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Aggregation of latex microparticles: simulation and experimental verification

Aglomeración de micropartículas de látex: simulación y verificación experimental

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Abstract

Manipulation of micrometric objects at the single level is one of the most important research fields because these techniques can be applied to handle biological material. The objective of this paper consists of presenting a microsystem designed for particle microhandling. The operating principle of the device hinges upon dielectrophoresis, which is the lateral motion of electrical neutral matter under the influence of non-uniform electric fields. In practice, the device was made on a silicon substrate onto which interdigitated castellated microelectrodes made of platinum were patterned by lift-off. Moreover, the microchamber walls were patterned in a photocurable resin which allows a constant sample volume during the experiments. Besides this, the chip was tested with polystyrene microspheres of 4.2 μm in diameter and some results of common dielectrophoresis and particle clustering are also presented. Microparticle aggregation patterns are consistent with the electric field profile calculated by the finite element method over the electrode surface.

----- *Keywords:* dielectrophoresis, microparticle clustering, microsystems, finite element method

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Resumen

La manipulación de objetos micrométricos a nivel individual es uno de los campos de investigación mas relevantes, ya que estas técnicas se pueden aplicar al manejo de material biológico. El objetivo de este artículo es el de presentar un microsistema diseñado para la micromanipulación de partículas. El principio de funcionamiento de este dispositivo es la dielectroforésis, la cual consiste en el movimiento lateral de material eléctricamente neutro bajo la influencia de campos eléctricos no uniformes. En la práctica, el dispositivo se fabricó sobre un substrato de silicio en el cual se depositaron microelectrodos interdigitados hechos de platino. Además, las paredes de la microcámara fueron fabricadas con una resina fotocurable, lo cual permite un volumen constante de la muestra bajo estudio durante la experimentación. Igualmente, el circuito integrado se probó con microesferas de poliestireno de 4,2μm de diámetro. En este trabajo se presentan algunos resultados de dielectroforésis común y aglomerado de partículas. El patrón de los microagregados es consistente con la distribución del campo eléctrico sobre los electrodos, el cual se calculó con el método de los elementos finitos.

----- *Palabras clave:* dielectroforésis, agregado de micropartículas, microsistemas, método de los elementos finitos

Introduction

Progress in biological and medical research owes much to investigations at the cellular and molecular levels. All the information about an organism, from bacteria and algae to higher plants, animals and humans is stored in single cells. This explains the growing research interest of medicine, pharmacology, biotechnology and food industry in new methods for handling cells, characterising cells by forming hybrids by fusion of selected cells and their cultivation, single or in groups, under optical and electronic observation [1 - 3]. In conventional biotechnological processes, cells are often treated as a mass in the form of suspensions in a medium. However, as cells have dimensions in the order of micrometers and they are generally fragile, conventional mechanical tools are too large and stiff to carry out an efficient cell handling [4].

A great amount of effort has been invested to develop new bioparticle-microhandling microtools, which involve the manufacture of devices whose size roughly matches the one of bioparticles, normally between 1 to 100 μm or less if viruses, bacteria or DNA macromolecules must be handled [5 - 7]. Nowadays, these dimensions and resolution levels may be achieved by using microsystem technologies. The advantages of miniaturisation of such microtools include reduction of the required sample volume and test times, cost reduction by mass production, the possibility of integrating multiple analytical functions in the same chip, as well as the minimisation of problems such as heating of the solution as a result of the voltages applied.

Our proposal is a microdevice dependent on electric-field mediated forces, which is fabricated by employing microsystem related to techniques such as silicon micromachining. Some aspects referring to the physical phenomenon, the microstructure modelling and simulation, the technological approach used to manufacture the device as well as a few relevant results obtained with polystyrene microbeads are presented in the sequel.

Methods and materials

Basic concepts

When an uncharged body is placed in a non-uniform electric field, it induces electrical charges upon the particle surface. This charge distribution will have equal quantities of positive and negative electric charges. The electric field will cause an alignment action on the dipoles with regard to itself. As the field is non-uniform, one dipole end will be in a weaker region than the other, which will originate a net force acting on all permanent or induced dipoles and cause them to be constrained to move towards or away from the region of highest field density.

Such a force imparted on uncharged particles as a result of polarisation induced by non-uniform electric fields is termed dielectrophoresis (DEP) [8] which may be towards (positive DEP) or away (negative DEP) from the electric field maximum, depending on the electrical properties of the particles and suspending medium respectively [9]

For a non-ideal insulating spherical particle of radius *r* the time-averaged dielectrophoretic force is given by [10]:

$$\mathbf{F_{DEP}} = 2\pi\varepsilon_0\varepsilon_m r^3 \mathrm{Re}[\mathrm{F_{CM}}]\nabla|\mathbf{E}_{rms}|^2, \qquad (1)$$

where ε_0 = 8.854x10^{-12} (Farad m^{-1}) is the free-space permittivity, ε_m is the effective medium permittivity, ∇ is the gradient operator, $\mathbf{E}_{rms}$ is the root-mean-square value of the electric field strength, and Re[$\mathrm{F_{CM}}$] denotes the real part of the Clausius-Mossotti factor defined by:

$$\mathrm{F_{CM}} = \frac{\left(\varepsilon_p^* - \varepsilon_m^*\right)}{\left(\varepsilon_p^* + 2\,\varepsilon_m^*\right)}, \qquad (2)$$

where the subscripts *p* and *m* stands for particle and medium, and ε* is the complex dielectric permittivity given by:

$$\varepsilon^* = \varepsilon_0\varepsilon - j\,\sigma/\omega, \qquad (3)$$

being ε the relative effective permittivity, σ the effective conductivity, and ω the angular frequency of the applied field.

From equation (1) it can be seen that $\mathbf{F}_{DEP}$ depends on the particle size, as well as on the magnitude of the electric field which is related to the electric potential applied. The gradient operator stands for the spatial non-uniformity of the electric field applied, which is a geometrical factor depending on the electrode layout. Equation (1) assumes that the electric field is harmonic in the temporal domain, that the field amplitude is inhomogeneous in the spatial domain and that it oscillates with a homogeneous phase of the space, which is valid for the biphasic electrode microarrays used in this work.

Equations (1), (2) and (3) also reveal that the magnitude and polarity of $\mathbf{F}_{DEP}$ depends in quite a complicated manner on the frequency of the field applied and on the relative values of the conductivities and permittivities of the particle and the surrounding medium.

Thus, if the particle polarisability exceeds that of the surrounding medium the arrangement of the induced charges produces a dipole moment with the same direction as the applied field, inducing p-DEP. Nevertheless, if the medium polarisability exceeds that of the particle the induced dipole moment is opposite to the field, originating n-DEP.

Electric field simulation

In view of the critical role played by field inhomogeneities in DEP, suggested by the ruling equations, calculating the field distribution is always of interest and insight. Thus, numerical calculation by means of the Finite Element Method (FEM) was extensively utilised to gain an insight about the electric field profiles over diverse electrode shapes.

Figure 1 depicts the interdigitated castellated microelectrode layout, which is one of the most popular electrode designs when particle collection or spatial separation at well-established locations are required.

To assess and visualise the electric field distribution, the 3-D model shown in figure 2 was studied by means of the commercial program ANSYS (Swanson Analysis Systems, Inc.), which is a general purpose software based on the FEM method to solve physical problems [11].

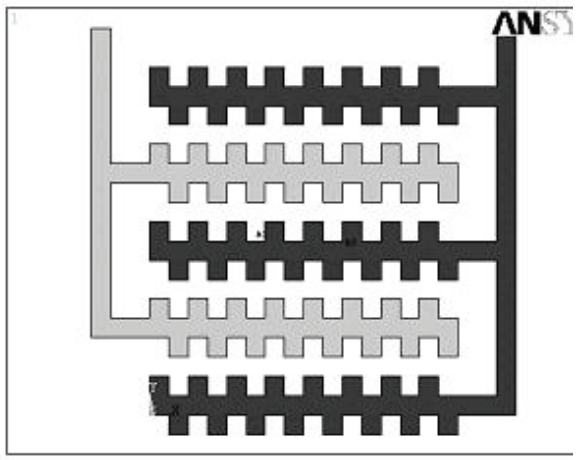

Figure 1 Shifted interdigitated castellated microelectrode pattern. Castellations of electrodes of one polarity are geometrically displaced with respect to the other ones

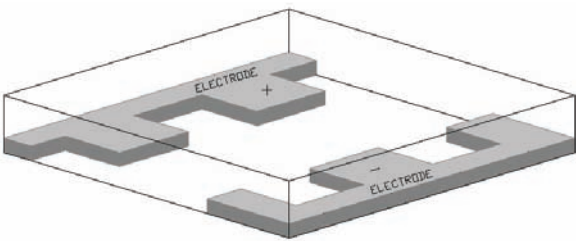

Figure 2 3-D model. Gray areas represent the electrode surfaces where the driving voltage is applied. The castellation size and medium height is 50 μm

Figure 3 represents the electric field distribution that corresponds to a pseudo-topographic colour-coded surface of the field. It can be seen that points located near to the electrode tips will experience the highest electric field strength, whilst this strength diminishes as the observation point moves towards the central part of the interelectrodic gap, towards the bays or onto the metallic surface of the electrodes.

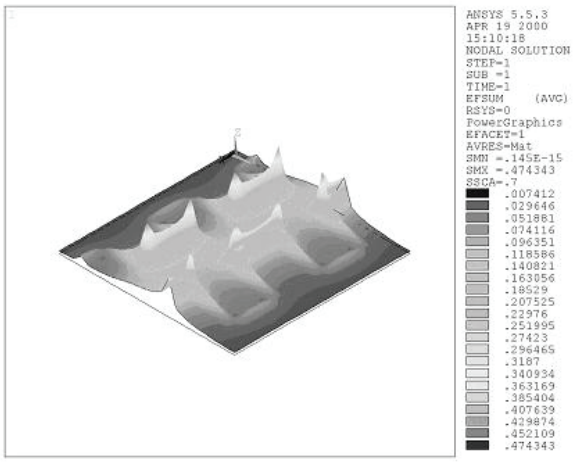

Figure 3 Pseudo-topographic profile of the electric field distribution (V μm^{-1}) in a plane located at 2 μm over the electrode surface, having applied a diference potential of ±5 V

As a result, particles more polarisable than the medium will be collected at the electrode tips, probably forming pearl-chains. On the other hand, particles less polarisable than the suspending medium will be repelled from the field maxima, clustering at the bay zones or on the electrodes.

Microsystem design and fabrication

When designing microsystems intended for bioparticle microhandling based on dielectrophoretic phenomena, one must bear in mind that the typical read-out of this technique is mainly performed with optical tools (microscopes, image analysis, etc.) rather than with electrical apparatus. Also, effort must be made to form a true microchamber by patterning the walls of a cavity with a known volume, i.e. a micropool should be fabricated to guarantee a constant volume of the suspending medium over the electrodes, avoiding possible experimental fluctuations due to this item. Taking into account these considerations, a whole microsystem was designed and fabricated as described below.

Proposed design

Figure 4 depicts a cross-section view of the proposed device, which includes a substrate in which electrodes are grown. Moreover, this substrate can be attacked to shape the fluid flow ports, employing silicon micromachining techniques. Holes patterned by this technique will serve to bring the suspending medium onto the electrode surface and, once the desired measurements have been taken, to carry away the mixture for further analysis if necessary. They can also be used to permanently recirculate the sample in order to refresh the suspending medium, bringing new specimens over the active electrode test area, which is advantageous when working with biological objects. Additionally, silicon or photoresin walls limit the microchamber working area where the microparticle solution is contained. Finally, a cover slide is placed on the top of the structure to close the cavity.

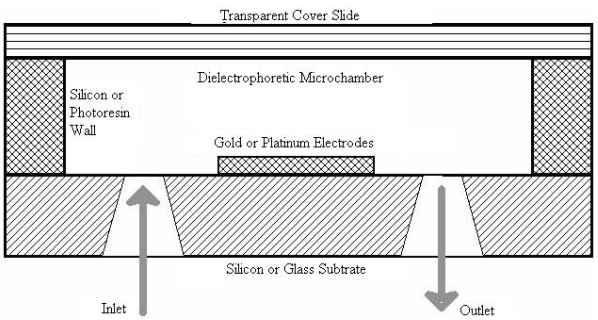

Figure 4 Cross-section view of the proposed microdevice

From the technological point of view, a 'mixed' approach was followed in order to manufacture the proposed microdevice. This option consists of growing the electrodes on a silicon substrate via standard photolithography techniques, then shaping the cavity walls by means of a photocured process initially conceived at the Microelectronic National Centre (CNM) in Barcelona, Spain, for packaging and rapid prototyping of differential silicon pressure microsensors and flow meters [12]

Fabrication process

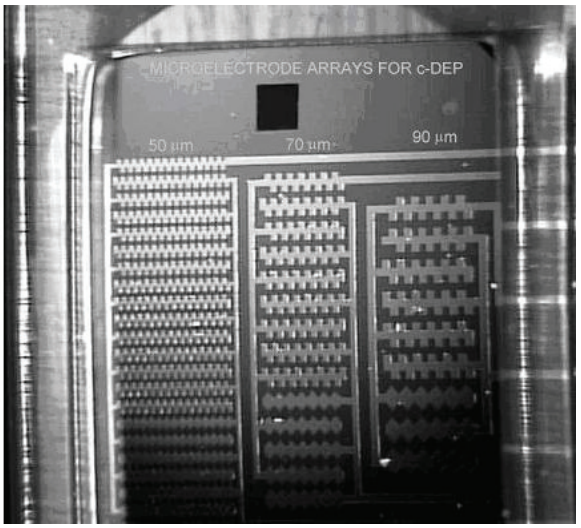

Figure 5 Partial view of the developed microsystem. Typical dimensions of the interdigitated castellated electrodes (from left to right) are 50, 70 and 90 µm, respectively. Polydimethylsiloxane, PDMS, walls and the inlet hole micromachined by tetramethylammonium hydroxide, TMAH, can also be seen

The technological process at the wafer level can be roughly divided into three stages. The first one is the platinum microelectrode patterning in which metal electrodes are defined onto a silicon wafer of 300 µm thickness by means of lift-off. After that, the wafer is drilled by silicon micromachining in order to shape the inlet and outlet holes. The wafer-level processing ends up with the photolithographic structuring of a UV-curable polymer (polydimethylsiloxane, PDMS) onto the component side of the wafer, to cast the microchamber walls. A partial view of the resulting microdevice is shown in figure 5.

Microsystem assembly

At this point of the fabrication process, the wafer was ready to be diced. Each die was glued onto a PCB (Print Circuit Board) especially designed for this purpose. The PCB was conveniently drilled to allow the assembly of the fluidic interface

(Teflon® tubes appropriately attached to the micromachined holes). Lastly, wire bonding will be produced in order to complete the electrical interface.

Figure 6 shows a photograph of a microsystem for bioparticle microhandling assembled as described in the preceding paragraph, with only the wire-bonding step remaining to be performed.

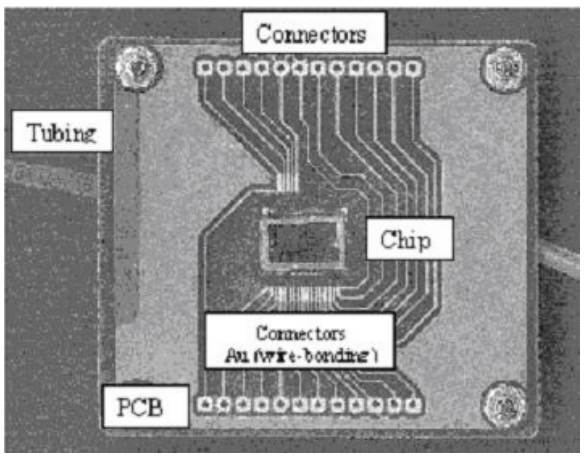

Figure 6 Photograph of the microsystem mounted on a PCB (5.5 cm in width and length) with wire-tracks metallised in gold to facilitate the chip wire-bonding procedure

Results and discussion

In the experimental stage, polystyrene microspheres of 4.2 and 15 μm in diameter (5% of solids in water, supplied by Molecular Probes Inc., Eugene, Oregon, USA) were used as test particles. Particles were resuspended in distilled water at a ratio of 1 to 100 (each particle size) with respect to the original concentration. The liquid conductivity, $\sigma_m = 2x10^{-4}$ S m^{-1} at 28°C, was measured by means of a Corning® 441 conductivity meter. The suspending medium was micropipetted onto the electrode active area, which is limited by the photoresin walls, by means of an Eppendorf® micropipette dispenser with variable volume between 10 and 100 μL.

Before the liquid was used, and being aware of the manufacturer's suggestion, the application of ultrasound between 3 and 5 minutes was routinely done. It helps to reduce particle aggregation due to the storage conditions keeping them in the monodisperse state. Once the solution had been dropped onto the chip and before the driving voltage was turned on, a waiting time of around 30 seconds was respected to allow the sample stabilisation while particles lost the kinetic energy gained during the fall.

Common dielectrophoresis

Results described below were obtained by testing interdigitated castellated both classical and shifted microelectrodes. These experiments illustrate the occurrence of both n-DEP and p-DEP, opening the possibility for using these arrays for particle separation. A signal generator system with amplitude variable between 0 and 10 V and a frequency range from 0 to 20 MHz was employed.

Having dropped the particle suspension into the micropool, electrodes of 70 μm were energised with a sinusoidal signal of 7 V in amplitude and 10 MHz. After a few minutes, particles were concentrated forming clusters located at the electrode bay regions of both classical and shifted interdigitated castellated arrays, i.e. particles were driven towards and agglomerated at regions corresponding to the electric field minima, as predicted by simulations of the electric field previously described. The aforementioned behaviour, i.e. particles of 4.2 μm in diameter affected by n-DEP, was observed till a frequency of approximately 1.2 MHz as illustrated in figure 7.

Figure 7 shows a whole view of the microelectrode array, in which clusters of particles (bright points) can be easily seen at the electrode bays, the electric field minima, of both classical and shifted interdigitated castellated microelectrodes.

When the frequency of the applied signal is decreased, maintaining unaltered the voltage amplitude, particle motion towards the electrode corners is observed. In other words, the particle clusters previously described are now attracted towards the highest electric field regions, veri-

fying the p-DEP phenomenon on the microelectrode array of 70 µm. This behaviour is evident for instance at 100 kHz.

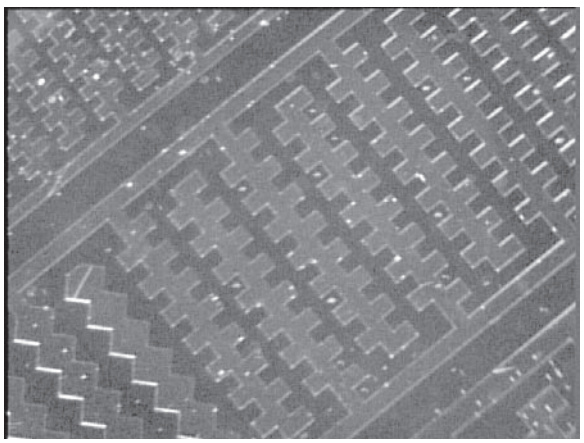

Figure 7 Clusters of polystyrene microbeads of 4.2 µm in diameter diluted in de-ionised water (σ_m = 2 µS cm^{-1}) in a ratio of 1 to 100, affected by n-DEP. A sinusoidal signal of 7 V amplitude and 1.3 MHz in frequency was applied to a microelectrode array of 70 µm

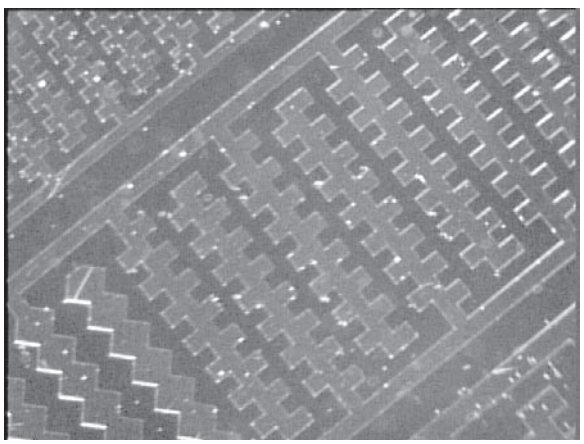

Figure 8 Clusters of polystyrene microbeads of 4.2 µm in diameter diluted in de-ionised water (σ_m = 2 µS cm^{-1}) in a ratio of 1 to 100, affected by p-DEP. A sinusoidal signal of 7 V amplitude and 100 kHz in frequency was applied to a microelectrode array of 70 µm

Interestingly, particles remain attached to the electrode corners till the frequency is risen again to a value in which n-DEP can stably occur, i.e. if frequency is increased, particles are repelled towards their original positions at the electrode bays. In other words, the occurrence of n-DEP and p-DEP can be successively achieved by simply changing and selecting the right values for the applied frequency signal. Figure 8, in which the observation area is the same as in figure 7, proves the occurrence of p-DEP in which particles are attached to the corners of both classical and shifted microstructures.

When comparing classical and shifted castellated electrode layouts in practice, see figures 7 and 8, it can be seen that as a consequence of the geometrical castellation displacement, particles also collect at displaced allocations. However, in this case more experimental work is required to prove that in a mixture of particles of diverse electrical properties, affected by the same frequency, they will collect at different places over the electrode geometry, following the electric field distribution dictated by the computer analysis.

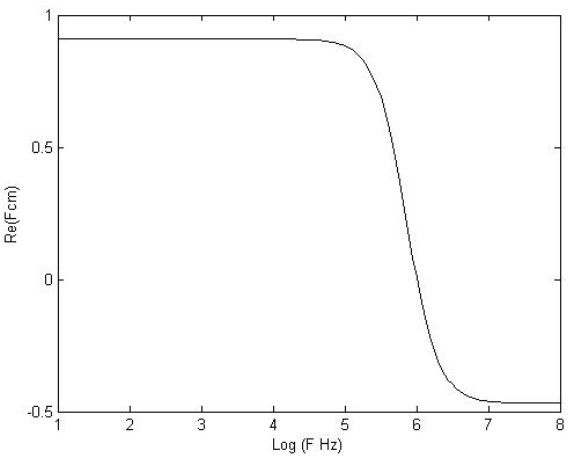

Figure 9 Real part of the Clausius-Mossotti factor for a polystyrene microsphere. The crossover frequency, f_c, is 1 MHz

As previously described, frequency variations produce particle motion towards well-defined regions on the microelectrode array. These results can be explained according to the homogeneous particle model described by equation (2). For the employed experimental values, i.e. σ_m =

$2x10^{-4}$ S m^{-1}, $\varepsilon_m = 80$, $\varepsilon_p = 3.5$, and considering $\sigma_p = 6.2$ mS m^{-1}, the crossover frequency, f_c, is 1 MHz, as illustrated in figure 9. It means that for frequencies above f_c, particles will experience n-DEP while for frequencies below f_c, the opposite is true, i.e. such particles will be attracted towards the electric field regions of maximum intensity.

In view of the experimental results one can say that the occurrence of common dielectrophoresis in polystyrene microbeads was successfully experimentally verified. From the point of view of frequency changes, the homogeneous particle model and its related Clausius-Mossotti factor explains coherently the observed particle behaviour. In other words, particle behaviour is consistent with that described by the particle model corresponding to an homogeneous sphere.

Microparticle clustering

Figure 10, a negative image of interdigitated castellated microelectrodes, shows the occurrence of n-DEP in a mixture of 4.2 and 15 µm particles, verified in the microelectrode array of 70 µm. Having dropped the mixture into the micropool, the electrodes were energised with a sinusoidal signal of 10 V in amplitude and 1.5 MHz.

Smaller particles were concentrated at the bay regions, forming clusters just at the same locations of the electric field minima on the interdigitated castellated arrays, as indicated by the electric field simulation carried out for this structure, see figure 11.

Particles of 15 µm in diameter (black dots on figure 10) were initially driven towards the centre of the electrode surface, which is another region of minimum electric field strength. Once settled there, these particles remained attached to the electrode surface. This can not be explained by the dielectrophoretic force or by the gravity effects because these forces scale proportionally to the particle volume and no significant differences can be found between them. This behaviour could be explained by the influence of unconsidered contributions to the force acting on particles suspended in a liquid, such as higher polar moments (quadrupolar, octupolar, etc) or hydrodynamic viscous drag forces due to electrohydrodynamic (EHD) effects, i.e. fluid motion as a result of thermally induced medium inhomogeneities. The review of Ramos and co-workers worked out an order-of-magnitude estimation of forces acting in DEP microelectrodes, concluding that for the low-frequency range the fluid motion moves particles away from the electrode edge and into well defined regions on top of the electrodes [13].

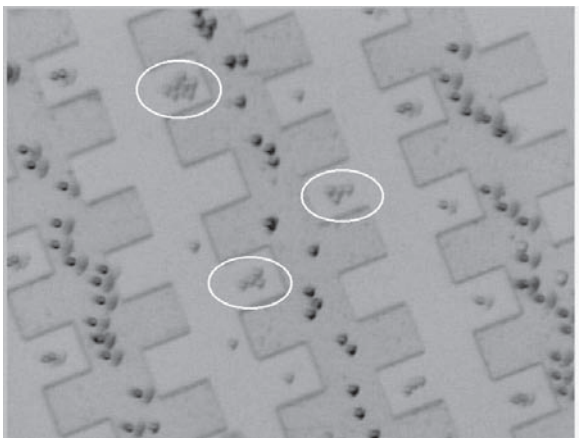

Figure 10 Clusters of polystyrene microbeads 4.2 µm in diameter diluted in de-ionised water (σ_m = 2 µS cm^{-1}) in a ratio of 1 to 100, can clearly be seen to be affected by n-DEP and collecting in triangular shapes at every electrode bay (yellow ovals). A sinusoidal signal of 10 V amplitude and 1.5 MHz in frequency was applied

As expected, electric field calculations and plots are useful to predict the particle distribution, as a result of the dielectrophoretic force, over the microelectrodes. For the case of interdigitated castellated shifted electrodes, figures 10 and 11 shows that latex microbeads under the influence of n-DEP will agglomerate at zones where the field intensity is minimum. Furthermore, particles of 4.2 µm can be seen to concentrate following a pattern dictated by the electric field minima allocation, resembling triangular shapes.

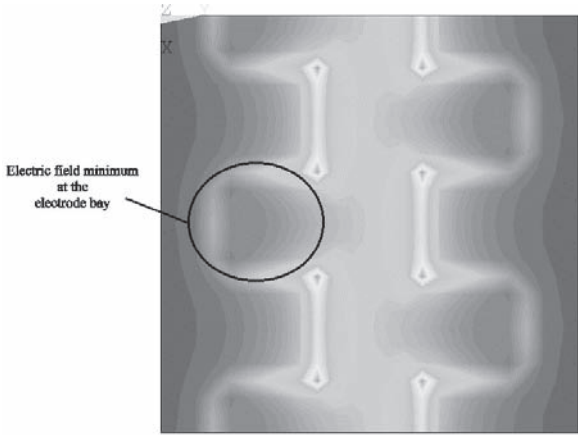

Figure 11 A top view of the electric field distribution shown in figure 3 is reproduced to illustrate that particles concentrate resembling triangular shapes, following the pattern dictated by the electric field minima locations

At this point, an additional force (for example hydrodynamic force) could be imposed to drag away the desired fraction of particles, collecting them for further assay. Additionally, microparticle aggregation can be used to manufacture biosensors in which colloidal particles have to be patterned in a presice manner [14, 15]. On the other hand, the ability to control the shape and geometry of microparticle clustering has a great potential to generate moulds or matrixes which could be used to culture and growth artificial tissues with the desired form.

Conclusions

A whole microsystem was designed and fabricated by means of microfabrication techniques. This microsystem hinges on the dielectrophoretic phenomena (p-DEP and n-DEP) and may be employed to perform diverse bioelectronic experiments such as separation and motion of microparticles.

The microstructure includes a silicon substrate in which electrodes of platinum were grown by the lift-off technique. Moreover, the substrate was drilled to shape the inlet and outlet fluid ports employing bulk silicon micromachining techniques. Holes patterned by this technique will serve to bring the suspending medium into the electrode surface and, once the desired measurements have been performed, to carry away the mixture for further analysis if necessary. Microcavity walls were moulded by means of a photopatternable resin (PDMS).

In view of the experimental results, one can say that the occurrence of common dielectrophoresis in polystyrene microbeads was successfully verified experimentally. From the point of view of frequency changes, the homogeneous particle model and its related Clausius-Mossotti factor explain coherently the particle behaviour observed. In other words, particle behaviour is consistent with that described by the particle model corresponding to a homogeneous sphere.

Additionally, particle distribution as a result of the electric field profile was successfully experimentally verified. The particle behaviour is consistent with that expected from the field distribution, as well as with the Clausius-Mossotti factor predictions. This fact validates the numerical results of the aforesaid electric field profiles, obtained by FEM analysis.

The reported experiments indicate the convenience of this kind of microdevices for handling microscopic objects, opening up the possibility of using these microstructures with other kind of particles such as biological cells.

Acknowledgements

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