3D field focusing and defocusing geometries for cell trapping on a chip

Olivier Français, G. Mottet, A. Ayina, Bruno Le Pioufle

3D field focusing and defocusing geometries for cell trapping on a chip

Olivier Français*, Guillaume Mottet, Alain Ayina, Bruno Le Pioufle
ENS Cachan, SATIE (UMR8029) - IFR d'Alembert, 61 Av du Prdt Wilson, 94230 Cachan cedex, France

*Corresponding author: Français Olivier, (33)-1 47 40 77 36, Fax: (33)-1 40 27 20 60, olivier.francais@cnam.fr

Abstract: **In MEMS, Electromagnetic field is a way to manipulate droplets, beads or cells without direct contact. In particular, dielectrophoresis is often used in BioMEMS applications, for cell handling operations such as moving, sorting, trapping or deforming cells. In this paper, a 3D focusing geometry for the electrical field is proposed, that permit the trapping of single cell, or cells pair, thanks to positive dielectrophoresis. The design is explained with highlighting the focusing effect versus insulator shape for trapping cells. A prototype based on classical MEMS process and results of first trapping are presented.**

Keywords: BioMEMS, Dielectrophoresis Forces, Microfluidic, Cells trapping,

PRINCIPLE OF THE BIOCHIP

Di-electrophoresis is the result of Coulomb forces applied on a dipole [1]. Here, the dipole is due to polarisation of a cell (or particle), put in a conductive media such as biological medium to which an AC electrical field is generated. The induced dipole has properties that depends on frequency, leading to the expression of the Dielectrophoresis Force (F_{dep}) [1] [2] :

$$F_{DEP} = 2\pi\varepsilon_m R^3 [K(\omega)] \nabla E^2 \qquad (1)$$

Here ε_m is the permittivity of the medium, R the radius of the cell and E the root mean square of the ac electric field applied on the cell. $[K(\omega)]$ is the real part of the Claussis Mossoti factor which evolution is given by :

$$K(w) = \frac{\left(\varepsilon_p^* - \varepsilon_m^*\right)}{\left(\varepsilon_p^* + 2\varepsilon_m^*\right)} \qquad (2)$$

With ε_p^* and ε_m^* are the complex permittivity of the particule and medium and is defined by the relation:

$$\varepsilon^* = \varepsilon - j\frac{\sigma}{\omega} \qquad (3)$$

With σ the conductivity and ω the angular pulsation.

It reflects the electrical properties of any materials which can be divided into two categories: conducting (case of low frequency) or isolating (case of high frequency). So, depending on the frequency, middle and particle properties, the F_{dep} can be attractive towards maxima of electric field (case of $[K(\omega)]$>0) or repulsive towards minima (case of $[K(\omega)]$>0) (1). It can be notice that $[K(\omega)]$ is a value between -0.5 and 1 (2).

In case of biological media, classical value of σ is between .001 to .1 Sm^{-1} with permittivity equal to $80\varepsilon_o$ with ε_o=8.82x10^{-12}F.m^{-1} . In case of polystyrene beads, σ=0 and inside permittivity is estimated to be $4*\varepsilon_o$. Due to large permittivity difference between medium and polystyrene bead, the $[K(\omega)]$ is considered to be equal to -0.5. For a biological cell, the electrical properties associate the membrane and the cytoplast to obtain the equivalent complex permittivity of the cells. For example in the case of Jurkat cells, $[K(\omega)]$ follows the evolution versus frequency presented in Fig.1 (case of a medium conductivity equal to 7x10^{-3}S.m^{-1}) [3] :

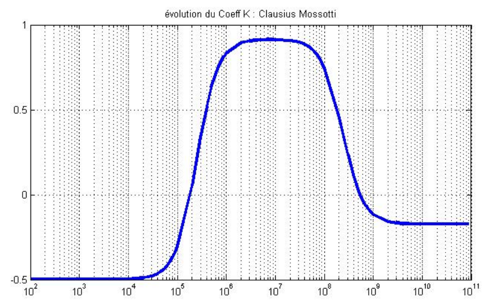


Figure 1: Real part of Claussius Mossoti factor (2) evolution versus frequency in the case of Jurkat cells

So by acting on the electric field frequency ($f = \omega / 2\pi$), it is possible to handle cells versus non uniform electric field distribution (1). In the case of Fig. 1, for a frequency between 1 MHz to 100 MHz, positive F_{dep} can be obtained which moves cells at maxima of electric field. In the case of Negative F_{dep}, cells are moving towards minima of electric field.

DESIGN OF THE CHIP FOR CELL TRAPPING

In order to obtain a non uniform electric field and generate the DEP, main devices use specific shape of electrode creating the field [4][5]. A promising alternative is proposed in

ISBN: 978-2-35500-009-6

this paper and is based on classical BioMEMS process using planar electrode deposition by sputtering and photolithography SU8 microchannel for the microfluidic part (Fig. 2). An electric field path is created by two planar and rectangular electrodes, separated by a microfluidic channel. First, the electric field is dispersed in the Z axis (direction perpendicular to the surface of the chip), prior to be concentrated in the Y axis (axis perpendicular to the fluidic flow corresponding to the X axis) thanks to insulating walls [6]. A first design based on this principle is presented on Fig.2.

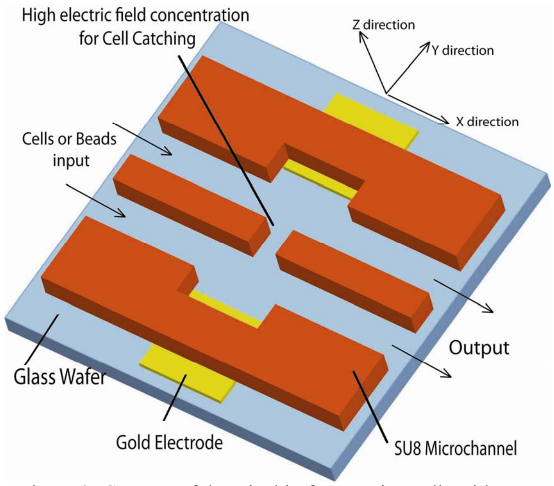


Figure 2: Concept of the Biochip for trapping cells with F_{dep}

The main microfluidic channel (X direction) is divided into two paths separated by an insulator. An opening is created in the insulator in order to define an electric path across the channel (Y direction) (Fig.2). Electrodes being in contact with the medium, these apertures in the insulator act as an electrical field focusing tool according to the local electric law, where current lines $\vec{J}$ follow the electric field $\vec{E}$:

$$\vec{J} = \sigma_m \vec{E} \qquad (4)$$

With σ_m conductivity of the middle

Between the two insulators, a maxima of electric field value is then obtained and may captures cell in case of positive DEP.

III. Simulation of the Principle

In order to highlight the defocusing/focusing effect, simulations have been achieved by using Comsol© in AC/DC mode, in a quasistatic mode by considering the middle as a conductive medium. A first approach is to simulate the chip in 2D (Fig.3). In this case, the electrodes are considered having a thickness equal to the channel. This device corresponds to the case of electrodes obtained by electrodeposition [7].

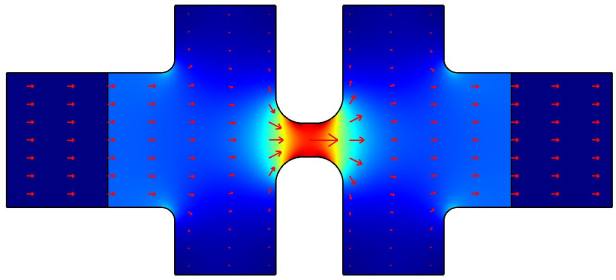


Figure 3 : Electric field distribution in 2D simulation

Simulation presented in Fig. 3 corresponds to a device based on channel of 150µm with insulator of 100µm large separated by a gap of 50µm. The opening for the electric path is 200µm and the electrodes are located at 100µm from the main channel. The focusing effect is developed at the middle of the insulator gap, following the shape of insulator.

A 3D model (Fig.4) had been extruded from the 2D device to take into account the flatness of our electrode to illustrate the defocusing effect.

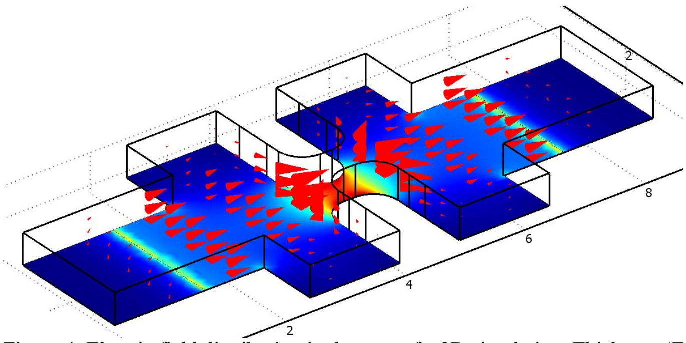


Figure 4: Electric field distribution in the case of a 3D simulation. Thickness (Z direction) of the device is equal to 50µm.

Both simulations (Fig.3 and Fig.4) highlight the focusing effect due to insulator position across the current path. One can notice the more the two insulators are close, the more the focusing effect will be high. In fact, the focusing increasing is equal to the ratio between insulator gap and electrode opening.

(Fig. 5) shows the electric field increasing toward Y direction across the channel up to the insulator versus X direction for several positions in Y direction. Y focusing of the electrical field, leading to high field concentration, is favourable to the cell trapping thanks to positive dielectrophoresis at the middle of the chip (Fig. 5). The thickness of insulator will permit to control the rate of increasing and location of F_{DEP}.

 ISBN: 978-2-35500-009-6

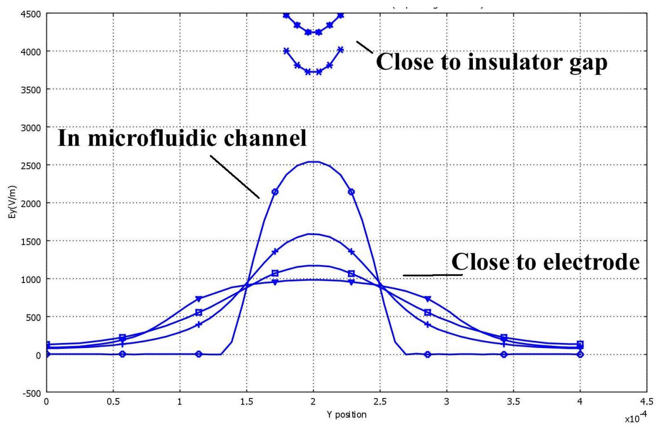


Figure 5 : Electric field value in Y direction for several position across the channel from electrode to insulator.

Thus, the geometry of the chip allows Z defocusing: The electric field is oriented towards Z direction above and near electrode (Fig. 6 case close electrode), then is turning in Y direction far from electrode to become constant versus Z direction in the channel (Fig. 6 case in the channel). If electrode are not to close from the channel, a behaviour similar to thick electrode are obtained in channel and around insulator given a DEP force equal to zero in Z direction. When approaching the insulator gap, the electric field value in increasing due to the focusing effect (Fig.6: case close insulator).

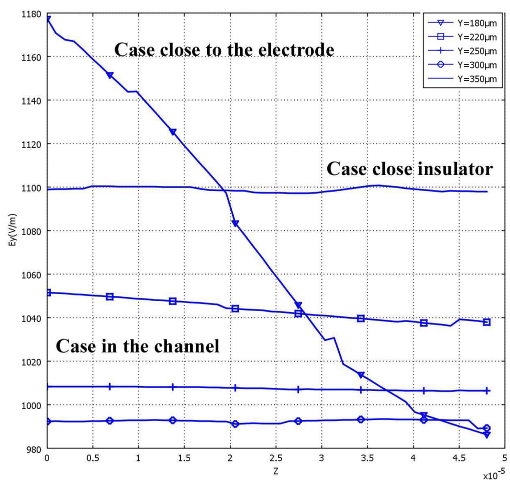
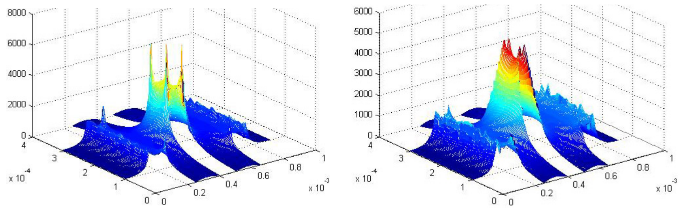


Figure 6: Electric field value in Y direction versus Z direction (from glass substrate to channel thickness) for several position across the channel (Y position).

So, good electric field homogeneity is obtained in the microfluidic path with a direction perpendicular to fluid path (Y direction). Starting from flatness electrode, behaviour similar to thick electrode is obtained and electric field reduction value is here compensated by the focusing effect close to insulator.

IV INFLUENCE OF THE GEOMETRY ON F_{DEP}

The shape of insulator influences the focusing effect and the electric field spatial distribution. Simulations, presented in Fig. 7 highlight this phenomenon and permit to optimize the F_{dep} for the cell trapping.



a: square insulator b : rounded insulator

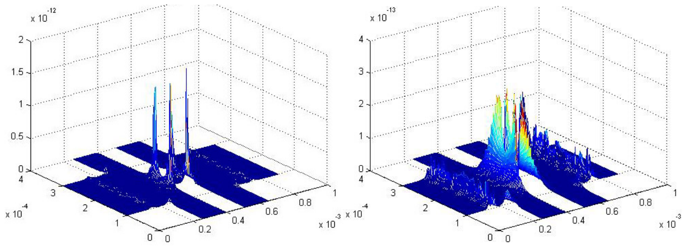
Figure 7: Electric field distribution versus insulator shape

As seen in Fig.7 the electric field is increased close to the insulator barrier. An electric field peak is obtained that can be positioned and controlled thanks to the geometry of the insulator. In the case of a rectangular shape, the peak is located at the corner (tip effect) (Fig.7 a). In the case of rounded edges, the peak moves to the middle of the opening, which is the desired position for the trapped particle (Fig. 7 b). A peak value is also obtained close to the electrode due to tip effect. It will also influence cells and may attract them if electrodes are close to the frontier with fluidic channel.

From the electric field value, calculation of the Fdep (1) has been achieved using a numerical program (Fig. 8). A compromise is shown due to:

1) the decreasing peak value of Fdep with the rounding of insulator (Fig. 8.b) compare to square insulator (Fig. 8.a).

2) the position of field maxima dependence on the rounding and being more in the middle with rounding (Fig. 8.b) compare to square electrode (Fig. 8.a) where the F_{dep} is maximum at the corner and not the insulator middle.



a: square insulator b : rounded insulator

Figure 8: F_{dep} calculation versus rounded insulator

So, a too heavy rounding decreases the F_{DEP} by reducing the rate of focusing effect but locates it at the middle of insulator.

V FABRICATION OF THE BIOCHIP

A first device has been fabricated (Fig.9 and Fig. 11) based on simulation presented above. In order to have a completely transparent biochip, the process is achieved on glass wafer as support. If transparency is not useful, silicon wafer can be also used. For avoiding complex alignment between electrode and microchannel (which is the case of using PDMS technology [9]), SU8 is used as photosensitive material for fabricating the microchannel. So, the process starts from a glass wafer where 15nm/150nm Cr/Au electrodes are deposited (by vacuum evaporation) (Fig.10.a) and patterned (wet etching KI solution and Cr etchant, after photolithography of a photosentitive resist layer) given the electrode shape (Fig.10.b).

 ISBN: 978-2-35500-009-6

Then a thick (depending on thickness wishes but between 25µm and 75µm in classical usage) SU8 layer is spin coated (Fig.10.c) and patterned with the shape of microfluidic part (Fig.10.d) (UV exposure through a mask, followed by developing and baking) for the micro fabrication of the microchannels (Fig.1) [8][9]. In order to package the device, a PDMS cap is reversibly bonded by Plasma O_2 technique. The bonding is sufficient to obtain good fluidic isolation for several hours. Accesses are obtained by hole previously done in the PDMS.

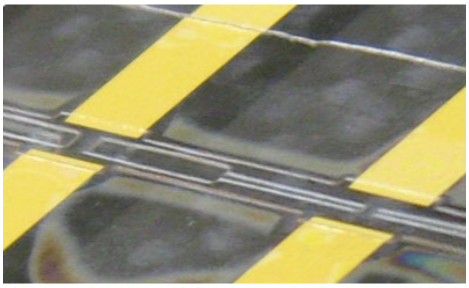


Figure 9: Photographic view of the chip: gold electrode are visible and the insulator geometries (case of 200 µm gap between electrodes).

During the process, practical care must be done during SU8 spin coating. Poor adhesion of SU8 may be obtained if substrate is not well clean and dry. Dehydrating treatment on oven at 150°C during one hour associated to plasma O_2 treatment will drastically enhance SU8 adhesion on substrate; a adhesion layer such as HMDS can be also used. During baking of the SU8, slow ramp (5°C/mn) reduces also constraints in the layer.

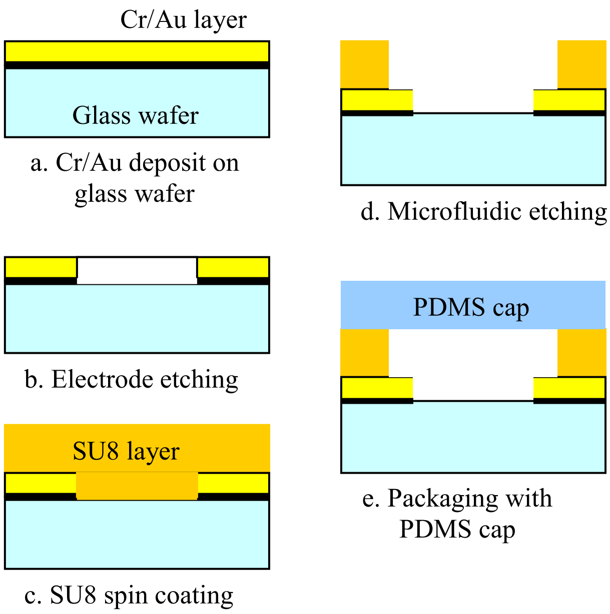


Figure 10: Generic process for achieving the biochip

Several designs had been achieved by modifying shape, size and number of gap insulator in order to increase the number of cells trapped (Fig.11).

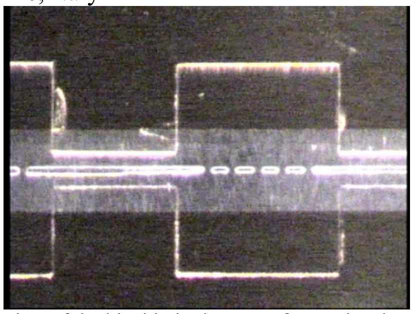
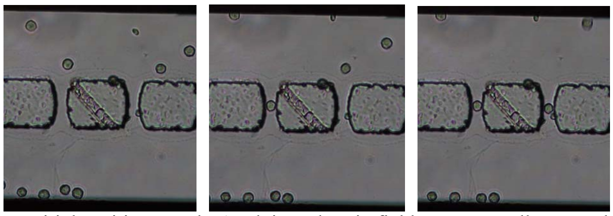


Figure 11: Top view of the biochip in the case of 5 gap insulator of 50µm each

VI Test on living cells

Tests with living cells (mouse cells DC3F) had been achieved for validating the catching principle. A conductive medium of 10mS.m^{-1} had been used.

As shown in figure 12, applying an AC electric field between the two electrodes induces DEP forces and moves cells and positioned them at the highest electric field value (near electrode or in the insulator gap). Here, using a frequency of 3Mhz with a voltage amplitude of 10V applied between two electrodes separated by 200µm, a positive F_{DEP} is obtained (fig.12.b), attracting cells towards electrode (depending on initial location of cells) or towards the focusing effect between two insulators (fig.12.c) as shown in modelling.



a. Initial position b. Applying Electric field c cells trapped

Figure 12 : Cell trapping by positive DEP forces

By using parallel and thin insulator this principle can be extended to generate high density trapping cells for further biological applications.

Conclusion

By mixing conductive media and insulator part in a microfluidic chip, we have shown that cells can be trapping using Dielectrophoresis forces. The process used for fabricating the Biochip is based on planar electrode associated to microchannel obtained by photolithography of SU8. By this way; a precise alignment between electrode and channel is obtained which is not possible simply with PDMS technique..

Due to focusing/defocusing effect, behaviour of the electric field is equivalent to thick electrode with amplification near insulator gap, where cells are trapped. 3D Modelling with a numerical software (Comsol ©) gives a good approach of the insulator shape influence on dielectrophoresis forces evolution and maxima/minima position.

Tests had been achieved for the design and trapping cells realised validating the study. The biochip is now ready for developing applications towards cells manipulating.

ACKNOWLEDGMENTS

The authors would like to thank Joseph Lautru for the help in ENS de Cachan clean room and Lionel Rousseau from ESIEE for his advices concerning the biochip fabrication.

REFERENCES

[1] H. A. Pohl, Dielectrophoresis, Cambridge University Press, London, 1978.

[2] N. Demierre, T. Braschler, P. Linderholm, U. Seger, H. Van Lintel, P. Renaud, *"Characterisation and optimisation of liquid electrodes for lateral dielectrophoresis" Lab On Chip, 2007, 7, pp 355-365*

[3] M. Frénéa-Robin, "Micromanipulation de particules par diélectrophorèse: application au rangement matriciel et au tri de cellules sur puce", Doctoral thesis, SATIE, ENS de Cachan, 17-12-2003.

[4] B. Le Pioufle, M. Frénéa, A. Tixier, « Biopuces pour le traitement de cellules vivantes : micromanipulation des cellules par voie électrique ou microfluidique », C.R. Physique, 5, (2004), pp 589-596.

[5] C.T. Ho, R.Z. Lin, W.Y. Chang, H.Y. Chang and C.H. Liu, « Rapid heterogenous liver-cell on-chip patterning via the enhanced field-induced deelectrophoresis trap" Lab on a Chip, 2006, Vol 6, pp 724-734.

[6] C.-F. Chou, J. O. Tegenfeldt, O. Bakajin, S. S. Chan, E. C. Cox, N. Darnton, T. Duke, and R. H. Austin. *"Electrodeless dielectroporesis of single- and double-stranded DNA", Journal of Biophysics, 83, pages 2170–2179, 2002.*

[7] G.Mottet, J. Villemejane, J.P. Lefevre, B. Le Pioufle, « A complet process to make a lab on a chip with complex fluidics and thick electrodes for biological applications" Microfluidique 2008, December 2008

[8] H. Sato, H. Matsumura, S. Keino1 and S. Shoji, "An all SU-8 microfluidic chip with built-in 3D fine microstructures » , J. Micromech. Microeng, 16 (2006) pp 2318-2322.

[9] O. Francais, M.C. Jullien, L. Rousseau, P. Poulichet, S. Desportes, A. Chouai, et al., "An active chaotic micromixer integrating thermal actuation associating PDMS and silicon microtechnology", DTIP 2006, MEMS & MOEMS, Stresa, Italy, 26-28 April 2006

 ISBN: 978-2-35500-009-6