Modeling of on-chip (bio)-particle separation and counting using 3D electrode structures

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Abstract In lab-on-a-chip applications, manipulation and quantification of (bio)particles is required in a variety of biomedical applications such as drug screening, disease detection and treatment. For manipulation of particles, electrical techniques such as dielectrophoresis (DEP) is very suitable. For the quantification or counting of the bioparticles, flow cytometer, fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) are common techniques. In this study, modeling of microfluidic (bio)-particle separation based on dielectrophoresis and counting based on capacitance measurement using COMSOL Multiphysics have been presented. The device performance with planar and 3D electrode structures have been compared. Microfluidic devices with an asymmetric pair (for separation) and a symmetric pair (for counting) of electrodes are considered. The effects of the geometrical parameters, material properties, flow rate, particle size and applied voltage on the device performance have been discussed. The fabrication procedure of 3D electrode structures is also addressed.

Keywords: Dielectrophoresis, Capacitance Measurement, Counting, Separating

1. Introduction

In many devices using lab-on-a-chip technology, the manipulation and quantification of (bio)-particles are required in a variety of biomedical applications such as drug screening, disease detection and treatment. For manipulation of particles at such small scales, electrical techniques such as electrophoresis and dielectrophoresis (DEP) are very suitable [1]. DEP is the movement of particles in a non-linear electrical field due to the interaction of the particle's dipole and spatial gradient of the electrical field. DEP can induce both negative and positive forces on particles depending on the dielectric properties of the particles and the suspending medium. For an AC field, DEP force is also a function of the frequency of the field which makes DEP force tunable for a given particle. Each (bio)-particle has a distinct morphology; hence, a distinct dielectric signature which is function of its type, interior structure, and state. The unique dielectric signature makes DEP a label-free and sensitive selection tool. Using this dielectric signature, DEP can be utilized to discriminate and identify (bio)-particles from other particles or to detect and isolate diseased or damaged (bio)-particles without any need for labeling [1]. DEP have been successfully implemented for many biomedical applications [1,2]. More recently, DEP has been implemented for the separation of *S.cerevisiae*, *L.casei*, *L.casei* and Jurkat cells [3], for trapping and lysis of RBC [4], and for differentiation micro-algal species with different lipid contents [5].

One major issue about the DEP-based microfluidic devices is the throughput. Their throughput is low compared to other conventional manipulation techniques [1]. One way to increase the throughput is to increase the channel dimensions. For the devices with planar and embedded electrodes, the electric field has fringe-like nature (see Figure 1). Therefore, the height of the device cannot be increased. The strong electric field and DEP force exists in a confined region over the electrodes (DEP force decreases drastically in height direction). The particles need to flow in the vicinity of this confined region. For trapping devices, the width of the channel can be increased to increase the throughput; however, this is not a solution for continuous flow devices. The same issue also exists for on-chip counting devices based on impedance measurements. One way to increase the performance of the DEP-based separation and the impedance-based counting is to use 3D electrodes at the sidewalls, which eliminates the fringe-like structure of the electric field lines.

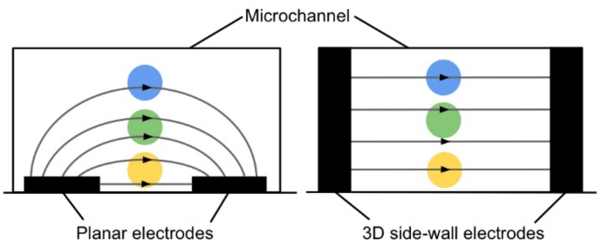


Figure 1: Electric field within a microchannel with planar and 3D side-wall electrodes

Particle counting has very high functionality in various areas from biological to environmental applications such as sampling, identification, filtering and decontamination procedures [3]. For the quantification or counting of the (bio)-particles, flow cytometer, fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) are common techniques used in conventional laboratory environment. Although these techniques are robust, they require complex and expensive instruments, trained personnel, use of bulky and expensive external hardware, and high-end microscope set-ups [4]. Moreover, these instruments have a very limited level of portability and integrability with other analysis tools [5]. One alternative to these techniques is the electrical sensing. Common practice for the electrical sensing based particle counting is to flow a particle in a channel whose size is comparable with the particle size and to monitor the impedance or capacitance of the channel. Since the electrical properties of the (bio)-particles are different than that of the buffer solution, a peak can be detected as the (bio)-particles are moving across the sensing section. Microfluidic particle counting has been studied in the past decade, and it has shown enormous potential for low-cost and portable applications in biological applications [3]. More specifically, the quantification of red blood cells [6] and immobilized cells [7] has been performed by sensing the impedance change within the buffer solution. Present microfluidic particle counters have a common limitation which is low throughput and needs to be improved in the future.

In this study, a numerical modeling using COMSOL Multiphysics for the separation and counting of (bio)-particles have been developed, and the performance of the planar and 3D electrode structures have been compared. Microfluidic devices with an asymmetric pair (for separation) and a symmetric pair (for counting) of electrodes are considered. The effects of the geometrical parameters, material properties, flow rate, particle size and applied voltage on the device performance have been discussed. The fabrication procedure of 3D electrode structures is also addressed.

2. Analysis and Simulation

2.1 DEP-based particle separation

DEP is the motion of particles within a non-uniform electric field due to interaction of the electrical field gradient with the dipole moment of the particle. Depending on the dielectric properties of the medium and the particle, DEP can be either in the direction of higher electric field gradient (positive-DEP, pDEP) or lower electrical field gradient (negative-DEP, nDEP). An electrical field can be generated within a microfluidic device by using two asymmetric electrodes as seen in Figure 2.

The simulation of the particle trajectory within a microchannel can be modeled by using point particle approach [1]. In this approach, particle is assumed to be a point-particle. Moreover, the effect of the particle presence on the flow and electric field is neglected. Utilizing this method, the velocity of a particle in a flow field and an electrical field can be written as [1]:

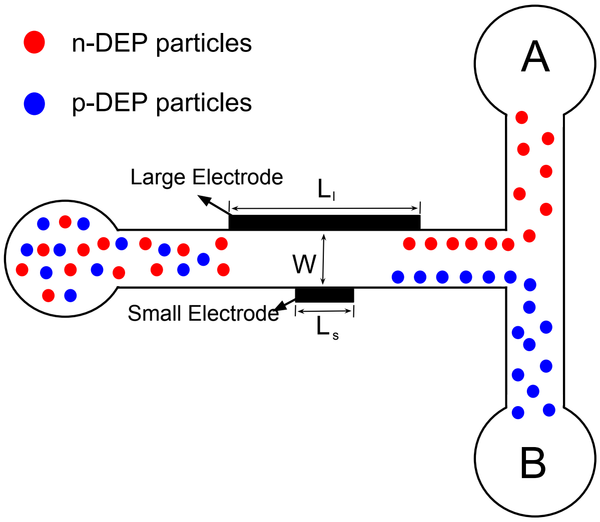


Figure 2: Schematics of DEP-based separation

$$\mathbf{u}_p = \mathbf{u} - \frac{\varepsilon_m R^2 \mathcal{R}e\left[f_{CM}(\omega)\right]}{3\mu} \nabla \mathbf{E}_{rms}^2 \quad (1)$$

In this equation, $\mathbf{u_p}$, $\mathbf{u}$, $\mathbf{E}$, R, μ, ε_m and f_{CM} are particle velocity, flow field, electric field, particle radius, viscosity of the fluid, permittivity of the fluid and Clausius-Mossotti factor, respectively. Theoretically, the real part of f_{CM} varies between -0.5 and 1.0. Negative means nDEP response, and positive means pDEP response.

To model the particle trajectory within the microchannel the flow and electric field needs to be determined. In the simulations, COMSOL Multiphysics software is utilized. For the flow field, Navier-Stokes equations together with no-slip boundary condition at the channel wall, predefined flow rate at the channel inlet, and zero pressure at the channel exit. For the electric field, Laplace equation is solved together with the insulated boundary condition at the channel wall, zero voltage at the small electrode and the predefined voltage at the larger electrode. Once the electric and flow field is obtained, the particle trajectories are obtained by using the streamline function of COMSOL and COMSOL-MATLAB interface. To simulate the random distribution of the particle flow at the device inlet, the initial locations of the particles at the channel inlet are assigned randomly by using normal distribution function of MATLAB. 100 pDEP and 100 nDEP particles are released, and the number of particles collected in reservoir A and B is determined. Cases with both planar and 3D sidewall electrodes are simulated for 5μm and 10μm particles. f_{CM} for pDEP and nDEP particles is assigned as 1.0 and -0.5, respectively. In an ideal case, all of the pDEP particles and nDEP particles need to be collected in reservoir B and A, respectively. Density and the viscosity of the fluid are assigned as *1000 kg/m*^{3}, and *1x10*$^{-3}$ *Ns/m*^{2}, respectively. The length of the small and large electrode is taken as *50μm* and *250μm*, respectively. The flow rate within the channel is assigned as *0.03 ml/min*. The channels with *50μmx50μm* and *100μmx100μm* are considered. The effect of different voltage values on the device performance is assessed.

2.2 Capacitive particle counting

The simplest capacitor consists of two conductive plates separated by air or other dielectric materials. Applying voltage to the capacitor plates produces electrical field and stores energy between plates. Electrical capacitance of a capacitor depends on its dimension and material. The simplest equation for electrical capacitance calculation is as following:

$$C = \frac{\varepsilon_0 \varepsilon_r A}{d} \quad (2)$$

ε_0, ε_r, A and d are the permittivity of air, relative permittivity of dielectric material, area of the capacitor plates and distance between two plates, respectively. Equation presents that any change in the physical parameter of the capacitor such as geometry and effective permittivity results in capacitance change of the system. Thus, this characteristic can be utilized for detection and counting of particles based on capacitance variation. In order to implement capacitance measurement for microfluidic application, two symmetric metal electrodes have been attached to both side of microchannel, and a constant voltage has been applied to these electrodes. When the particle passes through the microchannel, due to the variation in effective permittivity of bulk solution, the electrical capacitance of system changes and reaches a maximum value in the region between two electrodes. The number and amplitude of capacitance peaks provide the information about the number and size of (bio)-particles. The most important factors that affect the bulk solution capacitance are the size of electrodes, size of the particle (affects relative permittivity) and particle orientation. Capacitance of such systems is approximately independent of applied voltage on electrodes (by completely ignoring edge effects), but it strongly depends on the size of the electrodes embedded within the device. Electrodes with larger areas increase system capacitance and make particle detection and counting easier to detect. This size dependence of capacitance introduces an advantage associated with the implementation of 3D sidewall electrodes in capacitance measurement based particle counters and sorters.

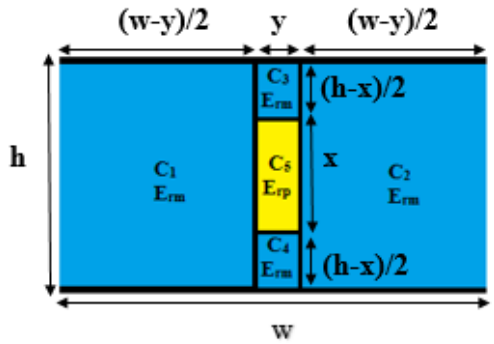


Figure 3: Capacitor modeling of the system with particle in vertical orientation

Most (bio)-particles do not have symmetrical structure like sphere, so it would be important to assess the particle orientation effect on the value of electrical capacitance. For instance; the system capacitance for an ellipse in horizontal position is different than the vertical orientation. 2-D analytical assessments for the capacitance dependence on particle orientation are presented for a particle with rectangular shape. The height and width of the rectangle are *x* and *y*, respectively. The rectangle has placed in the middle of microchannel in both vertical and horizontal orientations and the system capacitance is calculated for both configurations. The equivalent capacitance of the system in vertical orientation of particle is:

$$C_{eq} = C_1 + C_2 + (C_3 \square C_4 \square C_5) = \frac{l\varepsilon_0\varepsilon_{rm}w}{h} - \frac{l\varepsilon_0\varepsilon_{rm}yx}{h}\left(\frac{\varepsilon_{rm}-\varepsilon_{rp}}{h\varepsilon_{rp}+x(\varepsilon_{rm}-\varepsilon_{rp})}\right) = C_{Channel} - \frac{l\varepsilon_0\varepsilon_{rm}yx}{h}\left(\frac{\varepsilon_{rm}-\varepsilon_{rp}}{h\varepsilon_{rp}+x(\varepsilon_{rm}-\varepsilon_{rp})}\right) \quad (3)$$

The equivalent capacitance of the system in horizontal orientation of particle is:

$$C_{eq} = C_1 + C_2 + (C_3 \square C_4 \square C_5) = \frac{l\varepsilon_0\varepsilon_{rm}w}{h} - \frac{l\varepsilon_0\varepsilon_{rm}yx}{h}\left(\frac{\varepsilon_{rm}-\varepsilon_{rp}}{h\varepsilon_{rp}+y(\varepsilon_{rm}-\varepsilon_{rp})}\right) = C_{Channel} - \frac{l\varepsilon_0\varepsilon_{rm}yx}{h}\left(\frac{\varepsilon_{rm}-\varepsilon_{rp}}{h\varepsilon_{rp}+y(\varepsilon_{rm}-\varepsilon_{rp})}\right) \quad (4)$$

Theoretical analysis of the described simple system reveals that capacitance for particles with asymmetric shapes depend on particle's orientation inside the microchannel.

The microfluidic device for particle counting has been modeled based on capacitance measurement using COMSOL Multiphysics. The device is polymer based and consists of a microchannel with embedded two symmetric electrodes. The model has been solved by Electrostatics module: electrodes boundaries are defined as terminal and ground and the value of system capacitance is calculated and plotted as postprocessing. The simulation involves electrostatic equation in dielectric materials:

$$-\nabla.(\varepsilon_0\nabla V - \boldsymbol{P}) = \rho \quad (5)$$

In the equation, ε_0 (F/m) is the permittivity of vacuum, **P** (C/ m^2) is the electric polarization vector and ρ (C/m^3) is the space charge density. Defined materials in the model are water with relative permittivity of 80 as medium, and polystyrene particles with relative permittivity of 2.5. Modeling of the device has been implemented for two different types of electrodes, planar and 3D sidewall electrodes. Figure 4, shows the schematics of a micro-device with planar and 3D electrode structures.

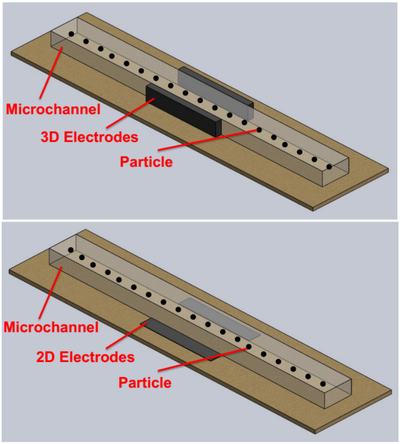


Figure 4: Schematics of a microdevice with planar and 3D electrode structures

In the cases of same electrode structures, system capacitance depends on the effective permittivity of the buffer solution mixture with particle. Any change in the size of particle may affect the system capacitance and this feature can be used for particle sorting in microfluidics applications. The device sensitivity to detect various sized particles has been assisted by changing the size of particles in the channel.

For asymmetric shaped particles, the capacitance of the system may be different for the particles with different configuration. In order to evaluate device functionality for asymmetric shape of particles, the modeling of the device is implemented for elliptic particles with two different orientations, namely horizontal and vertical orientation. Figure 5 shows the schematics of an ellipse in a microchannel with horizontal and vertical position.

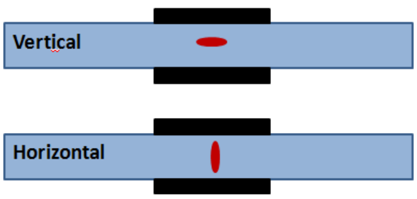


Figure 5: Schematics of an ellipse in a microchannel with different configuration

In this modeling, the functionality of the device depending on the electrodes structures and geometrical properties of particle is considered for both planar and 3D sidewall electrodes for different size particles. The channels with *50μmx50μm* and *100μmx100μm* and particles size of *10μm*, *15μm* and *20μm* are considered. To demonstrate the effect of the particle orientation, an asymmetrical particle (elliptic particle) with different orientation is also considered.

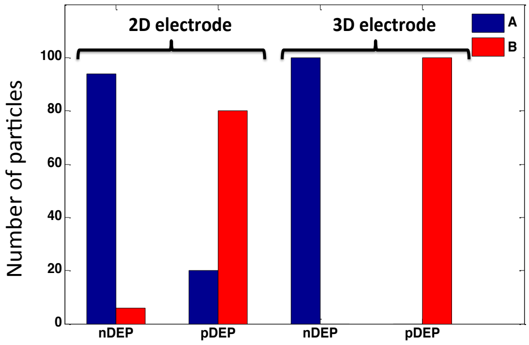
3 Results and Discussion

The simulations were carried out on a SuperServer Workstation (Intel Xeon X5687, Quad core, 3.60GHz, 96GB RAM). Finer meshes are generated within the microchannel. A mesh independence study is performed to get mesh independent solutions. It was observed that the element number of approximately 160K (with a degree of freedom of approximately 430K) for separation and the element number of approximately 1.1M (with a degree of freedom of approximately 1.5M) gives the mesh independent results. Typical run-time for the separation including the 200-particle release was about 2 minutes. For the counting simulations parametric sweep of COMSOL is utilized and the particles is moved inside the channel with 25 steps. At each step the electric field is determined and the capacitance value is determined. The total simulation time for each particle takes approximately 60 minutes.

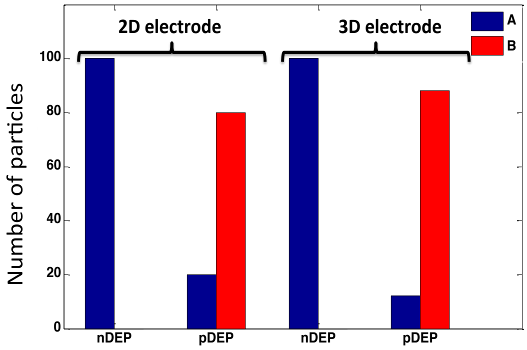
3.1. DEP-based particle separation

Figure 6 compares the device performance for planar (2D) and 3D electrodes. To achieve a more comprehensive comparison, the cases with microchannels and particles with different size are considered for different voltage values. Results show the total number of particles collected in outlet A and B (referring to Figure 2). A total of 100 pDEP and 100 nDEP particles are released from the inlet. As an ideal output, 100 pDEP and 100 nDEP particles are desired to be collected at outlet B, and A, respectively. As seen from the figure, the implementation of 3D electrodes improves the separation efficiency for the given flow field at the applied voltage. Figure 6-(a) clearly states that the microchannel

(*50µmx50µm*) with the 3D electrodes is able to reach the desired output, while the device with planar electrode cannot. For the microchannel with a size of *100µmx100µm*, to achieve a similar output for planar electrodes the output voltage needs to be increased. For nDEP particles, all the particles are collected at A, however pDEP particles are not separated successfully (i.e. some of the pDEP particles are collected also in outlet A). The use of 3D electrodes still cannot reach the ideal output, but clearly by the use of 3D electrodes, the number of particles collected at outlet A decreases.



(a) 50µmx50µm, D=10µm, φ=1V

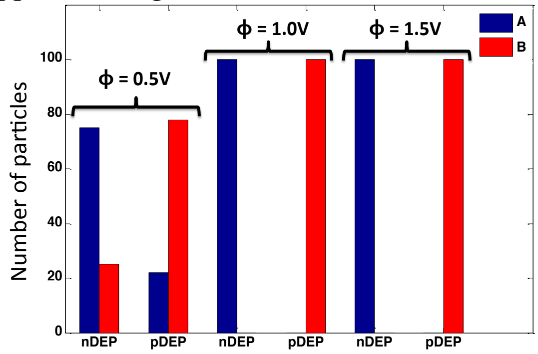


(b) 100µmx100µm, D=10µm, φ=4V

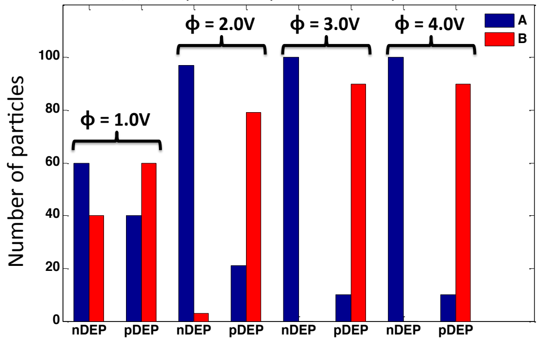
Figure 6: Separation with planar and 3D sidewall electrodes

Figure 7 shows the effect of voltage variation on the separation performance of the device with 3D electrodes. The modeling presents that the separation efficiency increases with the increasing voltage which is expected since the increasing voltage leads to enhanced DEP force. As seen from the figure, the required voltage increases with the increasing channel size. However, the increase in voltage also increases the electrical field which introduces a Joule heating within the channel [11]. Joule heating is not desirable, since it may cause some adverse effects which deteriorates the device performance, and especially for the application with live (bio)-particles, Joule heating may affect the (bio)-particle behavior and/or damage the (bio)-particles [11].

On possible way to increase the separation efficiency without any increase in the voltage is the implementation of multiple small electrodes (referring to Figure 2). Figure 8 shows the comparison of single-pair and multi-pair electrode configuration for *10µm* particles. With the introduction of the multiple tips, the separation efficiency enhances and the desired ideal output is achieved for the same at an applied voltage of 4V.



(a) 50µmx50µm, D=10µm



(b) 100µmx100µm, D=10µm

Figure 7: Effect of voltage on separation performance

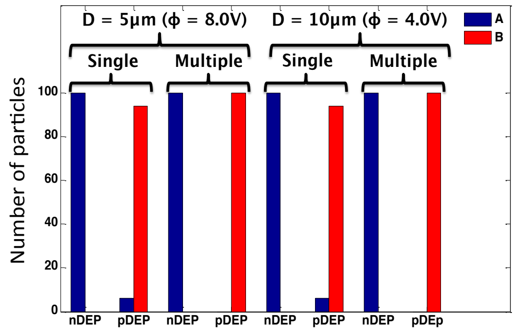


Figure 8: Comparison of single-pair and multi-pair electrode configuration

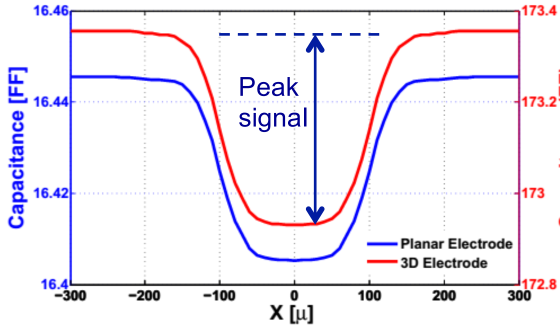
3.2. Capacitive particle counting

The sensitive portion of the microchannel (the region that encompasses electrodes) has been modeled. To discriminate 3D sidewall electrodes from planar electrodes, the microchannel with presented electrode features has been modeled. Flow of *10µm* polystyrene particles through the electrodes is considered, and the capacitance change on the electrodes are determined for planar and 3D sidewall electrodes case.

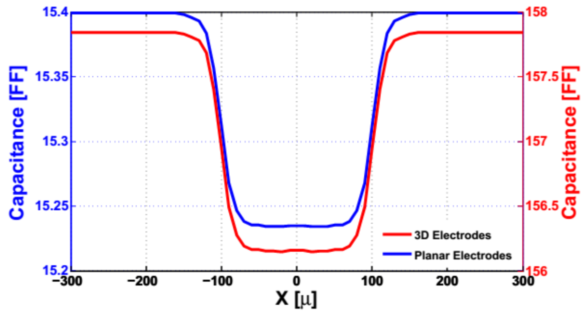
Figure 9 shows the capacitance change of the system due to particle movement within the microchannel for two different channels. According to simulation result, the capacitance signal for 3D electrodes is considerably larger than that of planar electrodes (10.5 times larger). The peak signal is 0.05 FF for a planar electrode, however it increases to 0.5FF. The result is much better when the size of the channel is reduced to *50µmx50µm* . The planar case gives a peak signal of ~0.15FF, and the 3D electrode case gives a peak signal of ~1.6FF. Both the based signal and the signal peak are increased with the introduction of the 3D electrodes which enhanced the detectability. Thus, detecting and counting process would be easier with 3D electrodes than that of planar electrodes.

Systems capacitance also varies with the particle size since the change in the size of the particle changes the effective permittivity of the system. To demonstrate this effect, the model is run for particles with different size: *10µm*, *15µm* and *20µm* particles. As seen in Figure 10, the peak signal increases significantly with the increasing size due to decreasing effective permittivity of the system. The increase is more significant for the channel with smaller cross-section. The peak signal increases from 0.5FF to ~3.5FF, and from 1.6FF to ~12FF for 20µm particles for the larger and the smaller channel size, respectively.

Capacitance of the system may also vary due to the orientation of (asymmetric) particles. To explore this issue, the flow of an elliptic particle with different configuration is simulated. Figure 11 illustrates the simulation result for an ellipse with semi axis's dimension of (20,0,0) for horizontal posture and (0,0,20) for vertical posture in the microchannel. As seen, although the orientation changes the peak of the signal, the change is not significant (only ~0.01FF change in the capacitance)

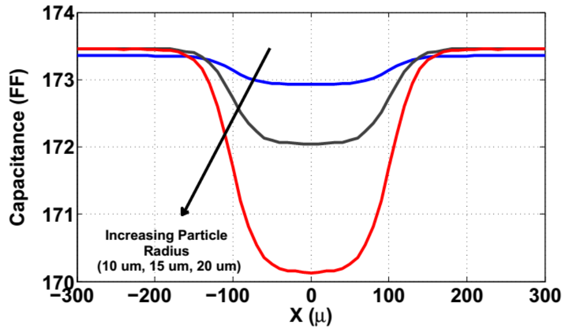


(a) *100µmx100µm (HxW)*

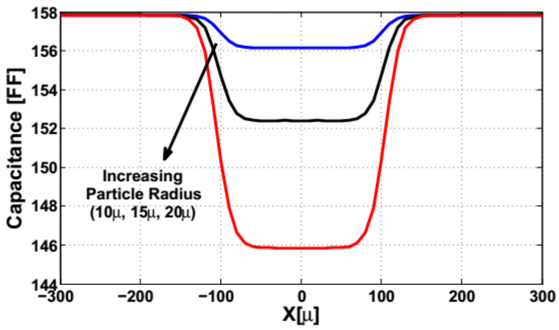


(b) *50µmx50µm (HxW)*

Figure 9: Capacitance change in the microchannel with planar and 3D sidewall electrodes



(a) *100µmx100µm (HxW)*



(b) *50µmx50µm (HxW)*

Figure 10: Capacitance variation of the microchannel due to size variation of particles

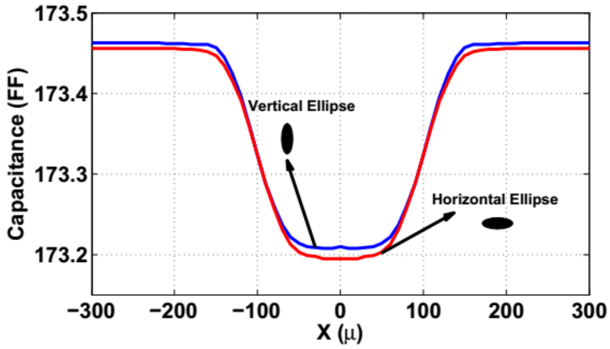


Figure 11: Capacitance variation for different orientation of elliptic particles

4. Conclusions

In this study, a numerical modeling using COMSOL Multiphysics for the separation and counting of (bio)-particles have been presented, and the performance of the planar and 3D electrode structures have been discussed. MATLAB interface of COMSOL is utilized for the simulation of the particle trajectories. To simulate the random distribution of the particles at the channel inlet, the built-in function of MATLAB is utilized. The proposed computational model can predict the particle separation and particle counting performance. The current computational model can be extended to take into account the particle size variation [12] and/or particle-wall and particle-particle interaction through some predefined correction factors which can be introduced in Eq. (1) [1].

For the separation device modeling results propose that to have efficient particle separation, 3D sidewall electrodes are preferable over planar electrodes due to the use of low voltage and having the same DEP force throughout the channel height. In addition to considering 3D electrode structure, in order to have more efficient separation especially in relatively large channels, it is preferred to consider multi-pair small electrodes. Like separation case, for counting device, 3D sidewall electrode structure is preferred intensifying capacitance peak which makes it easier for the particle to be detected. In counting case, the functionality of device for particle's posture is also considered and presented that for elliptic particles, orientation of particle affects the system capacitance.

Although 3D sidewall electrodes are seemed to be preferable, fabrication of 3D sidewall electrodes is not straightforward. Although some methods have been proposed in the literature [13], a fabrication process with high repeatability, and which enables application of small voltages is still a challenge. A robust fabrication of 3D sidewall electrodes, together with the verification of the proposed model with the experimental is an active, on-going research in our research group.

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References

1. B. Cetin, D. Li, Electrophoresis, 32, 2011, 2410–2427
2. R. Pethig, Biomicrofluidics, 4, 2010, 022811
3. S. van den Driesche, V. Rao, D. Puchberger-Enengl, W. Witarski, M. J. Vellekoop, Sensor Actuat B-Chem, 170, 2012, 207–214
4. S. K. Ameri, P. K. Singh, M. R. Dokmeci, A. Khademhosseini, Q. Xu, S. R. Sonkusale, Biosens. Bioelectron., 54, 2014, 462–467
5. M. Javanmard, S. Emaminejad, C. Gupta, J. Provine, R.W. Davis, R.T. Howe, Sensors and Actuators B: Chemical, 193, 2014, 918–924
6. H. Zhang, C. H. Chon, X. Pan, D. Li, Microfluid Nanofluid , 2009, 7, 739–749.
7. E.P. Dupont, E. Labonne, Y. Maruyama, C. Vandevyver, U. Lehmann, M.A.M. Gijs, E. Charbon, Senors and Actuators B: Chemical, 2012, 174, 609-614.
8. Y.-H. Lin, G.-B. Lee, Biosensors and Bioelectronics, 24, 2008, 572-578.
9. K. B. Kim, H. Chun, H. C. Kim, T. D. Chung, Electrophoresis, 2009, 30, 1464–1469.
10. X. Cheng, Y. Liu, D. Irimia, U. Demirci, L. Yang, L. Zamir, W. R. Rodrıguez, M. Toner, R. Bashir, Lab Chip, 2007, 7, 746– 755
11. B. Cetin and D. Li, Electrophoresis, 29, 2008, 994–1005
12. B. Çetin, S. Büyükkoçak, S. Zeinali, B. Özer, *ASME-2013 4th Microscale/Nanoscale Heat & Mass Transfer International Conference*, December 11-14, Hong Kong, China (Paper No: 22181)
13. M. Li, W.H. Li, J. Zhang, G. Alici, W. Wen, J. Physc. D: Appl. Phys., 47, 2014, 063001