Precise Positioning of Particles in Microwells within Horizontal DEP Cages

M. Lombardini*, M. Bocchi**, L. Giulianelli* and R. Guerrieri*

*ARCES University of Bologna, Viale Pepoli 3/2, 40123 Bologna, Italy. mlombardini@arces.unibo.it
**MindSeeds Laboratories, Bologna, Italy, massimo.bocchi@mindseedslabs.com

ABSTRACT

An efficient method to trap and analyze particles avoiding excessive electric field strength is to use negative dielectrophoretic cages, where particles find stability in the electric field minimum. We present a novel structure where particles are trapped and forced to interact within microwells opened at top and bottom sides by creating a cylindrical negative dielectrophoretic cage. This approach provides a controlled alignment of the particles along a predefined axis. An array of microwells is built by drilling through holes with a diameter of 150 µm on a flexible printed circuit board with two Cu/Au layers separated by one polyimide layer, providing a cheap and disposable device. Levitation of one or two polystyrene beads with a diameter of 25 µm in DI-water was experimented confirming the positioning predicted by physical simulations. Vertical position of the particles as a function of the amplitude of polarization signals was extracted from simulations and confirmed by experimental results.

Keywords: dielectrophoresis, microwell, microbeads, flexible PCB.

1 INTRODUCTION

The analysis and manipulation of one or few biological cells or artificial particles in the micron size range is a fundamental requirement in various life science applications such as isolation of rare cells [1,2] or high-efficiency transforming of microorganisms [3,4]. The use of dielectrophoresis for cell handling has received substantial attention as low voltages can produce significant and contactless forces on cells. With the appropriate electrode geometry it is possible to create cages for single particle trapping [1,5]. Additionally, characterization and manipulation of single cells or small cell aggregates can be achieved by adding specific electrodes to the microsystem for impedance sensing [6], electroporation [4] or electrofusion [3] applications. A strong advantage can be reached during experiments if the particles manipulated by the aforementioned electrodes are trapped and aligned along the axis where these electrodes are placed, where the efficiency of measurement or manipulation is generally maximum. The goal of this work is to deliver a structure that is able to stably trap particles aligned along the axis where, in a future implementation, additional electrodes will be fabricated.

Fig. 1: (a) 3D view of a microwell. Particles are trapped in the well by means of the DEP cage. (b) Top view of the well (xz-plane). Particles are trapped near the wall and aligned along the z axis of the well. (c) 3D isosurface plot of the modulus of the electric field in a microwell. Physical simulation gives the shape of the electric cage, h is the vertical distance between electrodes on different layers. (d) Top view of the well (xz-plane), where d is the diameter of the well. Electrodes on the same layers have a spacing s and a width w. From this view the cage shows its cylindrical shape.
2 STRUCTURE OF THE WELL

2.1 DEP theory

The structure that we present is a microwell whose 3D and 2D views are shown in Fig. 1a-d. The structure can be considered similar to a well of a standard microtiter plate, the main differences being the opened bottom side and the possibility to load, trap, analyze and release particles by means of negative dielectrophoresis (nDEP) forces. Depending on the frequency of the applied electric field and on the dielectric properties of both the liquid buffer and the particle, DEP force can be positive (pDEP) or negative (nDEP). The general formula for the time-averaged DEP force in the presence of an alternating electric field is [5]:

$$F_{DEP} = 2\pi R^3 \varepsilon_f \cdot \text{Re}[f_{CM}(\omega)] \cdot \nabla \left| \varepsilon \right|^2$$  \hspace{1cm} (1)

where $R$ is the particle radius, $\varepsilon_f$ the fluid permittivity, and $\nabla \left| \varepsilon \right|^2$ is the gradient of the square modulus of the electric field. $f_{CM}(\omega)$ is the Clausius-Mossotti factor [5] and is a function of the angular frequency $\omega$ and is calculated for a uniform sphere as:

$$f_{CM}(\omega) = \frac{\varepsilon_p - \varepsilon_f}{\varepsilon_p + 2 \cdot \varepsilon_f}$$  \hspace{1cm} (2)

where $\varepsilon_p$ and $\varepsilon_f$ are the complex permittivity of the particle and the fluid that can be both expressed by $\varepsilon = \varepsilon - j\sigma / \omega$ with $\varepsilon$ the permittivity and $\sigma$ the conductivity of the fluid and the particle. The sign of $f_{CM}(\omega)$ affects the direction of the force. In pDEP conditions ($\varepsilon_p > \varepsilon_f$) particles find stability in regions where the electric field is maximum, while in nDEP conditions ($\varepsilon_p < \varepsilon_f$) the stability is in the electric field minimum point. Consequently, by choosing the appropriate dimensioning of the dielectric properties of the buffer with respect to the one of the particles, we can assure that particles will find stability in the electric field minimum rather than in the maximum. For example, nDEP conditions are obtained when using polystyrene beads suspended in water, whatever is the frequency of the exciting signals.

2.2 Prototype fabrication

We fabricated an array of active microwells (8 wells) using a standard flex PCB technology. Therefore we obtained an active microtiter plate that is cheap and disposable. Each microwell is made of a through hole (diameter $d=150 \mu m$) that connects two copper layers (thickness $t=29.2 \mu m$) separated by a polyimide layer (thickness $h=131.5 \mu m$). A nickel-gold electroless metallization was then applied to guarantee the biocompatibility of the materials. A couple of electrodes (width $w=200 \mu m$ and spacing $s=100 \mu m$) is structured on each metal layer. Consequently, a non-uniform electric field is generated by the resulting four electrodes situated along the microwell boundary.

The microwells are opened at the bottom side and can be filled by capillarity from an underlying microfluidic channel providing the suspension buffer. Particles are delivered manually or using a dispenser positioned at the top of the well. The opened top side is suitable for the recovery of the manipulated material using a micropipette.

The main goal of the structure is to position particles, by means of nDEP, in a precise region near the boundary of the well, controlling the alignment along a horizontal axis. Consequently, electrodes for impedance sensing, electroporation or electrofusion applications will be added to a future implementation of the structure providing an easy way to characterize and manipulate cells as the new set of electrodes will result to be in contact with the particles.

2.3 Shape of the DEP cage

Since we need to collect particles in a small region inside the well, it is important to characterize the electric field distribution of the cage.

The four electrodes are excited with sinusoidal signals with the same amplitude and frequency. By polarizing the electrodes, as shown in Fig. 1c, a nDEP cage is formed, whose 3D shape was predicted using a numerical FEM simulator (Fig. 1c,d).

This polarization generates a distribution of the modulus of the electric field which is symmetric respect the vertical and horizontal axis and, in case of nDEP, pushes the particles towards the electric field minimum. Exciting electrodes as in Fig. 1c produces an electric field between
the electrodes E1-E3 and E2-E4, along the y direction (vertical), and E1-E2 and E3-E4 along the x (horizontal) direction. Because of the absence of the electric field between electrodes E1-E4 and E2-E3, the central region of the well coincides with the electric field minimum.

Referring to a vertical cross sections of the structure (Fig. 2), it can be observed that the circular shape of the electric cage is obtained by symmetrically dimensioning the well. In fact, by choosing a 1:1 ratio between the geometrical parameters $h$ and $d$, the distance between the electrodes along vertical and horizontal directions results the same, leading to a symmetric distribution of the field, in a xy cross section of the well. Further, Fig. 1c and Fig. 1d show that the nDEP cage does not have a 3D cylindrical shape. In fact, the diameter of the circular shape of the cage in a vertical cross section of the structure increases proportionally with the z-distance of the cross section from the centre of the well. The further is the vertical cross section from the center and the bigger is the diameter of the nDEP cage. Consequently, the cage features two local minima close to the microwell boundaries as visible in Fig. 1c,d. These two local minima are aligned along the horizontal axis of the cage. When multiple particles are trapped they align horizontally into the cage.

### 2.4 Particle positioning within the well

Vertical position of the particle inside the cage ($Y_{STAB}$) depends on the gradient of the electric field and consequently on the amplitude of the exciting signals. It is calculated as the position where the total effects ($F_{TOT}$) of the forces acting on the particle is zero:

$$F_{TOT}(Y_{STAB}) = 0$$ (3)

Regarding our structure, the $Y_{STAB}$ is the position where the vertical component of the nDEP force ($F_{DEP}$) equates the buoyancy force ($F_B$), as the Brownian ($F_{BROW}$) motion for particles in the micron size range is negligible [5] and the drag force ($F_{DRAG}$) is null because the buffer and the particle can be both considered initially still. Consequently:

$$F_{DEP} = F_B + F_{BROW} + F_{DRAG} = F_B$$ (4)

Besides, the buoyancy force is expressed by:

$$F_B = \frac{4}{3} \pi R^3 g (\rho_f - \rho_p)$$ (5)

Where $\rho_f$ and $\rho_p$ are, respectively, the density of the fluid and of the particle and $g$ is the gravity acceleration.

To sum up, the $Y_{STAB}$ depends on the density and the electrical parameters of the particle and the buffer as well as on the distribution of the electric field within the well. Consequently, considering a specific particle suspended in a fluid, it is possible to change its vertical position within the well by varying the amplitude of the exciting signals. The higher is the amplitude of the signals and the higher is the vertical position of the particle within the well.

On the other hand, physical simulations reported the existence of a minimum amplitude ($V_{MIN}$) for the excitation signals that corresponds to a minimum height of the cage, under which it is no more possible to trap particles.

### 3 RESULTS

#### 3.1 Horizontal alignment of particles within the well

The particle used in the experiments and modeled for simulations is a polystyrene bead of 25 µm diameter, with a relative permittivity of 2.5 and a density of 1062 Kg/m³ [7].

Using a microscope (Nikon eclipse 80i), optical observation from the top of the microwell was performed. Polystyrene beads were suspended in water and delivered into the well by dispensing from the top after filling the well with the supernatant. The sinusoidal signals...
employed for electrical polarization were generated by a custom circuit. The frequency was set to 600 KHz and the amplitude at 6V.

Afterwards, the particles were stably collected in a defined region within the well. Fig. 3a shows a single bead that is trapped near the wall of the cavity where there is one of the two electric field minima (Fig. 1d). Fig. 3b,c show two beads trapped at once. In both the cases the particles are positioned along the horizontal axis of the cage but, in Fig. 3b, particles are trapped inside two different electric field minima and consequently result to be separated while, in Fig. 3c, particles are forced in contact as they are trapped by the same electric field minimum. The particles are randomly trapped inside one of the two minima. In a future implementation, it will be possible to force the interaction between particles, by adding dedicated electrodes that push the particles into the same minimum.

By subtracting this distance from the experimental distance the particles tend to get closer to the center. On the other side, experiments reported particles coordinates that were sometimes higher than the median level. This is a consequence of the difficulty in focusing exactly the center of gravity of the bead.

3.2 Vertical position of the particles

With this series of experiments we wanted to quantify the effect of the variation of the amplitude of the exciting signals on the height of the cage. The experimental data, compared to the physical simulation data, contribute to confirm the model used to describe the structure.

The amplitude of the signals applied to the electrodes was changed by steps of 1V in a range between 2V and 6V with a constant frequency of 600 KHz. Vertical position of the bead was measured by adjusting the focus of the microscope using a motorized stage and reading the absolute value of the vertical position of the stage.

The upper side of the electrodes placed on top layer was chosen as reference point to extract the vertical coordinate of the particles inside the hole. Knowing the build-up of the device, we can extract the vertical distance between this reference point and the center of the structure ($\Delta Y_{TOP-MID}$). By subtracting this distance from the experimental distance between the top of upper electrodes and the position of the particle ($\Delta Y_{TOP-PART}$), we extract the position of the particle relative to the middle of the well ($Y_{PART}$):

$$Y_{PART} = \Delta Y_{TOP-PART} - \Delta Y_{TOP-MID}$$

In Fig. 4 it is reported the average of $Y_{PART}$, whose standard deviation is mainly caused by the effect of the evaporation that systematically shifts the focus of the particles. Zero height corresponds to the vertical center of the structure. We can notice that simulation results position the beads under this level whatever is the amplitude of the signals and that, for higher values of the voltage amplitude, the particles tend to get closer to the center. On the other side, experiments reported particles coordinates that were sometimes higher than the median level. This is a consequence of the difficulty in focusing exactly the center of gravity of the bead.

4 CONCLUSION

We demonstrated the possibility to trap beads within open microwells and align them along an axis where additional electrodes for additional purposes such as cell detection by impedance measurement, electrofusion or electroporation can be placed. Consequently, being the particles aligned along a predefined axis, the efficiency of the aforementioned methods could be increased by integrating specific electrodes aligned along the same axis.

Measurements on the particle height confirmed the values expected from simulations, thus confirming the correctness of the model used for three-dimensional FEM simulations. The array of microwells was designed using materials that are biocompatible, as to be suitable for being used with cells. Ongoing and future work will be focused on the integration of additional electrodes for the manipulation of trapped cells.

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REFERENCES