$ µm) were held constant among simulations. The average $F_R$ for every post shape was normalized with respect to the optimal designs from Figure 5.5a (where $L_S = 50$ µm). The longitudinal spacing between posts has a larger effect on
the square when compared to that of the circle and diamond. An increase in $L_S$ from the initial value (50 µm) to the optimal (80 µm) for the square results in an increase of 45% in $F_R$. In contrast, increasing $L_S$ to the optimal value for the circle (90 µm) results in a $F_R$ increase of 11%. The optimal longitudinal spacing for the diamond resulted the initial value itself (50 µm). Overall, closely-spaced posts ($L_S < 140$ µm) increase the average force ratio. There is a critical spacing, however, after which $F_R$ starts to decrease. This can be either caused by the perturbed electric field not being able to fully return to its relaxed state or by the net particle velocity being increased by streaming iDEP from the previous post. A closer examination of such critical spacing shows that there is a broad tolerance for the parameter design in the three post shapes. The longitudinal spacing can be varied by ±10 µm with reductions in $F_R$ of only 1% for all post shapes.

5.5 Effect of the lateral spacing between posts

The lateral spacing ($W_S$) between consecutive posts partially defines the cross-sectional area reduction limits. Figure 5.7 presents the average force ratio ($F_R$) for different levels of lateral spacing. The length and longitudinal spacing of each post shape were set according to their optimal values in Figures 5.5a and 5.6, respectively. The width was the same for all posts ($W = 200$ µm). The average force ratio ($F_R$) for every post shape was normalized with respect to the optimal designs from Figure 5.6 (where $W_S = 50$ µm). An increase in $W_S$ from the initial value (50 µm) to the optimal, being 30, 20 and 20 µm for the square, diamond and circle posts, respectively, results in significant $F_R$ increases of 33%, 80% and 102%, respectively. In general, closely-spaced posts ($W_S < 40$ µm) increase the average force ratio until a critical spacing is reached. Interestingly, such increase is almost linear for a spacing of 20 µm larger than the critical one (between 20 and 40 µm for the diamond and circle posts, and 30
Figure 5.6: Normalized average force ratio ($F_R$) for the diamond, circle and square insulator posts as a function of the longitudinal spacing ($L_S$), where $L_S \in \{10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 400, 600, 800\}$ µm. The length ($L$) corresponded to the optimal length for each post shape from Figure 5.5a. The width ($W = 200$ µm) and lateral spacing ($W_S = 50$ µm) were the same for all post shapes. Results for each post shape were normalized with respect to the optimal designs from Figure 5.5a (where $L_S = 50$ µm), shown with an arrow. The left-hand side of the figure represents closely-spaced structures, while the left-hand side represents distantly-spaced structures.

and 50 µm for the square post). The largest increase in $F_R$ obtained by decreasing the lateral spacing is achieved within this range. However, there is a limit: when the posts are more closely spaced than the critical spacing, $F_R$ decreases 2%, 8% and 25% for the diamond, square and circle posts, respectively. Since a decrease in the lateral spacing increases the perturbed state of the electric field and results in a higher rate of Joule heating [111], a reasonable compromise should be selected considering the given application.
Chapter 5. Optimization of the insulating structures

5.6 Effect of the microchannel lengthscale

Finally, the lengthscale or size of the microchannels was examined. For this, the post width \( W \) was systematically varied from 40 to 1,000 \( \mu \text{m} \) and the rest of the parameters were scaled in the same proportion. For example, the circle post device resulting from Figure 5.7 has a post width \( W = 200 \mu \text{m} \), a post length \( L = 80 \mu \text{m} \), and each post is longitudinally spaced by \( L_S = 90 \mu \text{m} \) and laterally spaced by \( W_S = 20 \mu \text{m} \). If the post width is reduced to half \( W = 100 \mu \text{m} \), each parameter is also reduced in half \( L = 40 \mu \text{m}, L_S = 45 \mu \text{m} \) and \( W_S = 10 \mu \text{m} \). The percentage in the reduction of the microchannel cross-sectional area remained constant among all simulations with the former methodology.

Figure 5.7: Normalized average force ratio \( (F_R) \) for the diamond, circle and square insulator posts as a function of the lateral spacing \( W_S \), where \( W_S \in \{10, 20, 30, 40, 50, 60, 70, 80, 90, 100\} \mu \text{m} \). The length \( L \) and longitudinal spacing \( L_S \) corresponded to the optimal values for each post shape from Figures 5.5a and 5.6, respectively. The width \( W = 200 \mu \text{m} \) was the same for all post shapes. Results for each post shape were normalized with respect to the optimal designs from Figure 5.6 (where \( W_S = 50 \mu \text{m} \)), shown with an arrow. The left-hand side of the figure represents closely-spaced structures, while the left-hand side represents distantly-spaced structures.
Figure 5.8 shows the value of the average trapping condition ($T_C$) as a function of post width. The post length, longitudinal and lateral spacings were set according to the optimal values shown in Figures 5.5a, 5.6 and 5.7, respectively. For every post shape, $T_C$ was normalized with respect to the optimal designs from Figure 5.7 (where $W = 200 \ \mu m$). The average trapping condition for the three post shapes is similar when the post width exceeds $180 \ \mu m$, while small devices ($W < 120 \ \mu m$) tend to increase $T_C$. The reduction in the lengthscale is, however, bounded by a critical post width, after which the average trapping condition decreases. Interestingly, the square post shows two critical points in such region. Decreasing the lengthscale of the device seems to be more beneficial for the circle and diamond posts (increases in $T_C$ of 34% and 37%, respectively, for their optimal value of $80 \ \mu m$) than for the square post (an increase in $T_C$ of 6% for its optimal values of $90 \ \mu m$ and $160 \ \mu m$).

5.7 Particle trapping performance of the optimal devices

The parametric variation in this work can be used altogether to select an optimal design for each post shape. It is important to state that the optimal designs identified in this study are the result of parametric variations that allowed detecting local optiumns for the explored solution space. Formally, these optimal designs are actually pseudo-optimal designs, since it is not possible to guarantee that they are the globally optimal designs. The optimal designs, as identified in this study, have a width of $W = 80 \ \mu m$ for the diamond, $80 \ \mu m$ for the circle and $90 \ \mu m$ for the square post; a length of $L = 48 \ \mu m$, $32 \ \mu m$ and $18 \ \mu m$ for the diamond, circle and square, respectively. The insulator posts are spaced by $\{L_S, W_S\} = \{20 \ \mu m, 8 \ \mu m\}$ for the diamond, $\{36 \ \mu m, 8 \ \mu m\}$ for the circle and $\{36 \ \mu m, 14 \ \mu m\}$ for the square.

A set of experiments was performed to evaluate the effectiveness of the microchannel
Chapter 5. Optimization of the insulating structures

Figure 5.8: Normalized average trapping condition ($T_C$) for the diamond, circle and square insulator posts as a function of the post shape ($W$), where $W \in \{40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 200, 300, 400, 600, 1000\}$ µm. The length ($L$), longitudinal ($L_S$) and lateral spacing ($W_S$) corresponded to the optimal values for each post shape from Figures 5.5a, 5.6 and 5.7, respectively. Results for each post shape were normalized with respect to the optimal designs from Figure 5.7 (where $W = 200$ µm), shown with an arrow. The left-hand side of the figure represents small devices, while the left-hand side represents large devices.

Designs identified as the best configurations for enhancing the particle trapping performance. In assessing the trapping performance of these designs, an appropriate criteria of the experimental trapping performance must be applied. The criterion should permit the comparison of the distinct designs under dissimilar experimental conditions (e.g., different number of post columns in the region of interest). A common parameter for evaluating a separation in high-performance liquid chromatography is the separation impedance [112]. This parameter accounts for the efficiency of the separation (in terms of number of plates, $N$), but it also accounts for the cost of performing the separation (in terms of pressure drop, $\Delta V$). In our system, the particle trapping efficiency is determined by the area occupied by particles once they are trapped ($A$), while the cost of particle trapping takes the form of the electrical potential that is
necessary to apply in order to achieve well-defined bands of trapped particles \((V_{LEP})\). Hence, analogous to the separation impedance, we define a performance impedance parameter:

\[
P_I = \frac{V_{LEP}}{A},
\]

The defined performance impedance evaluates the trapping performance considering the thickness of the band of trapped particles, but also considers the cost of particle trapping in terms of the required electric potential. A smaller value of \(P_I\) indicates a system with a higher trapping performance. Hence, an increase in either the average trapping capacity \((T_C)\) or the average force ratio \((F_R)\) should be reflected as a decrease in the performance impedance \((P_I)\).

Figure 5.9 illustrates 2-\(\mu\)m particles trapped as bands of enriched particles in each parametric variation step performed towards the optimal designs. Each subfigure shows the electric potential at which the picture was taken, corresponding to the \(V_{LEP}\), and the respective performance impedance, \(P_I\). Figures 5.9(a-c) show the initial diamond, circle and square post devices, which have impedance values of 47, 47 and 111, respectively. The square shape has a significantly lower performance than the diamond and circle structures. According to the numerical simulations (Fig. 5.5a), the optimization of the post length is more beneficial for the square post, followed by the circle and diamond posts. The trend can be observed experimentally in Figures 5.9(d-f), where the optimal post length results in a decrease of 80, 13 and 7 impedance units for the square, diamond and circle posts, respectively.

Devices after the optimization of the longitudinal spacing are introduced in Figures 5.9(g-i), where a decrease of 7, 9 and 0 units in \(P_I\) for the square, circle and diamond posts, respectively, was achieved. This trend matches the order suggested by the numerical simulation results (Fig. 5.6). Notice how the experimental band of trapped particles in the circle and square posts is more uniform for the devices with an optimal longitudinal spacing (Figs. 5.9h and 5.9i), where the front region is flatter.
Figure 5.9: Experimental bands of trapped 2-µm particles for (a-c) the initial devices, (d-f) devices with an optimal length included, (g-i) devices with an optimal longitudinal spacing included, (j-l) devices with an optimal lateral spacing included and (m-o) devices with an optimal lengthscale (or post width) included. Each subfigure shows the electric potential at which the picture was taken, and corresponds with the lowest electrical potential ($V_{LEP}$) that was necessary to apply in order to achieve well-defined bands of enriched particles. Moreover, each subfigure shows the performance impedance ($P_I$). The suspending medium corresponds to DI water with a pH of 8 and a conductivity of $2 \times 10^{-3}$ S/m.
in comparison with the devices having the initial longitudinal spacing (Figs. 5.9e and 5.9e). Since the front region is defined by the shape of the DEP velocity profile [74], this observation indicates an enhancement in the DEP force exerted on particles when the posts are optimally spaced. The good agreement between simulations (Fig. 5.7) and experiments can be also observed for the devices with an optimal lateral spacing (Figs. 5.9(j-l)), whose performance impedance decreased in 7, 3 and 2 units for the circle, square and diamond, respectively. The experiments performed with an optimal lengthscale (or post width) resulted in decreases of 15, 13 and 6 impedance units for the circle, square and diamond posts, respectively. While simulations depicted the diamond as the most benefited post shape (Fig. 5.8), followed by the circle and square posts, the experimental results show the diamond post having a lower performance than expected. This observation is related to corner rounding effects. The fabricated diamond posts have significantly smaller average widths ($W = 59.5 \, \mu m$) and lengths ($L = 38.1 \, \mu m$) than the designed values ($W = 80.0 \, \mu m$ and $L = 48.0 \, \mu m$), for an average difference of 23.1%. This situation contrasts with the circle and square posts, that only present deviations of 5.7% and 6.2%, respectively.

Overall, decreases of 103, 38 and 21 impedance units were observed for the square, circle and diamond post shapes, respectively, in the final optimal designs. The $V_{LEP}$ for these devices was decreased by 840, 630 and 200 V, respectively. These are significant decrements in terms of electric potential requirements, which directly translate to simpler and less expensive equipment to achieve particle separations. Just to cite an example, a voltage of at least 500 V was required to achieve well-defined bands of enriched particles in a recent study by our group [106], where different post shapes were experimentally tested. In the present work, the same degree of particle enrichment and trapping was obtained with voltages below 200 V (Figs. 5.9(n-o)). This is a clear quantitative illustration of how improving the channel design truly enhances particle trapping.

The optimization of the post length and width are the steps that have the largest benefits on the applied electric potential. Interestingly, both geometrical parameters
can be tuned independently of the mobilities ratio using the average trapping condition, $T_C$. Hence, the results for such parameters remain constant for a different set of particles and suspending medium, when negative DEP is considered. The optimal longitudinal and lateral spacing, however, should be reevaluated for a given particle under analysis. The achieved reduction in the $V_{LEP}$ to dominate particle movement is important for the integration of iDEP devices in lab-on-a-chip platforms, since the power consumption of fully-operational devices may be dramatically decreased. In addition, decreasing power consumption enables the incorporation of less-sophisticated voltage suppliers within a device. Since such equipment is generally affordable and less bulky than specialized high voltage power suppliers, the results from this work may provide an accessible framework for studying the application of iDEP devices.
Chapter 6

**Design of asymmetric posts to reverse the elution order**

The separation and enrichment of particles of interest is one of the main applications for DEP devices, where the manipulation of cells and biomolecules are particularly attractive. Biological applications using iDEP devices are abundant in literature. Binary mixtures of four species of bacteria (*E. coli*, *B. subtilis*, *B. cereus* and *B. megaterium*) where concentrated and separated in glass devices by Lapizco-Encinas et al. [3] using DC fields. The same authors demonstrated the separation and concentration of live and dead bacteria using similar devices [65]. Under similar experimental conditions, Moncada-Hernández and Lapizco-Encinas [32] demonstrated the separation of *E. coli* and *S. cerevisiae* cells in less than two minutes. The sawtooth channel designed by Pysher and Hayes [34], fabricated in polydimethylsiloxane (PDMS), was able to separate a mixture with *B. subtilis*, *E. coli* and *S. epidermis* cells. Kang et al. [91] showed the continuous separation of human white blood cells and breast cancer cells using a microchannel with an insulating hurdle. Although these studies employed DC fields, recent studies reported the application of DC-biased AC or cyclical electric fields [57, 113, 114]. For instance, Gencoglu et al. [57] used a DC-biased, low frequency AC field to concentrate and selectively release particles and cells from an iDEP device with diamond-shaped insulating posts.

In these studies, as commonly occurs in iDEP devices, particles in the sample were first trapped and enriched with negative DEP by applying DC or low frequency AC
electric potentials [32, 57, 74]. Next, the applied potential was lowered, resulting in a decrease of the DEP force experienced by particles. The smaller particles in the sample were consequently released as a “peak” of concentrated particles. By further lowering the applied potential, larger particles were subsequently released. A potential disadvantage of this approach is that larger particles stay trapped in the device for longer times. This situation can be problematic when dealing with samples containing biological particles. For instance, a prolonged exposure of eukaryotic cells to electric fields can lead to significant cell damage [83]. Cell damage is mainly caused by an imposed potential on the cells membrane [115], by the rising of Joule heating [116] and by pH changes caused by electrolysis at the electrodes surface [86]. Many studies have demonstrated that both high electric field magnitudes and long exposures can lead to significant cell damage. In consequence, the design of an iDEP device with the ability to invert the order of particle elution is needed. If larger particles can leave the system before smaller particles, larger cells would be subjected to the electrical treatment for shorter time periods, and cell damage can be largely avoided.

The presented characterization and optimization of the post array allowed the identification of geometries that have a maximum and a minimum trapping capacity and which are feasible to fabricate. The incorporation of these two geometries in a single asymmetric post (Fig. 6.1) induces a difference in the DEP experienced by particles at both sides of the posts. More importantly, this asymmetry can displace the location of the trapping or enrichment regions within the device. These observations are exploited in the design of a system where large particles are eluted first, which is counter-intuitive for iDEP devices with DC and low frequency fields. The elution order is reversed by coupling the asymmetric posts with DC-biased AC electric fields (which are asymmetric with respect to time). The proposed device was able to separate a mixture of 500-nm and 1-µm polystyrene particles (a difference of 0.5 µm in diameter), demonstrating its capability to separate particles by exploiting relatively small differences in particle size. One of the objectives that boosted the development of this work is the quick elution of large and fragile biological particles before smaller
particles. Hence, a second mixture containing yeast cells (6.3-µm) and polystyrene particles (2-µm) was also studied. The larger yeast cells were successfully enriched and eluted before the smaller 2-µm polystyrene particles by applying a DC-biased AC electric field for only 40 seconds and maintained an overall cell viability of 85%. In this separation, the asymmetric insulating posts acted as “check valves” for the large yeast cells, allowing for a quick elution of the cells first.

6.1 MECHANISM BEHIND THE ASYMMETRIC POSTS EFFECT

The two sides of the asymmetric insulating posts distort the electric field differently. Figure 6.2a shows the magnitude of the gradient of the square of the electric field ($\nabla E^2$) obtained between two posts when a potential of 500 V was applied. As observed, the intensity of the two maxima regions located in the short side of the post (in red) is higher than that of the long side regions (in blue). In addition to the

Figure 6.1: (a) Schematic illustration of the microfluidic channel with an array of asymmetric insulating posts, along with their geometrical parameters and measurements. The upstream and downstream directions are the directions towards the inlet and outlet, respectively. The figure shows the regions of interest where fluorescence measurements were made.
difference in intensities, using asymmetric posts results in different locations of the maxima zones. By looking at the division between the two halves of the posts (magenta dotted line), it can be seen that the maxima zones of the short side are located closer to the division than those of the long side. Since the DEP force acting on a particle is proportional to $\nabla E^2$ (Eq. 2.15), these differences in intensity and location between the maximum regions can be exploited to exert dissimilar DEP forces on particles. The level of asymmetry of the posts is an extra parameter that can be used to fine tune a system. When AC potentials are applied to a microchannel, the linear EK force changes direction at each half-cycle, resulting in a back and forth motion for the fluid and particles [117]. Under an AC electric potential, and in the absence of insulating posts, the suspending medium and particles would oscillate between two endpoints, with no net displacement (Eq. 2.11) and no DEP effects (Eq. 2.16). Since DEP depends on the gradient of the square of the electric field, its direction does not change with the electric field direction. A unique situation develops when asymmetric posts are employed; where particles present inside the posts array are exposed to a weaker DEP force from the long side of the post and a stronger DEP force from the short side of the post. Experiments with a mixture of 500-nm and 1-µm particles were used to test this new iDEP technique employing a square DC-biased AC electric potential. The selected signal had a positive peak voltage ($+V_p$) of 500 V, a negative peak voltage ($-V_p$) of -700 V and a frequency ($f$) of 400 mHz. The use of a DC-biased AC signal was chosen in order to produce a net particle movement in the upstream direction (towards the inlet), and to induce DEP trapping on both sides of the posts. The design of the electric signal is an additional parameter that can also be employed to customize an asymmetric post iDEP system.

Figures 6.2(b-e) show a schematic representation of the forces acting on particles over one period of the applied signal. Figures 6.2(f-i) illustrate the experimental system response when the electric signal is applied to the particle mixture. During the positive half-cycle, the larger 1-µm particles coming from the upstream direction face a barrier created by the strong DEP force in the short side of the posts (Figs.
Figure 6.2: (a) Regions between two posts where the gradient of the square of the electric field ($\nabla E^2$) has local maximum values when 500 V are applied. (b-e) Schematic representation of the forces acting on particles upon the application of a square DC-biased AC signal ($+V_p = 500 \text{ V}$, $-V_p = -700 \text{ V}$ and $f = 400 \text{ mHz}$) to a binary mixture with red 500-nm and green 1-µm polystyrene particles. The arrows show the migration direction for the red 500-nm and green 1-µm polystyrene particles. (f-i) Sequential time response of the experimental system when the electric potential signal was applied for (f) 1.85, (g) 1.98, (h) 2.12 and (i) 3.57 seconds. The sequential images show the operational principle of the asymmetric posts device. The suspending medium corresponds to a 0.2 mM $\text{K}_2\text{HPO}_4$ solution with a pH of 7.2 and a conductivity of $6 \times 10^{-3} \text{ S/m}$.

6.2b and 6.2f). This is a barrier that particles are not able to penetrate. The DEP force promotes the formation of a band of trapped 1-µm particles close to the division line (magenta line), forming an enrichment region (Figs. 6.2b and 6.2f). The DEP force experienced by the small 500-nm particles, in contrast, is not enough to trap them; and the 500-nm particles freely cross the constriction moving downstream due to EK migration (Figs. 6.2b and 6.2f).

When the electric field direction is reversed in the negative half-cycle, the DEP force direction does not change. However, since the amplitude of the negative half-cycle is higher than that of the positive half-cycle (negative DC offset), the DEP barrier created by the short side of the posts is instantly displaced in the upstream direction (Fig. 6.2c). This displacement, which is the key mechanism of the proposed technique, causes the negative DEP force to push the 1-µm particles towards the downstream direction at a high velocity (Fig. 6.2g). Although the 1-µm particles also experience the EK force and a DEP force from the long side of the posts (both pushing in the...
upstream direction), these forces are considerably smaller than those of the short side, and the 1-µm particles easily cross the constriction moving downstream. Once the 1-µm particles have crossed the narrowest part of the constriction, and the DEP force exerted by the short side of the posts decreases, the DEP barrier generated by the long side of the posts causes the 1-µm particles to be trapped (Fig. 6.2d); this results in the creation of a second particle enrichment region in the downstream side of the post (Fig. 6.2h). As the signal reaches its positive half-cycle again, the 1-µm particles migrate downstream to the next column of posts (Figs. 6.2e and 6.2i) and the cycle starts over. This movement continues until the 1-µm particles reach the outlet of the post array, where they accumulate over time. In contrast, the 500-nm particles, whose movement is always dominated by the EK force, are not trapped during the process. Since the applied signal has a negative DC offset, the migration of the 500-nm particles towards the inlet during the negative half cycle is greater than migration towards the outlet during the positive half cycle. Thus, the net displacement of the 500-nm particles is towards the inlet (upstream direction).

Fluorescence measurements were performed to assess particle concentration and movement. The corrected total fluorescence (CTF) was employed to report these measurements since it offers an enhanced accuracy by integrating spatially the fluorescence intensity and by subtracting the mean fluorescence of the background readings [118]. Figure 6.3a shows the CTF signal obtained from the 500-nm and 1-µm particles with respect to time when the DC-biased AC potential was applied for 60 seconds. These fluorescence measurements were performed at the outlet of the post array (Fig. 6.1). The band of enriched and trapped 1-µm particles oscillates and becomes thicker at each cycle of the electric potential, since new particles are eluted and join the band of trapped particles at each cycle. The fluorescence signals plotted in Figure 6.3a clearly show significant oscillations, which are a result of particles arriving and leaving the region of interest, as well as fluorescence variations when particles aggregate together. To produce a simpler representation of these measurements, it was opted to sample fluorescence in a time sequence when particles were likely to be in the same
location; that is, we measured fluorescence approximately every 10 seconds (results in Fig. 6.3b). This sampling frequency was estimated considering the frame rate of the videos and a manual inspection using a program developed in-house employing the R language for statistical computing [119]. As observed, the CTF measurements presented in Figure 6.3b are a more adequate and simpler representation than the data in Figure 6.3a. During every cycle of the DC-biased AC electric signal, the larger 1-µm particles keep moving forward, until they ultimately reach the outlet of the post array and remain trapped there. As a result, the 1-µm particles gradually concentrate at the outlet of the post array, thickening the particle band and increasing the green fluorescence signal (Figs. 6.3(c-f)). The quick increase in particle concentration can be visualized by the steep increase in magnitude of the green CTF versus time (Fig. 6.3b). The DEP force experienced by the 500-nm particles in both sides of the post is small when compared to the EK force. Therefore, the small red 500-nm particles move along with the fluid with a net motion in the upstream direction, with no apparent concentration increase, as observed by the almost constant magnitude of the red CTF (Fig. 6.3b). The progressive enrichment of the green 1-µm particles at the outlet of the post array is shown at four different times in Figures 6.3(c-f). By comparing Figure 6.3c with Figure 6.3f, it is observed how the band of trapped green 1-µm particles becomes much thicker as time progresses, demonstrating that the 1-µm particles are being successfully enriched and retained at the outlet of the post array. In contrast, the smaller 500-nm particles are gradually depleted by motion towards the inlet of the post array.

6.2 Separation of inert polystyrene particles

In order to assess the potential of the proposed asymmetric posts approach, the separation of the 500-nm and 1-µm particle mixture was further evaluated employing
Figure 6.3: (a) Corrected total fluorescence (CTF) of the red 500-nm and green 1-µm particles with respect to time when a square DC-biased AC signal (+Vp = 500 V, -Vp = -700 V and f = 400 mHz) was applied for 60 seconds. (b) CTF of the red 500-nm and green 1-µm particles resulting when fluorescence was sampled at the same time period during each cycle. (c-f) Sequential time response of the red 500-nm and green 1-µm polystyrene particles mixture when the electric potential signal shown in (a) was applied for (c) 18.65, (d) 28.97, (e) 38.75 and (f) 48.67 seconds. The sequential images show the increasing fluorescence concentration at a subset of the sampled times in (b). The suspending medium corresponds to a 0.2 mM K$_2$HPO$_4$ solution with a pH of 7.2 and a conductivity of $6 \times 10^{-3}$ S/m.
a customized sequence for the applied electric potential (Fig. 6.4a). This evaluation comprised four consecutive steps: (i) enrichment and elution of the 1-µm particles at the outlet of the post array (Fig. 6.4b), (ii) release of the 1-µm particles as a “peak” of concentrated particles (Fig. 6.4c), (iii) enrichment and elution of the 500-nm particles (Fig. 6.4d) and (iv) release of the 500-nm particles as a peak of concentrated particles (Fig. 6.4e). The customized DC/AC electrical signal required in each step (Fig. 6.4a) is described next. The elution and enrichment of the green 1-µm particles was achieved with a square signal with +Vp = 450 V, -Vp = -600 V and f = 450 mHz. Since particle size is smaller, a square signal with +Vp = 1,000 V, -Vp = -1,200 V and f = 850 mHz was employed to elute and enrich the red 500-nm particles. The release of both particles as peaks of enriched particles was achieved by applying a DC signal of 40 V, which produced a stable EO fluid flow transport towards the outlet.

Figures 6.4(b-e) show the CTF signals from the particles versus time for each consecutive step. For this experiment, a sample of 7.5 µL of the 500-nm particle suspension (7.28×10^8 beads/mL) was first introduced in the channel, followed by 7.5 µL of the 1-µm particle suspension (7.28×10^8 beads/mL). Both samples were introduced at the inlet reservoir. This resulted in a large number of 500-nm particles and a scarce amount of 1-µm particles present at the beginning of the experiment (Fig. 6.4b, t = 0 s), allowing the observation of the net displacement for both types of particles. During the early cycles of the first step, the 1-µm particles progressively concentrate at the outlet of the post array, as noted by the steep initial increase in the green CTF signal (Fig. 6.4b, t < 30 s). The 500-nm particles, due to the negative DC offset, keep migrating towards the inlet, producing particle depletion as depicted by the decay of the red CTF (Fig. 6.4b, t < 30 s). When t = 23 s, the concentration of both particles is equal. The enrichment of 1-µm particles and the depletion of 500-nm particles asymptotically approached a limit. After approximately 70 s, the variation in the enrichment and depletion of particles is small, and the post array is depleted of both types of particles (Fig. 6.4b, t > 70 s). When t = 120 seconds, the applied
Figure 6.4: (a) Characteristics of the electric potential signals applied during the four steps of the elution-release experiments for the green 1-µm and red 500-nm polystyrene particles mixture. (b-e) CTF of the red 500-nm and green 1-µm particles with respect to the overall time during the (b) 1-µm particles elution, (c) 1-µm particles release, (d) 500-nm particles elution and (e) 500-nm particles release. Fluorescence measurements for the elution and release steps were performed at the outlet of the post array, as depicted by the regions of interest drawn in Figure 6.1. The suspending medium corresponds to a 0.2 mM K₂HPO₄ solution with a pH of 7.2 and a conductivity of 6×10⁻³ S/m.

Signal was switched to a DC potential of 40 V for 30 seconds, which produced the EK migration towards the channel outlet of all particles present in the channel. The peak of enriched 1-µm particles is shown in Figure 6.4c. This peak has a well-defined Gaussian shape with a relatively small variance, exhibiting the successful release of the 1-µm particles. The plot depicts a region poor in 500-nm particles, as observed in the low magnitude of the red CTF signal (Fig. 6.4c). This demonstrates the potential that the asymmetric posts iDEP technique offers for particle enrichment and separation, with the added plus that larger, and perhaps more fragile, particles are eluted first.
Once the 1-µm particles were eluted and released, at $t = 150$ s, the applied potential was switched to the second DC-biased AC signal (Fig. 6.4a). This AC signal features higher amplitudes ($+V_p = 1,000$ V, $-V_p = -1,200$ V and $f = 850$ mHz), which were required in order for the DEP forces to enrich and drive the red 500-nm particles towards the outlet of the post array (as it was done with the green 1-µm particles). Although the employed amplitudes are quite high and have the potential to trap both particles, there is a region rich in 500-nm particles and poor in 1-µm particles caused by the net movement of the small particles and the previous elution of the large ones. The enrichment of red 500-nm particles is demonstrated by the increase in magnitude of the red CTF signal versus time (Fig. 6.4d). There were a few green 1-µm particles still present in the post array. These particles were eluted as well, as illustrated by the low magnitude and constant green CTF signal (Fig. 6.4d). Similar to the case of the 1-µm particles elution, the red CTF signal of the 500-nm particles asymptotically reached a maximum 32 seconds after the elution process was started. The oscillatory variations in fluorescence observed after 32 seconds are just the result of the bands of trapped particles having different sizes. In the final step of this separation, the applied signal was switched to a DC voltage of 40 V for 30 seconds to release the red 500-nm particles as a peak of enriched particles using EO flow (Fig. 6.4e). This second released peak also has a well-defined Gaussian shape with relatively small variance. Note that the green CTF signal of the 1-µm particles is scarce this time. These results demonstrate that the technique has not only the potential to elute large particles first, but to be adequately tuned to achieve both selective enrichment and separation of particle mixtures.
6.3 Separation of yeast cells and polystyrene particles

To test the effectiveness of the technique with a biological sample, a mixture of yeast cells (average diameter of 6.3 µm) and 2-µm polystyrene particles was studied and the yeast viability was assessed after experimentation. Since other iDEP systems can easily handle separations with such a relatively large size difference (around 4 µm [32, 57]), we focused on achieving the elution of yeast cells first and quantifying the cell damage caused by the electrical treatment. For this, a square electrical signal with +Vp = 200 V, -Vp = -400 V and f = 1.2 Hz was initially applied (Fig. 6.5, notice the negative DC offset), followed by a DC signal of 40 V employed to release the yeast cells as a peak of enriched cells (Fig. 6.5c).

During the first cycles of the elution step (t < 25 s), yeast cells start to quickly concentrate at the outlet of the post array, as observed by the steep increase in the green CTF versus time (Fig. 6.5b). During that time, the 2-µm particles are not enriched, as the red CTF remains constant (Fig. 6.5b). After 25 seconds, however, the concentration of 2-µm particles at the post outlet slightly increases, while the enrichment of yeast cells at the post outlet slows down. After approximately 40 seconds, the size variation in the bands of trapped yeast cells was small (i.e., the bands remained almost the same size), which indicated that it was time to switch the applied signal to a simple DC voltage in order to release the enriched cells as a peak. Although still unimodal, the yeast peak shows a positive skewed spatial distribution (Fig. 6.5c). This is the result of a large velocity distribution during the application of the DC voltage, where the once compact band of trapped yeast cells was quickly dispersed into several clusters. The first cluster had the majority of the yeast cells and is the one composing the sharp peak. The subsequent clusters arrived later on and are responsible for the smooth curve after the main peak. By comparing the post outlet region during the initial (Fig. 6.5d) and final (Fig. 6.5e) stages of this separation, it can be seen that mainly only green yeast cells were enriched, with almost no presence of red 2-µm particles. This confirms that the observed clusters
were composed of yeast cells.

The viability of the yeast cells used during experimentation was quantified and compared to a control group of yeast cells that were suspended in the same medium but were not subjected to any electrical treatment. The viability test was suitable for supporting three important aspects. First, this technique is gentler with cells because it allows for a much quicker elution of cells. Elution of yeast cells is achieved after only 40 seconds of applying the DC-biased AC signal, where $85 \pm 9\%$ of the yeast cells remained viable, which is a positive outcome. In a previous study by our group [83], yeast cells were manipulated under conventional iDEP conditions in a device with diamond-shaped posts under a potential of 300 V, which is close to the root mean square amplitude ($V_{RMS}$) of the DC-biased AC signal applied in this work (316 V). In that study, after 60 seconds of the DC electric potential application, only $67 \pm 2\%$ of the yeast cells remained viable [83]. Assuming an exponential decay in cell viability with respect to time, $73\%$ of the yeast cells would be expected to remain viable after 40 seconds. The reason behind the gentler manipulation of cells when using asymmetric posts and DC-biased AC potentials resides in the operational principle of the device. Once the yeast cells arrive to the outlet of the post array, they are only subjected to DEP forces during the negative half-cycle of the electric potential signal, and this force comes from the weaker long side of the post. During the positive half-cycle of the signal, cells just migrate along with the fluid in the downstream direction. Therefore, cells are only subjected to DEP forces during half of the time period of the electric signal.

In addition to exposing cells to high electric fields for only half of the effective time of the signal, the technique also reverses the elution order. Thus, cells are exposed to the electric fields for a shorter time and are eluted quickly (40 seconds). The successful separation of yeast cells from a mixture with 1-µm and 2-µm polystyrene particles was previously reported using diamond-shaped insulating structures [57]. The authors applied a DC-biased AC signal ($+V_p = 900 \text{ V}, -V_p = -600 \text{ V}$ and $f = 20 \text{ Hz}, V_{RMS} = 765 \text{ V}$) for 60 seconds to elute the 1-µm particles, followed by a DC
voltage of 200 V for 45 seconds to elute the 2-µm particles. The yeast cells, which were eluted last, were released after 105 seconds of operation. By using the same exponential decay assumption, only 32% of the yeast cells would be expected to be viable after such longer electrical treatment and high electric field intensities. By using the proposed approach, since yeast cells are the larger particles, they would be eluted first (after 40 seconds) with an average of 85% of viable yeast cells.

Our method also offers the capability of concentrating particles at a single location (at the outlet of the post array). Using conventional iDEP approaches, yeast cells would be trapped at every single column of the post array, decreasing the potential for cell enrichment. If trapping at a single location is desired in conventional iDEP devices, a higher voltage should be applied, so that cells are trapped at the inlet of the post array; which results in an increasing number of non-viable cells after experimentation. Importantly, since the elution order is inverted and the DEP force scales with the cube of particle radius, lower amplitudes of the applied electric potential are required for a separation to be successful. Using the conventional iDEP approach, high electric potentials need to be applied to trap all particles within a mixture. The voltage is then systematically decreased to release each particle type. That is, the minimum potential that needs to be applied is constrained by the smallest particle in the mixture, increasing the potential requirements. With this enhanced approach, low potentials are initially applied to trap the largest particle and the voltage is systematically increased to separate each particle type. In this case, the largest particle in the mixture constraints the separation, which greatly decrease the potential requirements. In summary, the asymmetric posts iDEP systems allow for larger particles to be quickly concentrated at a single location (e.g., at the outlet of the post array) and released first when in a mixture with smaller particles, both aspects being counter-intuitive in traditional iDEP systems. As a result, the proposed asymmetric posts DEP technique produces effective particle enrichment and separation, offering high cell viability as a plus.
Figure 6.5: (a) Characteristics of the electric potential signals applied during the elution and release of the green yeast cells from a mixture with red 2-µm polystyrene particles. (b-c) CTF of the green yeast cells and red 2-µm particles with respect to time during the (b) yeast elution and (c) yeast release. (d-e) Sequential time response of the binary mixture when the yeast elution signal was applied for (d) 4 and (e) 40 seconds. The sequential images show the increasing green yeast concentration and the small variation in the red 2-µm particles concentration. Fluorescence measurements for the elution and release steps were performed at the outlet of the post array, as depicted by the regions of interest drawn in Figure 6.1. The suspending medium corresponds to a 0.2 mM K₂HPO₄ solution with a pH of 7.2 and a conductivity of 6×10⁻³ S/m.
Chapter 7

Design and fabrication of a hybrid DEP device

As a stand-alone technique, DEP is a very versatile force that can be used to manipulate and discriminate between different particle and cells. Examples of DEP applications include the separation of heterogeneous populations of cells into homogeneous subpopulations [120] and the discrimination between ill and healthy cells [121]. The large applicability of DEP is driven by a number of advantages over other separation techniques. For instance, DEP does not require the use of tagging, labeling or binding of particles that are needed in flow cytometry, FACS and MACS. Moreover, the polarization mechanism employed in DEP allows the differentiation of particles and cells based on their dielectric properties, which are determined by the phenotype of the respective particles [122]. The same polarization mechanism enables DEP to exploit the frequency-dependent dielectric properties of particles. This is a clear advantage over other techniques that discriminate particles solely based on their size, like inertial microfluidics and microfiltration. The characterization of the frequency dependence of cells can be used to assess the dielectric parameters of their internal components, revealing underlying membrane altering mechanisms related to cell death, drug-tolerance, and drug-resistance [123]. More recently, DEP has been used for the manipulation and characterization of individual cells, which is not easily achieved by other separation technologies [124].

The current DEP technologies to fabricate devices use arrays of either metal-based
microelectrodes (eDEP) or polymer-based obstacles (iDEP). Each one of these technologies have some advantages and disadvantages associated. For instance, most eDEP devices have planar electrode configurations deposited at the bottom of the channel, separated by a distance in the order of micrometers [2]. This results in the generation of a strong DEP force that is localized on the immediacy of the electrodes. Hence, only particles moving close to the bottom of the channel can be manipulated using DEP, resulting in low throughput devices [125]. In contrast, the insulator structures employed in iDEP devices transverse the entire depth of the channel, creating a three-dimensional DEP effect that leads to a higher throughput [125]. These insulator structures distort the electric field distribution, which is exerted by two external electrodes separated by the channel length. The relatively large distance between the external electrodes results in a significant decrease in the electric field magnitudes achieved in iDEP devices. However, the use of external electrodes also minimizes the contact between electrodes and sample. This is a main issue in eDEP devices, because it leads to the accumulation of unwanted material on the electrodes surface when biological samples are studied [126]. A second drawback of using external electrodes is the fact that the electric field is generated through the whole channel. Considering that high electric potentials usually need to be applied in iDEP devices [127], significant amounts of Joule heating can be generated [128]. In order to prevent a temperature rise in the suspending medium, iDEP devices are limited to use low conductivity media. This represents a min limitation on the applicability of iDEP devices, since the conductivity of physiological fluids are considerably above the conductivity limits of iDEP devices.

It has been demonstrated before that the use of DC and low-frequency electric fields are effective for DEP particle separation [127]. A major disadvantage of employing low frequencies is that Faradaic reactions occur at the electrodes to maintain a constant electric field [129]. These reactions generate bubbles and harmful compounds that may diffuse into the channel, damaging cells and affecting the suspending medium properties. Is is important to note that mammalian cells are particularly sensitive
to the generated free radical species [129]. Therefore, eDEP devices are restricted to employ high frequencies, because these harmful compounds do not have time to diffuse away from the internal electrodes and are eliminated on the next field reversal [129]. In contrast, the minimal contact between electrodes and sample offered in iDEP devices enables the use of DC and low-frequency electric fields.

Several groups have focused on combining the two DEP fabrication technologies to yield devices that combine the best characteristics of them. For instance, Kilchenmann et al. [130] fabricated 3D electrodes by initially patterning photoresist on a silicon wafer in the form of pillars, as usually observed in iDEP devices. Since photoresist is an electrical insulator, the micropillars were coated with platinum and titanium layers to electrically activate them after the lithography step. Using this technique, the authors fabricated devices with both side-wall integrated and free-standing electrodes. Although no DEP application was reported, the authors provided a detailed characterization of the fabrication process and electrical properties of the resulting electrodes. In a similar approach, Martinez-Duarte et al. [131] and Jaramillo et al. [132] fabricated 3D electrodes by patterning photoresist in different geometrical arrangements. However, the arrays of self-standing patterned structures were later thermally degraded (pyrolysis) to form glass-like carbon electrodes, avoiding the use of metallic electrodes. Although glass-like carbon has a lower conductivity than most metals, the authors demonstrated the generation of suitable electric fields for DEP tasks. By applying tens of volts, viable and non-viable yeast cells were separated [131], *E. coli* cells were isolated from *B. cereus* cells [132] and intact *M. smegmatis* cells were identified in a mixture with antibiotic-treated cells [133] using glass-like carbon electrodes, at flow rates up to 35 µL/min. To further decrease the fabrication complexity of 3D electrodes, Lewpiriyawong et al. [134] proposed to mix PDMS with silver powder to fabricate sidewall electrodes in a channel. The PDMS-Ag composite material, whose conductivity was reported to be $2 \times 10^4$ S/m, was restricted to limited regions of the channel walls by cavities created in a positive photoresist. The authors applied AC electric potentials (below 26 V) to demonstrate
the continuous size separation of 10 from 15 µm particles, the separation of *E. coli* cells from polystyrene particles, and the selective separation of live from dead yeast cells. Since the composite electrodes were placed at the channel walls, flow rates in the order of 0.15 µL/min were employed. In a related study, Choi et al. [135] proposed to apply the ion-implantation technique to implant electrodes onto the sidewall and top-wall of a PDMS channel. Gold ion was implanted on the PDMS, acquiring an estimated conductivity of $2 \times 10^5$ S/m. The authors demonstrated the effectiveness of the proposed approach by aligning *E. coli* cells with an electric field applied from different directions at a flow rate of 4 nL/min. In another study, Jen et al. [136,137] proposed a novel device that combined several characteristics of eDEP and iDEP devices. First, the authors deposited chrome and gold on a glass slide to create a pair of planar microelectrodes, similar to eDEP. Next, the channel walls and an X-patterned insulating structure were produced by polymer casting, as is usually done in iDEP. The two pieces were aligned and sealed together, and AC electric potentials were applied to the pair of electrodes to focus particles showing both positive and negative DEP behaviors. The device was reported to be successful for flow rates up to 20 µLx/min. There is a need for devices that can combine the best characteristics of each fabrication technology, with a particular emphasis on customizable particle trapping regions.

This work focuses on the use of iDEP devices for microscale particle separation, because iDEP offers an inexpensive and easy-to-fabricate solution for particle manipulation. However, there are several issues that result from the limitation of iDEP devices of using low conductivity media and low-frequency electric fields. First, electrode polarization is known to cause problems when low conductivity media is employed (below 1000 µS/cm) [129]. When low conductivities are coupled with low frequencies, electrode polarization can lead to a profound reduction in the particle manipulation capabilities of iDEP devices [129]. A second issues relies in that charge double layer effects are important at these experimental conditions [129]. Double layer effects increase significantly as particle size decreases [63], and may limit the expected ability
of DEP to discriminate between particles.

In this work, the fundamental concepts of iDEP and eDEP devices are combined together to create a hybrid DEP device. In this novel device, insulating and conducting posts coexist together to selectively incorporate the main features of iDEP and eDEP devices, while overcoming their individual limitations. For instance, the composite electrodes can be easily patterned inside the microchannel, creating a three-dimensional DEP force that can generate high electric field magnitudes. While there exist other fabrication techniques that successfully created 3D electrodes, those require more complex procedures than the ones proposed in this work. Since the composite electrodes are patterned inside the microchannel, high conductivity media can be employed. Moreover, carbon-based electrodes are more chemically stable than metallic electrodes, so they are more robust during electrolysis [138]. Therefore, carbon-based electrodes can withstand the use of low-frequency electric potentials, and even DC fields [138]. The combination of high conductivity media and low-frequency electric fields is one of the main advantages offered by the hybrid device. This allows the use of conductivity gradients in the suspending medium and frequency gradients in the electrical signal to selectively concentrate and release particles. In addition to these advantages, the regions where particles are concentrated can be highly customizable by the addition of insulating structures. This allows the selective guidance of particles inside the device under physiological and high throughput conditions. Further, since the device is completely made of PDMS, the strong PDMS-PDMS bonding prevents any leakage of fluid while pumping through the microchannel.
Chapter 7. Design and fabrication of a hybrid DEP device

7.1 Motivation for the hybrid device

A main application of DEP is cell analysis. For example, identifying the type of cells in a sample and assessing the number of captured cells. Many detection techniques are based on immunoaffinity techniques, which target specific markers to selectively manipulate cells. In continuous flow microfluidic devices, immunoaffinity-based techniques rely on the immobilization of antibodies on the sensor surface for capture and specific surface interaction for detection [139]. These techniques offer a high specificity and low chance of cell damage. However, one of the factors restricting the detection limit of biosensors based on immunoaffinity methods is the low immunocapture efficiency of the immobilized antibodies on the solid chip surfaces. This is especially true in the case of bacterial cell capture, where the immuno-capture efficiencies range from 0.01% to 16% [140].

The low efficiencies are caused because bacterial cells in suspensions do not diffuse as quickly as small molecules. Hence, cells do not have sufficient opportunities to reach the surface of the chip and react with the immobilized antibodies. Only a small population of cells can settle down to the chip surface by sediment and be captured by the antibody molecules on the chip surface. Since the hybrid device specializes on cell manipulation, it can be used to enhance the immunocapture efficiency. Suehiro et al. [139,141] reported a microchip that used DEP trapping and antibody-capture for the capture of \textit{E. coli} cells. Planar castellated electrodes were employed for achieving the DEP manipulation, and the surface of these electrodes was immobilized with anti-\textit{E. coli} antibody. In an initial approach, both target and non-target cells were trapped by positive DEP onto the electrode surface. The portion of cells that were in contact with the antibody molecules immobilized on the electrode surface were consequently bounded to the surface [141]. After an incubation time, the DEP force was released and fresh buffer was injected to wash away unbound and non-target cells. In a more elaborated approach, the frequency of the electrical signal was adjusted to attract and retain only the target cells, while non-target cells were directly washed away by the flow [139]. This improvement eliminated the necessity of the washing
Using an interdigitated array to create electric field gradients, Yang et al. [142] integrated DEP with cross-flow biochips to aid the immuno-capture of *Salmonella Typhimurium*, a foodborne pathogenic bacteria. Two main steps of the chip operation involved the use of DEP: the pre-concentration of bacteria cells from suspension, and the attraction of bacteria cells to immobilized antibodies located on the chip surface. The frequency of the applied electrical signal was adjusted so that the target cells were attracted to the electrodes surface using positive DEP, while any non-target cells was attracted to the regions between electrodes by negative DEP. The immuno-captured cells were later detected using fluorescence measurements. The authors reported that using DEP enhanced the immuno-capture efficiency over five times (from 10% to 56%). On a related study, Yang et al. [143] proposed a DEP-enhanced immuno-assay for the detection of *E. coli*. The authors noticed that the trapping of cells by DEP is rapid but is not always highly efficient. Moreover, the authors reported that, depending on the design of the device, the trapping efficiency can range from as low as 1-3% to over 50% to as high as 90% [142].

These studies offer evidence of the potential of DEP to enhance the immuno-capture efficiencies. However, the proposed chips have a complicated and time-consuming fabrication procedure, since they need eight individual layers stacked over each other. Moreover, the proposed chips employ planar electrodes, which enable the manipulation of cells only close to the electrodes. The observations of Yang et al. [143] regarding the importance of the channel design may be attributed to a limited DEP effect caused by the planar electrodes. The hybrid device offers an attractive framework for this application. First, the electrodes generated in the hybrid device transverse the entire depth of the channel, creating a three-dimension DEP effect. This effect can enhance the pre-concentration of cells inside the device before being exposed to the immuno-assay. It has been reported earlier how the use of three-dimensional electrodes has a large impact on the trapping performance of DEP devices [144]. Second, the hybrid device enables the immobilization of capture antibodies on selected elec-
trode surfaces. Third, the DEP force created by the hybrid device can be used to select selectively attract cells to the electrodes surface. Since the hybrid device allows the use of broader media conductivity and applied frequencies it has the potential to be more selective for particle manipulation.

7.2 Combination of insulating and conducting structures

Figure 7.1 shows the distribution of the DEP force and the regions where particles are attracted or repelled considering their relative polarization in devices with cylindrical structures for three cases: when the posts behave as electrodes (Figs. 7.1a and 7.1d), when the posts are insulators and the electric potential is applied at the inlet/outlet reservoirs (Figs. 7.1b and 7.1e) and when insulating and conducting posts coexist within the same post array (Figs. 7.1c and 7.1f). When the posts are electrically active, the regions of high electric field gradient are located at the electrodes edge (Fig. 7.1a). The central electrode post presents the largest DEP force for this electrode design. Because of the higher polarization, particles that experience a positive DEP behavior are attracted towards these regions, and disperse over the whole electrode edge (Fig. 7.1d). This situation can be undesirable considering that particles are exposed to a strong fluid flow in this region. In contrast, particles that are less polarizable than the suspending medium are repelled from the electrodes edge and are pushed towards the regions between the electrodes. In this region, however, particles are even more exposed to the strong fluid flow and they cannot be effectively trapped (Fig. 7.1d).

When all posts are insulators, the electric field is pinched by the presence of the insulating structures. As a result, particles with a positive DEP behavior can be enriched in the constrictions between neighboring posts (Fig. 7.1e), where the electric
Figure 7.1: (a-c) Distribution of the DEP force and (d-f) regions where particles are attracted or repelled according to their relative polarization in devices with cylindrical structures when (a,d) posts behave as electrodes, (b,e) posts are insulators and the electric potential is applied at the inlet/outlet reservoirs and (c,f) insulating and conducting posts coexist within the same post array. The DEP force magnitude was standardized with respect to (a). Particles showing positive and negative DEP behaviors are shown in magenta and green, respectively.

field has a high magnitude (Fig. 7.1b). However, the high EO flow in these regions has the potential to prevent particle trapping [145], making the enrichment of these particles a challenging task. Even when an AC electric potential is applied and there is no net EO flow, the back-and-forth EO motion of particles can disrupt their trapping in this region [145]. The particles that are repelled from the regions with high electric field gradients can be enriched before each constriction, when DC electric potentials are applied, or both before and after each constriction, when AC electric fields are employed (Fig. 7.1e). However, the DEP force magnitude is reduced by a factor of \(\sim 1000\) (Fig. 7.1e) because of the longer distance between electrodes.

When insulating and conducting structures coexist together within the same array, the regions where particles are likely to be trapped due to DEP are a combination of the previous cases (Fig. 7.1f). While particles exhibiting a positive DEP behavior are retained at the electrodes edge, they are almost equally attracted to all electrode posts, resulting in more surface area to retain the particles (Fig. 7.1c). Moreover, the fluid flow is not the same in all these regions, so particles may gather in regions where
the posts shield particles from the fluid. Particles showing a negative DEP behavior are repelled from the electrodes edge and tend to cluster between the electrodes. Although this is similar to the case where all posts behave as electrodes, two main considerations should be noted. First, the particles that are more exposed to a strong fluid flow are the ones with a negative DEP behavior, which are usually larger than the particles with a positive DEP behavior. Hence, these particles experience a larger DEP force. Second, compared to the case where all posts are insulators, the DEP force experienced by all particles is significantly larger (an increase of $\sim740$ times). A larger DEP force competes with the fluid flow to dominate particle movement.

The main advantages of combining insulating and conducting polymeric structures is that electrodes can be closely spaced between each other, generating high electric field magnitudes, and insulators can be added to manipulate the regions where particles are likely to be trapped. Consider, for instance, the case of two triangular-shaped electrodes facing each other (Fig. 7.2a). As expected, the highest DEP force is concentrated at the tips of the electrodes. Particles with a positive DEP behavior are attracted to these small regions (Fig. 7.2b), where they are exposed to a strong fluid flow. Consider now the addition of four insulating structures in the shapes depicted in Figure 7.2c. The attraction regions are significantly modified. While the electrodes tips still attract particles, four new regions with a strong attractive DEP force are generated next to the insulating structures (Fig. 7.2d). Particles are thus attracted to these internal regions, where they can be successfully shielded from fluid flow.

The combination of insulating and conducting posts results in the development of trapping regions that can be highly customizable for a particular application without significantly increasing the fabrication complexity of iDEP devices.
Figure 7.2: (a) Distribution of the DEP force when two triangular-shaped electrodes face each other. (b) Particles showing a positive DEP behavior are attracted to the electrodes edge. (c) Distribution of the DEP force when four insulator structures are placed between the electrodes shown in (a). (d) The same particles are attracted to the inner region of the insulating structures, where they are protected from the fluid flow. The DEP force magnitude was standardized with respect to (a). Particles showing a positive DEP behavior are shown in magenta.

7.3 ELECTRICAL CHARACTERIZATION OF THE COMPOSITE MATERIAL

The fabrication of the device with polymeric insulating and conducting structures relies on the doping of PDMS to make it electrically conductive. To achieve this, multi-walled carbon nanotubes (MWCNTs) were added to PDMS and mixed in proportions ranging from 1 to 6% v/v. The MWCNTs had lengths ranging from 5 to 15 µm, and outside diameters ranging from 20 to 40 nm. The aspect ratio ($L/D$) of these MWCNTs ($\sim 333$) allowed them to be adequately dispersed in the PDMS matrix using shear mixing [146]. To produce the composite material, both PDMS precursor
and curing agent were mixed using a planetary mixer for 1 min at 2000 rpm. Next, the MWCNTs were added and the solution was mixed using the planetary mixer. The mixing recipe was optimized with respect to the samples conductivity, as follows: 2 mins at 800 rpm, followed by 3 mins at 1400 rpm and 15 mins at 2000 rpm. Samples mixed with this recipe showed the best conductivity. After the mixing process, the composite material was casted on aluminum molds and cured at 85°C for two hours using a convection oven. The rectangular-shaped molds were 50 mm long, 10 mm wide and 1 mm thick. The electrical conductivity of the cured samples was measured using the 4-point probe technique [146], as shown in Figure 7.3. A high voltage sequencer was used to produce an electrical current flowing through the samples by means of alligator clamps. The voltage was measured at two inner points of the sample, separated by a distance \( D = 1 \) inch. Silver conductive epoxy was used at each connection to decrease the contact resistance. The electrical current \( (i) \) and voltage \( (V) \) were measured using a digital multimeter, and the bulk composite conductivity was measured considering the cross sectional area \( (A) \) of the sample:

\[
\sigma_{\text{composite}} = \frac{iD}{VA}. \tag{7.1}
\]

Figure 7.4 shows the electrical conductivity as a function of the MWCNTs content. The lowest amount of MWCNTs content that resulted in a measurable conductive material was 3% v/v, showing a conductivity of \( 2.73 \times 10^{-5} \, \text{S/m} \). As the MWCNTs content increases over 3% v/v, the electrical conductivity grows exponentially \( (R^2 \approx 0.99) \) up to a value of 1.02 S/m at 5% v/v. This electrical behavior can be explained in terms of the percolation theory [147]. For lower concentrations of MWCNTs, there are no percolation paths in the nanotubes network, and samples do not conduct a significant amount of electrical current. As the MWCNTs content increases, nanotubes get closer to each other and form conducting networks that significantly increase the electrical conductivity. The percolation threshold indicates the composite transition between the insulator and conductor states. Since the electrical conductivity does not increase with the addition of more MWCNTs (1.06 S/m
at 6% v/v), the threshold is $\sim$5% v/v for the employed experimental setup. The PDMS with a MWCNTs content of 5-6% v/v was still viscous enough to be selectively casted on the desired regions. Figure 7.5 shows the casting of the composite material on predetermined regions. A 5% v/v MWCNTs content was used as the standard for the devices fabricated in this work. The conductivity values obtained in this work are comparable to other studies that use shear mixing to disperse the MWCNTs [148–150], which range from 0.5 to 10 S/m. However, these values are significantly lower than reports that use solvent assisted methodologies [146,147], which range in the order of $\sim$100 S/m, a 100 times increase. However, these techniques usually require 24 hours of processing time [146], to avoid leaving solvent residues in the composite material [147].
Figure 7.4: Electrical conductivity of the composite material as function of the MWCNTs content.

Figure 7.5: Experimental images of the connection lines (a) close to the terminal end and (b) below the conducting posts. The images show the selective casting of the conductive composite material.
7.4 Fabrication of the Insulating Structures

The fabrication of a hybrid DEP device started with the making of two master molds (top and bottom) using the SU-8 3050 photoresist and standard soft-lithography techniques [151]. Since this section focuses on the general fabrication procedure, a simple design will be described for illustration purposes. The top mold (Fig. 7.6a) contained the SU-8 structures that formed the channel walls (∼95 µm thickness) and the cavities that formed the insulator posts (32 cavities, ∼95 µm deep). The bottom mold (Fig. 7.6b) contained the cavities that formed the connection lines (∼67 µm deep) and the conductive posts (32 cavities, ∼157 µm deep). All posts (i.e., conductive and insulated) were cylindrical-shaped, with diameters of 200 µm. The dimensions of the master molds were measured using a profilometer.

A 2-mm thick layer of PDMS was casted onto the top mold to produce the microchannels and insulating structures (Fig. 7.6c). The channel was 10.16 mm long, 1 mm wide and ∼95 µm deep. The composite material was poured on the bottom mold to produce the connection lines and conductive structures. The excess of composite material was removed using a razor blade, and a 2-mm thick layer of PDMS was casted onto the mold to serve as the substrate for the connection lines and conductive structures (Fig. 7.6d). After the casting process, both molds were cured at 85 °C for two hours using a hot-plate. Once both PDMS pieces were cured, they were peeled-off from the master molds. The insulating structures and channel walls in the top PDMS piece had a height of 95 µm (Fig. 7.6c). For the bottom PDMS piece, the connection lines and conductive posts had heights of 67 and 90 µm, respectively (Fig. 7.6d). The height difference between the insulating and conductive posts (∼5 µm) prevents having posts that are taller than the channel height, which may cause leaking issues. A thin layer of PDMS was then spin-coated over the bottom PDMS piece (the one containing the conductive structures, Fig. 7.6d) to cover/insulate the connection lines (Fig. 7.6e). The spin-coating recipe was fine-tuned to obtain a thickness of PDMS of ∼70 µm [152] and verified using SEM. In order for this layer to have a uniform thickness, PDMS was diluted with toluene (1:1 ratio) and was later spin-
Figure 7.6: Schematic of the fabrication process for the hybrid device. The (a) top and (b) bottom master molds were fabricated using SU-8 on a silicon wafer. (c) Native PDMS was casted on the top mold to produce the channel walls and insulating structures. (d) The conductive composite material was casted on the bottom mold to produce the connection lines and conductive posts. (e) A thin layer of PDMS was applied to insulate the connection lines form the channel. (f) SEM image showing the conducting posts not being covered by PDMS.

covered. After the coating process, high-pressure air was blown over the conductive posts array to remove the excess of PDMS, and the device was left to rest for 40 min in order for the PDMS to re-flow into a uniform layer thickness. In this manner, PDMS was prevented from covering the conductive posts, as evidenced by the SEM image in Figure 7.6f. The device was finally cured at 85 °C for 30 min in a convection oven. The top and bottom PDMS pieces were then aligned and sealed by using a plasma corona wand.
Figure 7.7 shows experimental images of the fabricated device. The bottom piece of the prototype device before the application of the thin PDMS layer is presented in Figure 7.7a. Two pieces of copper sheet were added to the terminal ends of the device using silver conductive glue to connect the voltage supply (7.7b). Inlet/outlet ports were punched on the top piece, and was sealed to the bottom piece. The copper sheets were connected to the voltage supply using alligator clamps, with the aid of silver conductive glue (7.7c).
Figure 7.7: (a) Bottom piece of the device employed for cell lysis and fractionation. The channel is surrounded by the terminal ends that will connect the device with the voltage supply. (b) Copper sheets were glued on the terminal ends to facilitate the electrical connections. (c) Inlet/outlet ports were punched in the device, and the bottom and top PDMS pieces were sealed together. The inlet/outlet ports were connected to the syringe pump, and the copper sheets were connected to the voltage supply.
Chapter 8

Conclusions

Since the spatial interplay between DEP and EK forces in iDEP devices can hinder the regions where particles are trapped, also studied the particle trapping mechanism in iDEP devices for 1-µm and 2-µm polystyrene particles and prolate-shaped *E. coli* cells. Experimental work allowed the observation of the DEP trapping regions, while mathematical modeling was employed to predict the DEP barrier regions. The dielectrophoretic barriers that promote particle trapping (i.e., the barriers that particles are not able to penetrate) correspond with the regions where the EK and DEP velocities are in equilibrium. A series of experiments demonstrated that the location and shape of the trapping regions are strongly influenced by the electric field distribution, as well as particle size and shape. A correction factor was estimated to match the model predictions with the experimental results. This approach showed that particles are trapped in bands that can be described by DEP iso-velocity lines for particles trapped close to the center of the constriction and by EK iso-velocity lines for particles trapped away from this region.

The analysis of the particle trapping mechanism and the formation of DEP barrier regions enabled the development of objective functions that describe particle behavior within iDEP devices. This work demonstrates the importance of the geometrical parameters (e.g., shape, width and length) and arrangement (e.g., lateral and longitudinal spacing between posts) of the post array using a parametric variation study. Numerical simulations, where the average trapping condition (*T_C*) and the average lateral-to-longitudinal force ratio (*F_R*) were analyzed, allowed the identification of the
best designs that enhance the particle trapping performance. This parametric study comprised two main stages; the first stage was focused on identifying the best post shapes employing $T_C$ as the design parameter. The second stage allowed identifying the best spacing between posts utilizing $F_R$ as the design parameter. Experimental work with polystyrene particles was conducted to reflect and validate the parametric variation study, showing the superior performance in terms of particle manipulation gained at each optimization step. It was shown that there is a sweet spot in terms of post shape and spacing that produces the best performance. The fabrication feasibility was also analyzed, with a special focus on the effect of corner rounding on $T_C$ for each post shape. Ultimately, this methodology allowed the identification of the best designs that can be fabricated with standard microfabrication techniques and equipment. The lowest electrical potential (LEP) necessary to generate a strong band of trapped particles was decreased in 31, 79 and 84% for the optimal diamond, circle and square designs, respectively. These decrements are significant, since they result in simpler and less expensive equipment to achieve particle separation, which are necessary for real lab-on-a-chip applications.

The parametric variation study also allowed the identification of geometries that have a maximum and a minimum trapping capacity. These two limiting geometries were incorporated in a single asymmetric post design to induce a difference in the DEP experienced by particles at both sides of the posts. Importantly, the posts asymmetry can displace the location of the trapping or enrichment regions within the device. This novel dielectrophoretic approach has the characteristic of allowing quick enrichment and elution of larger, and perhaps more fragile, particles in a sample. This is particularly important when dealing with particle samples that contain eukaryotic cells, which can be directly harmed by electroporation [81] and an imposed potential to the cells membrane [115], as well as damage to the cells membrane due to Joule heating [111] and pH gradients in the suspending medium [86]. Coupling the asymmetric posts with DC-biased AC potentials creates dissimilar DEP forces that selectively enriched the larger particles in the sample at the outlet of the post array, while driving
the smaller particles towards the inlet. Subsequently, the enriched particles can be released as a peak of concentrated particles, achieving effective particle separation and enrichment. A key aspect of this separation is the use of the negative DC offset, which promotes an instantaneous displacement of the DEP barrier generated by the short side of the posts in the upstream direction. The same DC offset also produces a net displacement towards the inlet for the smaller particles. It was demonstrated that this novel DEP-based system can discriminate between particles with diameter differences as low as 500 nm. The system was also tested with biological cells, where yeast cells were successfully separated from inert polystyrene particles in under 40 seconds, while retaining a high cell viability of 85%. These results demonstrate that the use of asymmetric insulating posts combined with low frequency DC-biased AC potentials may be a preferable iDEP separation technique when the viability of larger cells in biological and clinical samples is a priority. In addition to offering a system where large particles are eluted first, the proposed approach offers an extra set of parameters (AC amplitude, DC offset, post asymmetry and shape) that can be adequately exploited to fine tune and customize a system to achieve challenging particle separations.

8.1 Future work

This work opens several doors to continue improving the performance of iDEP devices. Although we have designed a systematic methodology to characterize and optimize the manipulation of particles by iDEP, the study focused on systems with a single particle type. Similar to the selection of an optimal frequency to perform a DEP separation based on the dielectrophoretic signature, the proposed objective functions ($T_C$ and $F_R$) can be combined to select a geometry of the post array that maximizes a given separation based on the DEP force. That is, the selection of the geometry
that maximizes and minimizes, respectively, the manipulation of two particles in a binary mixture. The proposed methodology can also be enriched with constraints that restrict the solutions space of the geometries for specific separations. For example, when biological particles are employed, constraints in the electric field magnitude, such as decreasing the imposed potential in cells membrane [115] and the rising of Joule heating [111], can be added to the model in order to select the more appropriate design for a given application.

The asymmetric posts geometry has already been proved to have the potential to evolve as a standard for the separation of bioparticles, since it enriches particles quickly, in a single location and with a high viability. The key aspect of this device is the manipulation of the trapping regions, rather than the manipulation of the magnitude of the DEP force itself. This unique manipulation technique can exploit extremely small differences in the physical properties of the particles, possibly beyond the traditional approaches. Its application on complex biological mixtures is still an open area for research. For instance, the detection and isolation of circulating tumor cells (CTC), although intensely studied during the last few years, represents a research area facing numerous challenges [153]. One of the most prevalent challenges is the scarcity of CTCs in a blood sample for analysis [154]. It has been reported that most patients with metastatic cancer have fewer than 10 CTCs per mL of blood [155]. In addition, there are over $1 \times 10^6$ white blood cells (WBC) and $1 \times 10^9$ red blood cells (RBC) within the same blood volume [155]. The relatively large size difference that the three types of cells exhibit (median diameters of $\sim 10 \, \mu m$ for WBCs [156], $\sim 7 \, \mu m$ for RBCs [48] and $\sim 15 \, \mu m$ for epithelial CTCs [155]) makes DEP-based techniques strong candidates for performing this analysis, since DEP scales with the cube of particle radius. However, a successful isolation should not only trap the CTCs, but it should also retain the scarce number of CTCs in the sample while a massive number of other cells are constantly streaming through the trapping region. Since WBCs and RBCs have the potential to disrupt the trapping of the low abundant CTCs, the large concentration difference poses a practical challenge for the development of effective
separation technologies [153]. In addition, the isolation process of CTCs should be
gentle enough to avoid cells damage, so the scarce number of CTCs can be used for
further analysis. Given the ability to manipulate the trapping regions and the DEP
force simultaneously, the asymmetric posts device has the potential to address these
challenges.

Overall, the future work described so far has a common aspect: the manipulation of
biological particles. Since particle shape has a major effect on the magnitude of the
DEP force and on the experimental band of trapped particles, higher order moments
than the dipole should be considered in the forecasting and numerical simulations
of non-spherical particles. Green and Jones [157] developed a numerical approach
that can be used to extract the linear multipoles up to any arbitrary number. The
development of approaches that incorporate higher order moments can be used in two
main directions: to enhance the simulations or to characterize the dielectrophoretic
signature of particles. The first one is straightforward to follow and apply: increase
the complexity of the numerical simulations to provide a more accurate description of
the experimental system. The second one, however, follows an inverse scheme: using
data acquired from the experimental system to estimate physical properties of the
particles. Recently, several research groups have adopted the use of well-established
stochastic models [158,159] to estimate properties such as particle mutual interactions
[160], the relative polarization of several DNA conformations [159] and cells [158].
These models have the potential to allow the derivation of these physical properties
on an individual basis, rather than as averages of a whole population [158]. Since both
the magnitude of the DEP force and the trapping barriers can be manipulated with
the use of asymmetric posts and hybrid devices, they provide an adequate platform
for the characterization of the physical properties of particles under more controlled
experimental conditions.
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