RECENT TRENDS IN DIELECTROPHORESIS

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Key words: dielectrophoresis, BioMEMS, dielectrophoretic devices, dielectrophoretic chip, DEP 3D filter

Abstract: The paper presents the recent development of one important BioMEMS application: dielectrophoresis. A classification of dielectrophoretic devices, recent work in the field as well as theoretical consideration and numerical simulation will be presented in the first part of the paper. The second part of the paper presents some dielectrophoretic chip developed by the authors. Different structures of DEP devices such as DEP devices with 3D electrodes, DEP chip with asymmetric electrodes as well as an isolating DEP 3D filter are presented. The main biological applications of these microfluidic devices are: cell trapping and separation of two cellular populations. Some field-flow separation methods performed on the above mentioned chips are described.

Najnovejši trendi pri dielektroforezi

Kjučne besede: dielektroforeza, BioMEMS, dielektroforezne naprave, dielektroforezni čipi, DEP 3D filtri

Izvleček: Članek opisuje razvoj ene najpomembnejših BioMEMS aplikacij-dielektroforezo. V prvem delu članka bo predstavljena klasifikacija naprav za dielektroforezo, kakor tudi numerične simualcije samega pojava. V drugem delu je predstavljeno nekaj dielektroforeznih čipov, ki smo jih razvili na inštitutu za bioinženiring in nanotehnologijo v Singapurju. Predstavljene so tudi različne strukture DEP vezij: DEP vezja s 3D elektrodami, DEP čipi z asimetričnimi elektrodami in izoliran DEP 3D filter. Glavne biološke aplikacije teh mikrofluidnih naprav so: ujetje celic in loÄevanje dveh celičnih populacij. Opisanih je tudi nekaj ločevalnih metod, ki se izvajajo na zgoraj omenjenih čipih.

1. Introduction

Lab-on-chip devices are essential elements for biomedical instrumentation /1/. Microfluidic dispensing and controlling devices /2/, also the afferent technologies for microfluidic chip fabrication on glass /3/, polydimethyl-siloxane (PDMS) /4/, plastic /5/ or even UV curable polymer /6/ were developed.

In our group the research was focus on few directions: devices for drug delivery /7/, cell cultures on microfluidic platforms /8/, characterization of biological samples using impedance spectroscopy /9/ separation of white blood cells using magnetophoresis /10/ and dielectrophoresis /11/. The paper presents the recent trends in dielectrophoresis and main achievement of our team.

2. General consideration about dep

The term "dielectrophoresis" was first coined by Pohl /12/. DEP is a motion of dielectric particles caused by polarization effects in a non-uniform electric field. A particle suspended in a medium of different dielectric characteristics became electrically polarized. Due to the difference in electric field strength on the two sides of the particle, a net dielectrophoretic force pulls it towards the higher electric field gradient region (positive DEP) or pushes it towards the lower electric field gradient region (negative DEP).(Figure 1)

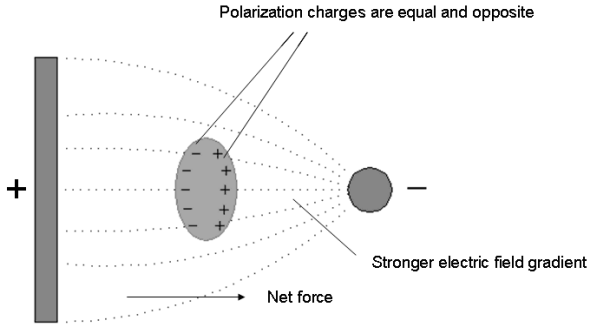

Fig. 1: Principle of dielectrophoresis

Early dielectrophoretic devices were made from thin electrical wires or other machined metal electrodes /13-15/. For many years, their applications were limited to the micro-scale particles, due to the size of the electrodes, which were not small enough to generate high electric field gradient. After 1990, with the development of microfabrica-

tion technologies, a large number of micro- or nano-scale complex electrode arrays designed for particle manipulation were precisely fabricated and integrated to form miniaturized dielectrophoresis chips.

2.1 Analytical consideration

The time-average dielectrophoretic force for the general field was defined as: /16/

$$\left\langle \bar{F}(t) \right\rangle = 2\pi\varepsilon_1 R^3 \left\{ \mathrm{Re}[K]\nabla E_0^2 + 2\,\mathrm{Im}[K](E_{x0}^2\nabla\varphi_x + E_{y0}^2\nabla\varphi_y + E_{z0}^2\nabla\varphi_z \right\} \quad (1)$$

E_{i0} and $\varphi_i (i = x; y; z)$ represented the magnitude and phase, respectively, of the field components in the principal axis directions. This expression contained two terms that contributed to the DEP motion.

1) The first term related the *real* component of the induced dipole moment in the particle and to spatial non-uniformity of the field magnitude. This force directed the particle towards higher or lower electric field regions, depending on whether the $\mathrm{Re}[K]$ was positive or negative. This was the conventional DEP term. The classical DEP force can be given by:

$$F_{DEP} = 2\pi R^3 \varepsilon_1 \,\mathrm{Re}[K]\nabla E_0^2 \quad (2)$$

2) The second term related the *imaginary* component of the induced dipole moment and to spatial non-uniformity of the field phase. This force directed the particle against or along the direction of travel of the electric field depending on whether the phases of the field components are larger $(\mathrm{Im}[K] > 0)$ or smaller $(\mathrm{Im}[K] < 0)$. This was called Travelling Wave Dielectrophoresis (TWD). The expression can be given by /16/:

$$F_{TWD} = \frac{4\pi^2 \varepsilon_m r^3 \,\mathrm{Im}[K(\omega)] E^2}{\lambda} \quad (3)$$

When $\mathrm{Re}[K] = 0$ or $\mathrm{Im}[K] = 0$, the particle experienced no positive or negative DEP force. The frequency at which the particle showed no DEP force was called crossover frequency. The crossover frequency depended on dielectric properties of particle and medium.

K was the well-known complex Clausius–Mossotti factor, defined as:

$$K = \frac{\varepsilon_2^* - \varepsilon_1^*}{\varepsilon_2^* + 2\varepsilon_1^*}, \quad \varepsilon^* = \varepsilon - j\frac{\sigma}{\omega} \quad (4)$$

ε_2^* and ε_1^* were the complex permittivity of the particle and medium respectively. The complex permittivity for a dielectric material can be described by its permittivity ε, conductivity σ, and angular frequency w of the applied electrical field E_0. Taking the example of conventional DEP item, the DEP force acting on a spherical particle was a function of Clausius–Mossotti factor Re (K), which determined the direction of the DEP force. The Clausius-Mossotti factor was a function of electric field frequency, as shown in Figure 2. At a range of frequencies, the particle experienced positive DEP while it showed negative DEP at another range of frequencies. The frequency where the particle showed no positive and negative DEP was called crossover frequency. The change of the permittivity and conductivity of the particles and medium caused the shift of the crossover frequency. Thus at a selected frequency range, one population of particles experienced positive DEP while the other population exhibited negative DEP. This provided a possible basis to separate the mixture of different particles.

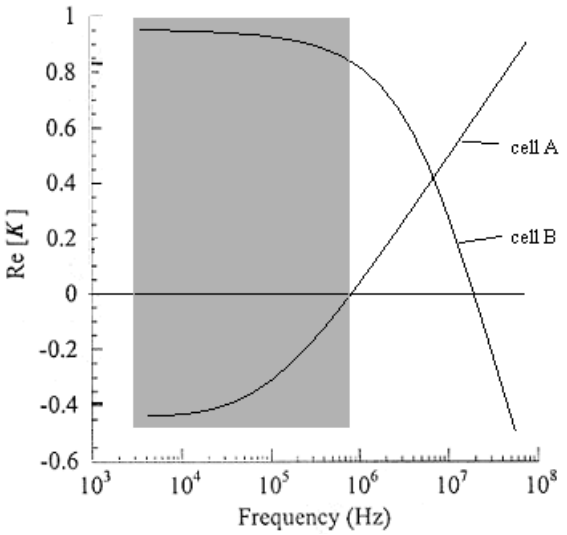

Fig. 2: Variation of the Re (K) as a function of frequency

A particle suspended in a solution was not subjected to DEP force only. It was exposed to many other forces such as hydrodynamic force, gravitational force, and electro-hydrodynamic force. For sub-microparticles, Brownian force must be considered. It was still a big challenge to overcome the Brownian force for manipulation of the sub-microparticles. A detailed calculation of various forces was described in /16/. The movement of the particle in a fluid was determined by the resultant force of many factors. Considering the influence of different types of forces, different separation mechanisms can be performed based on different microfabricated DEP devices.

However, quantification methods of the real value of DEP force on individual cell/ particles was just started. Therefore, pico-Newton resolution of the force was required. A first step was performed by Wei et al /18/ using optical tweezers as a force sensor. They claimed not only the sensitivity of the method for DEP force measurements, but also a selectivity that clearly distinguished the DEP force from all the other forces in the system, such as: electrophoresis, electro-osmosis, heat-induced convection, and Brownian forces. The method was suitable for quantifying the frequency-dependent DEP force and the crossover frequency of different particles.

2.2 Classification of DEP devices

Lately, a special attention was given to dielectrophoresis. This was proved by the increased number of publications especially over the last 5 years /18/. Recent reviews regarding dielectrophoresis were presented in /19-23/.

The dielectrophoretic force that generated the movement was strongly dependent on the gradient of the electric field. According to the methods used for achieving this gradient, different solutions were proposed.

Travelling wave DEP (Figure 3) was the method of changing the phase of the applied electric field /24, 25/ to achieve the gradient of the electric field between two parallel electrodes. Recent work performed by Choi et al in /26/ proved the potential of travelling wave DEP for high throughput separation of particles. An interesting application was presented by Lei et al in /27/, where the traveling wave dielectrophoresis was used for blood pumping in a microfluidic channel.

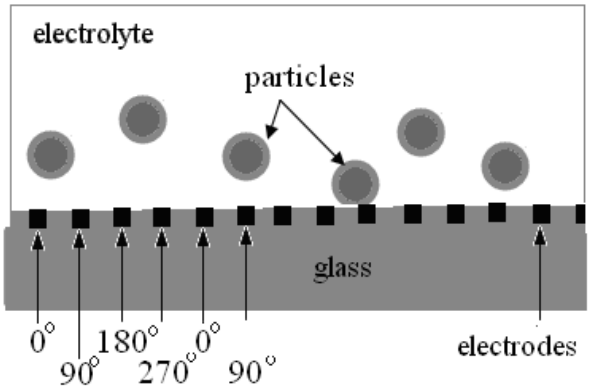

Fig. 3: Working principle of travelling wave dielectrophoresis

Isolating DEP (illustrated in Figure 4), the method presented in Figure 3b, consisted of generating electric field gradient of using of non-homogenous dielectric medium between two parallel-plate electrodes /28, 29/. Parikesit et al /30/ demonstrated that i-DEP can induce size-dependent trajectories of DNA macromolecules. Lewpiriyawong et al proposed in /31/ a conventional microfluidic H filter fabricated in PDMS. This filter induced a non-uniform electric field and generated a negative DEP force on the particles. Jen *et al* /32/ proposed an iDEP microchip to selectively trap dead HeLa cells and collect the viable cells by the hydrodynamic flow in the outlet with four isolated pillars that generated distortion in an uniform electric field.

Chiou *et al* proposed an optically induced DEP method where the gradient of the electric field was generated with a help of an optical image on a photodiode surface /33/. In the same direction, Lee *et al* proposed in /34/ a separation technique of particles under light-intensity gradient. A photoconductive layer was illuminated and a dielectrophoresis force was generated. This force competed with the hydrodynamic force. Hwang *et al* in /35/ discussed an optoelectrofluidic platform for differentiation of normal oocytes using a positive-DEP force that was induced by dynamic image projected from a liquid crystal display.

Moving DEP, presented in /36/ by Kua *et al* was a method where particles, initially trapped in a non-uniform electric

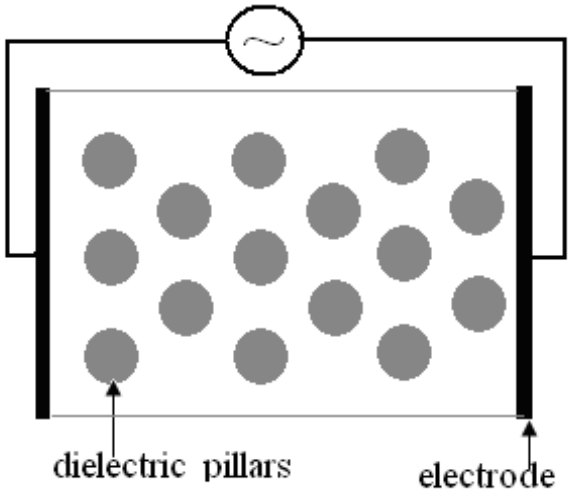

Fig. 4: Working principle of isolating dielectrophoresis

field, were moved using a travelling electric field. The mathematical modeling of the method was presented in /37/.

Another method used the spatial or temporal conductivity gradient. The medium conductivity was gradually increased generating an electric field gradient /38, 39/.

Another method described the gradient of the electric field which was generated by the non-uniform shape of the electrodes. The electrodes can be thin films /40, 41/ - Figure 3c, 3D pillars /42-44/, 3D electrodes that simultaneously defined the microfluidic channel /45/ or were embedded in the wall of the microfluidic channel /46, 47/ and also combination between a thin electrode and a 3D electrode /48/. H.C. Chang's group improved the dielectrophoretic force that acted on different particles in microfluidic device by buffer selection and cross linking /49, 50/. Cheng *et al* /51/ presented an integrated AC dielectrophoretic microfluidic platform based on planar electrodes. 3D DEP were formed gates by placing the electrodes on top and at the bottom of the microfluidic channel. A 3D-DEP cage was presented by Choi *et al* in /52/. Gradient of the electric field can be generated also in vertical direction using interdigitated electrodes; in this case the particles were either trapped at the bottom of the microfluidic channel by positive DEP or levitated using negative DEP /53/. Interdigitated electrodes were also used an interesting application proposed by Flanagan *et al* in /54/: they characterized the stem cells and their different progeny using dielectrophoresis (which induced a frequency-dependent dipole in cells).

There were three groups of applications of DEP phenomenon on cell:

- cell separation,
- cell lysis,
- cell patterning.

A main focus in DEP research was cell sorting- separation of two or more population, or even separation of rare cells

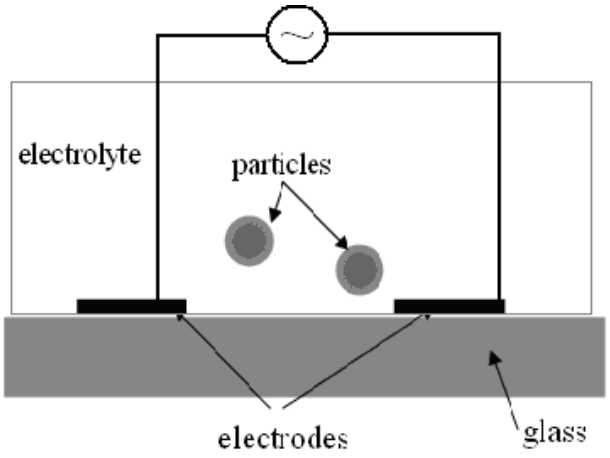

Fig. 5: Schematic representation of classical DEP (electrified gradient is generated by the irregular shape of the electrodes.

from a cell population. Reviews on cell separation were presented by Hughes /55/ and Gascoyne and Vykoukal /56/. These techniques can be summarized as: (a) flow separation, (b) field flow fractionation, (c) travel wave dielectrophoresis.

The flow separation method consisted of trapping, at different locations, the population that exhibited pDEP and nDEP. It used the hydrodynamic force to collect one of these cell populations flowing as a particle suspension solution over an electrode array. Flow separators were reported and demonstrated in /57-60/. The field-flow fraction /61, 62/ used a fluid velocity gradient to separate particles while DEP force was used to locate particles from different regions of the microfluidic channel. Traveling wave DEP can be also used as a separation technique. Related work was reported /63, 64/.

Lysing cells was an important step in the analysis of intracellular contents. This operation can be performed on microfluidic chip in correlation with cell concentration using dielectrophoretic force. Related work was presented in /65-68/.

Cell patterning was another application of dielectrophoresis with two main directions: single cell and multiple cells. In /69/ Ho *et al* proved patterning of hepatic and endothelial cells in a microfluidic chamber using DEP traps. **Rosenthal** and **Voldman reported in /70/** microfabricated DEP traps designed to pattern large arrays of single cells. Suzuki *et al* used nDEP and an interdigited array with four independent microelectrode subunits for cellular micropatern (C2C12 cells) /71/. Mittal *et al* /72/ used nDEP microwells for single-cell patterning in physiological media. Thomas *et al* /73/ proposed two complementary ring electrodes on different layers insulated by a dielectric layer that captured single cell inside the inner ring electrode.

3. Dep chip with 3d electrodes

3.1 Structure of the device

The DEP device structure, presented in Figure 6, consisted of three main layers: two glass wafers (top and bottom) and an inter-layer of conductive silicon that formed the microfluidic channels and the two DEP electrodes. A metallization layer on the bottom glass surface was connected to the bulk silicon electrodes via holes for easy assembly to a PCB. Inlet and outlet tubes were glued on the devices for fluid access.

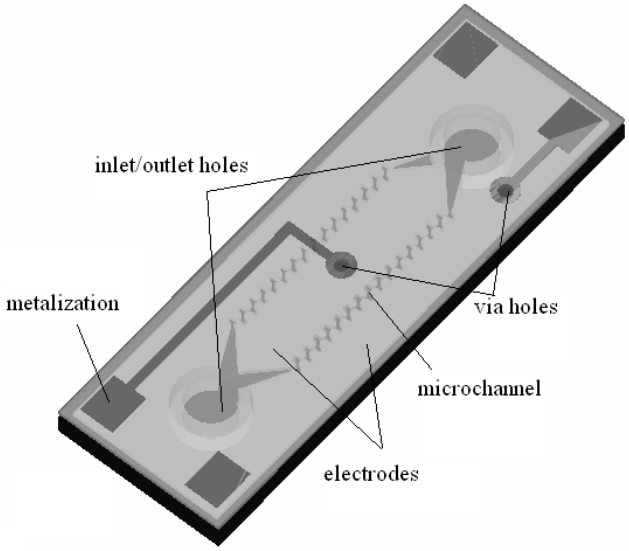

Fig. 6: DEP device with 3D electrodes

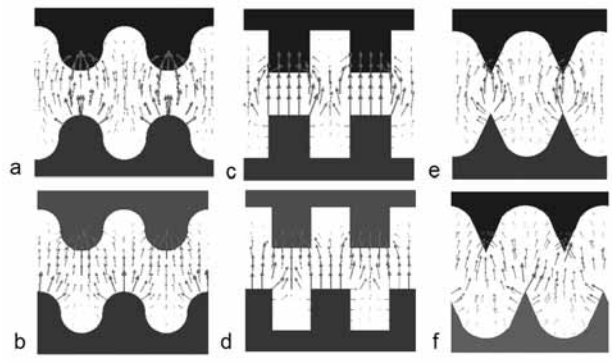

Fig. 7: Configurations of electrode for FEA analysis

Table 1: Electric field strength and gradient for different electrode configura-tions with 10V_{pp} actuation voltage (the distance between electrodes was 100μm)

Design	a	b	c	d	e	f
E [x 10^4V/m]	4.22	4.07	3.68	3.75	8.87	3.61
∇E^2 [X10^5 V^2/m^3]	2.91	1.76	2.29	2.38	12.8	5.53

To generate the DEP force for cell manipulation an AC voltage with a frequency of 1 kHz-100 MHz must be applied

to the electrodes so that an electric field and its gradient can be generated. From equation (1), the DEP force was proportional to the gradient of the square of electric field, which was determined by the geometry of electrodes and the insulator.

For the presented DEP device, the electrode arrangement was developed to maximize the electric field while minimizing the electrical dead volumes. This ensured that the DEP force was always sufficient to overcome Stokes' force and concentrate the cells at a relative low actuation voltage while minimizing fluidic dead volumes. This was a unique requirement for the design described since the electrode geometry also defined the fluidic geometry.

To estimate the electric field generated, triangular, rectangular and semi-circular type electrodes were analysed using the Maxwell electrostatic finite element analysis (FEA) software. Figure 7 showed examples of electrode configurations. Table 1 listed the maximum electric field and its gradient generated by each electrode configuration. A detail study regarding the gradient of the electric field was presented in /74/.

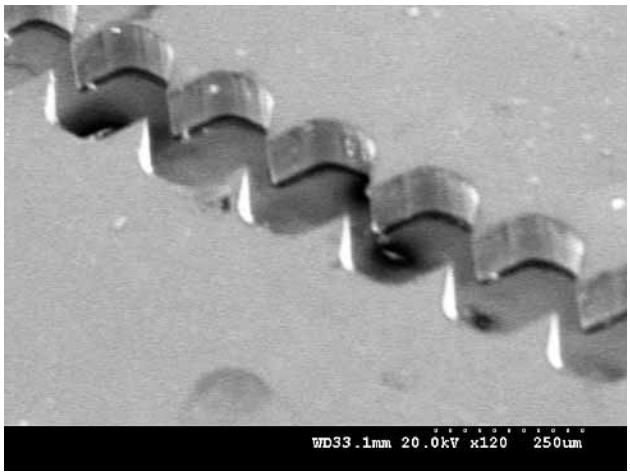

Fig. 8: SEM picture with the microchannel

3.2. Cell trapping in a DEP device with 3D electrodes

The testing of the device was performed using yeast cells. 100 mg of yeast, 100 mg of glucose and 2 ml DI water were incubated in an Eppendof tube at 37°C for 2 hours. The cells were then concentrated by centrifugation at 1000 rpm for 1 minute. The supernatant solution was removed and the cell pellet was washed by adding into the tube 2 ml of DI water. The centrifugation and washing process was repeated three times before the cells were collected and re-suspended in the separation buffer. The separation buffer was a mixture of phosphate buffered saline (PBS) and DI water. The conductivity of the separation buffer was

Fig. 9: Photo with DEP chips

adjusted to about 50 μS/cm using NaCl. The final concentration of the cells was $1x10^7$ cell/ml.

A function generator with a sinusoidal wave output was used to generate the drive signal. A linear amplifier was used to amplify the output voltage of the function generator in order to drive the DEP device. The drive signal was increased gradually from 0 to 25 V. The signal frequency was anywhere in the range of 20 kHz to 100 kHz.

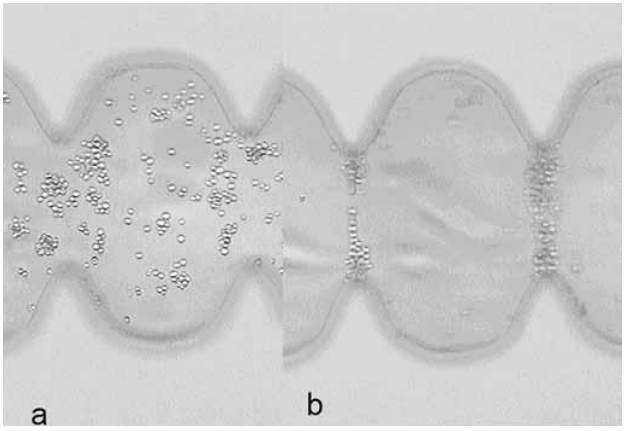

Fig. 10: The results of the trapping. Optical image with the trapping by positive DEP of yeast cells: (a) Before applying voltage, and (b) after.

The main advantage of the device with 3D electrodes consisted of a reduced Joule effect. A detailed analysis regarding Joule effect in different DEP devices was presented in /74/.

3.3. Sequential field-flow cell separation method in a DEP chip with 3D electrodes

The method was described in /75/ and consisted of four steps as illustrated in Figure 11. First, the microchannel was filled with the mixture of the two populations of parti-

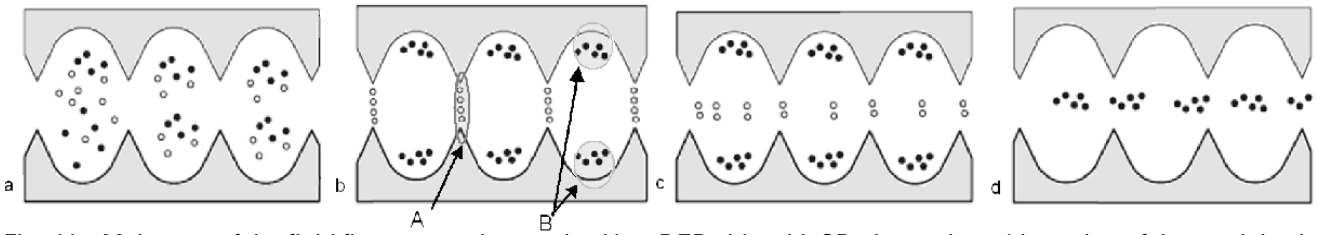

Fig. 11: Main step of the field-flow separation method in a DEP chip with 3D electrodes: a) insertion of the particles in the chip, b) cells separation using pDEP and nDEP, c) moving the first population by increasing the velocity of the fluid, d) the second population is released after removing the electric field.

cle (Figure 11a). Second, the particle populations were trapped at different locations along the microfluidic channels (Figure 11b). The population which exhibited positive dielectrophoresis was trapped in the area where the distance between the electrodes was the minimum while the other population that exhibited negative dielectrophoresis was trapped in places where the distance between electrodes was at its maximum. In the next step, increasing the flow in the microchannels resulted in an increased hydrodynamic force that swept the cell population trapped by positive dielectrophoresis out of the chip (Figure 11c). In the last step the electric field was removed and the second population was swept out and collected at the outlet (Figure 11d). The testing was performed with live/dead yeast cells.

3.4 Continuous field-flow separation of two cell populations in a DEP chip 3D electrodes

The method was similar to the previous one with the observation that the separation process was performed under continuous flow. The method was presented in detail in /76/ and consisted of two cell populations (live/dead) which flow through a microfluidic channel. The vertical walls of the channel were the electrodes of the DEP device. The irregular shape of the electrodes generated both electric field and fluid velocity gradients. As a result, the particles that exhibited negative DEP were trapped in the fluidic dead zones, while the particles that experienced positive DEP were concentrated in the regions with high velocity and thus collected at the outlet. The device was tested with dead and living yeast cells.

3.5 Bidirectional field –flow separation method

For the above presented methods the main disadvantage was the collection of the population trapped in the wells. This disadvantage can be eliminated using a chip with 2 inlets and two outlets, method described in /77/.

The methodology, illustrated in Figure 14, was as follows: first the solution with the mixture of two particle population was injected into the microfluidic chamber (Figure 14a); next the two populations were separated in different locations according to their electrical properties (Figure 14b);

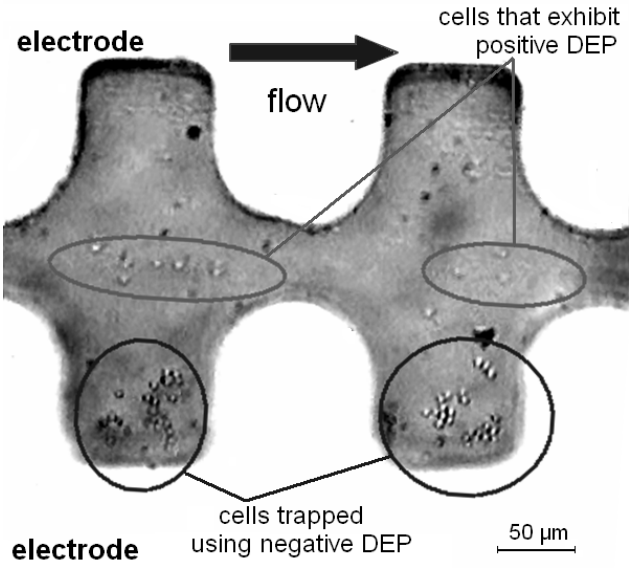

Fig. 12: Optical image of the separation process

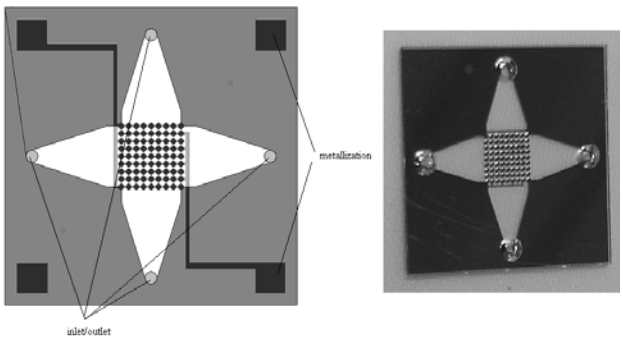

Fig. 13: Schematic drawing and optical image with the device use

subsequently, one population was collected at one of the outlet by flowing a fresh buffer solution (Figure 14c) while the other one was collected at the second outlet in the perpendicular direction (Figure 14d).

The two populations were separated using positive and negative DEP at different locations.

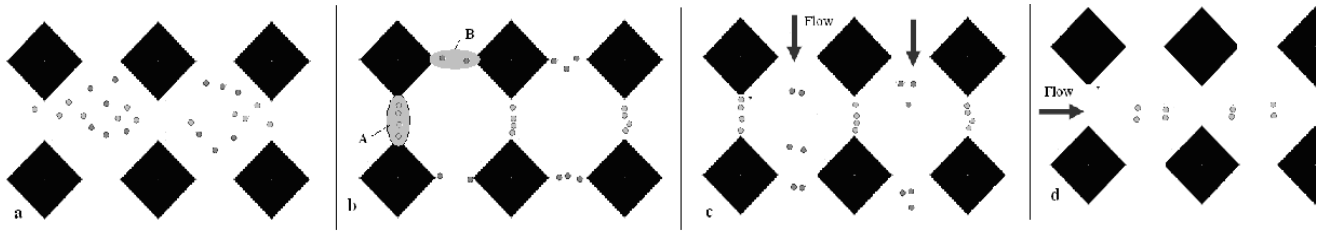

Fig. 14: Bidirectional field-flow separation method: a) insertion of the cell mixture, b) trapping of the cell in different location of the device, c) collection of the first cell population, d) collection of the second population.

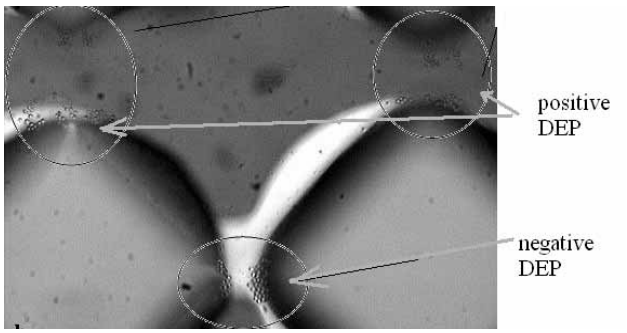

Fig. 15: Optical image taken during the separation process

4. Dep chip with asymmetric electrodes

4.1 Structure of the DEP chip with asymmetric electrodes

An asymmetric distribution of the electric field in the vertical plane can be possible using a special configuration of the electrodes: a bulk silicon electrode and a thin electrode (Figure 16).

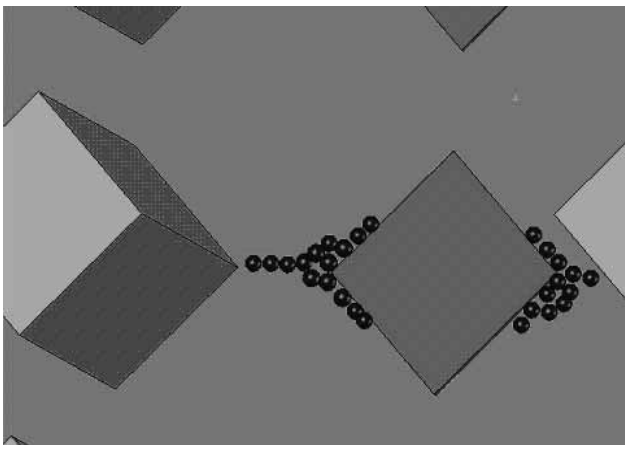

Fig. 16: DEP chip with 3D gradient

The thick electrode defined, at the same time, the walls, while the two glass dies formed the ceiling and floor of the microfluidic channel. The top glass die presented two etch-through inlet/outlet holes of the microfluidic channel. In the bottom glass die isotropic via-holes were performed through the glass for the lead-outs. The proposed DEP structure, with thin and thick electrodes, generated an asymmetric distribution of the electric field in the vertical plane. Therefore it created an enhanced electric field gradient. As a result, for positive DEP, the particles were trapped near the thin electrode, while for negative DEP the particles were levitated. Compared to the typical planar DEP devices, the proposed DEP structure presented an increased DEP force in the vertical direction. Details of the fabrication process of the device were presented in /48, 78/.

4.2 Cell trapping in a DEP device with asymmetric electrodes

The fabrication process of the DEP chip with asymmetric electrodes was similar to the process described previously. For the thin electrode a 1 µm-thick amorphous silicon layer (doped with Al) was used. The stress in the layer was measured to be less than 100 MPa compressive. The result of the testing of the DEP device using yeast cell was presented in Figure 16. The testing was performed in conditions similar to the testing of the DEP chip with 3D electrodes.

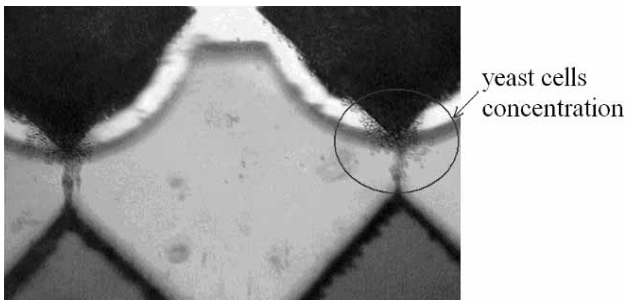

Fig. 16: Yeast cells trapped in the highest electric field regions around thin electrode tips due to positive DEP.

4.3 Continuous field-flow separation method in a DEP device with asymmetric electrodes

Figure 17 illustrated the separation method in a DEP chip with asymmetric electrodes. The mixture with two particle

populations was flowed through the microfluidic device. The magnitude of the electric field, its frequency and the medium properties were selected in such a way that one population exhibited positive DEP while the other one negative DEP. The electric field was applied as a continuous flow. Two results were recorded: (a) the particles that exhibited negative DEP were levitated due to a strong DEP force in a vertical direction that overcame the buoyancy force (b) the particles that experienced positive DEP were collected at the bottom of the device in the vicinity of the thin electrode. As a result, the cells that experienced negative DEP were collected at the outlet. When the electric field was released and the flow in the microfluidic channel was increased, the second population of cells was collected at the outlet.

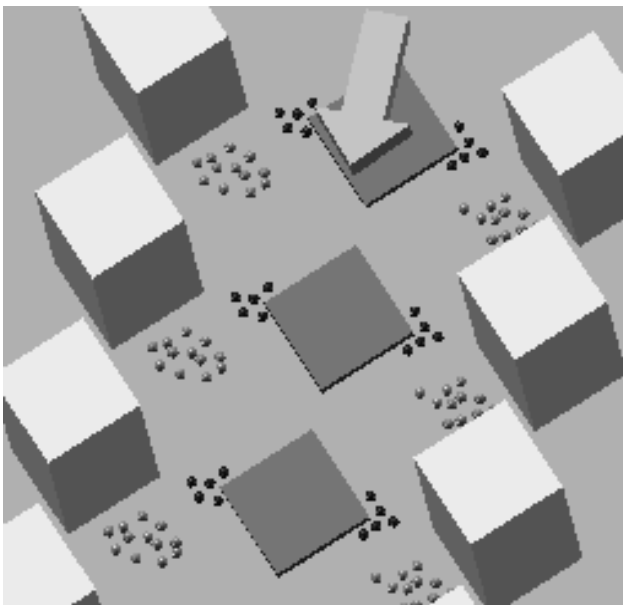

Fig. 17: Schematic view with the separation method: the population that exhibited positive DEP was trapped at the bottom of the device, while the population that experienced negative DEP was levitated and flowed out.

5. Conclusions

The paper presented an overview of the trends in dielectrophoresis as well as the contribution of the authors in this field with a special attention to structure of DEP devices with 3D electrodes or combinations between 3D and planar electrodes. The main advantages of the structures described were: (1) the electrodes defined the walls of the microfluidic channel, (2) usage of classical microfabrication techniques, (3) fabrication of the chip at wafer level, (4) the devices can work with a small volume of the samples, (5) a reduced Joule effect comparing with the DEP device with planar electrodes, (6) due to the reduced Joule effect the devices are suitable for biological applications

6. References

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