Supplementary Material (ESI) for Lab on a Chip

Horizontal nDEP cages within open microwell arrays for precise positioning of cells and particles

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Supplementary information

Calculus of the trans-membrane potential

A relevant parameter describing this effect is the trans-membrane potential (TMP)[23,24] induced by an external field across the cell membrane. For a spherical cell in suspension the following equation holds:

$$TMP = 1{,}5ER\cos(\theta)\left|\frac{1}{1+i\varpi\tau}\right| \qquad (3)$$

where E is the value of the field in the fluid, R is the radius of the cell, ϑ is the polar angle measured with respect to the direction of the field, ω is the angular frequency of the external field and τ is given by:

$$\tau = RC_m(\rho_i + \rho_a / 2) \qquad (4)$$

where C_m is the capacitance of the membrane per unit area which we determine from other works[25] to be 15,6mF/m^2 while ρ_a and ρ_i are respectively the resistivity of the interior of the cell and of the fluid (typical values[23] are 1 Ωm).

Effect of geometrical parameters on the shape of the nDEP cage

The spacing between the electrodes *s* and the thickness of the electrodes *t* both affect the distribution of the electric field inside the well.
We simulated the structure varying the *s* parameter between 75µm and 12.5µm (see Fig. S5), whilst fixing h=d=150 µm, t=35 µm and w=230 µm.
Decreasing the *s* parameters causes two different effects: an overall increase in DEPy and generation of a relatively central minimum (negative DEPz) that pushes particles to the centre instead of to the well wall.
Hence the best choice for a strong cage is setting *s* as small as possible; on the contrary, decreasing *s* generates a DEP cage that could attract particles to the exact centre of the well. In Fig. S5, the force that leads particles to the minimum along the z axis (DEPz) proves, as expected, to be negative inside a region near the center ($0<z<30$ µm) when the *s* parameter dimension is smaller than 50 µm. Again, the strength of DEPz changes with the s parameter. Fig. S5 shows that for $0<z<30$ µm the trend of the force is to increase with *s*, while for $z>30$ µm the force decreases with *s*.
Taking all these effects into account, one may summarize that s=50 µm corresponds to a good tradeoff between intensity of the DEPy force and stable collection of particles near the well wall.
We accordingly simulated the structure varying the *t* parameter between 35µm and 4,375µm (Fig. S5c,d), choosing the same geometrical parameters as before and keeping s=50 µm. Fig. S5c shows how DEPy increases proportionally with *t*.
Likewise, Fig. S5d shows that t= 35 µm prevents DEPz from becoming negative in the central part of the well while for smaller values of *t* the cage is less efficient in leading particles to the wall. Consequently the best choice for the *t* parameter is as big as possible which in our case is fixed by technology at t=35 µm.

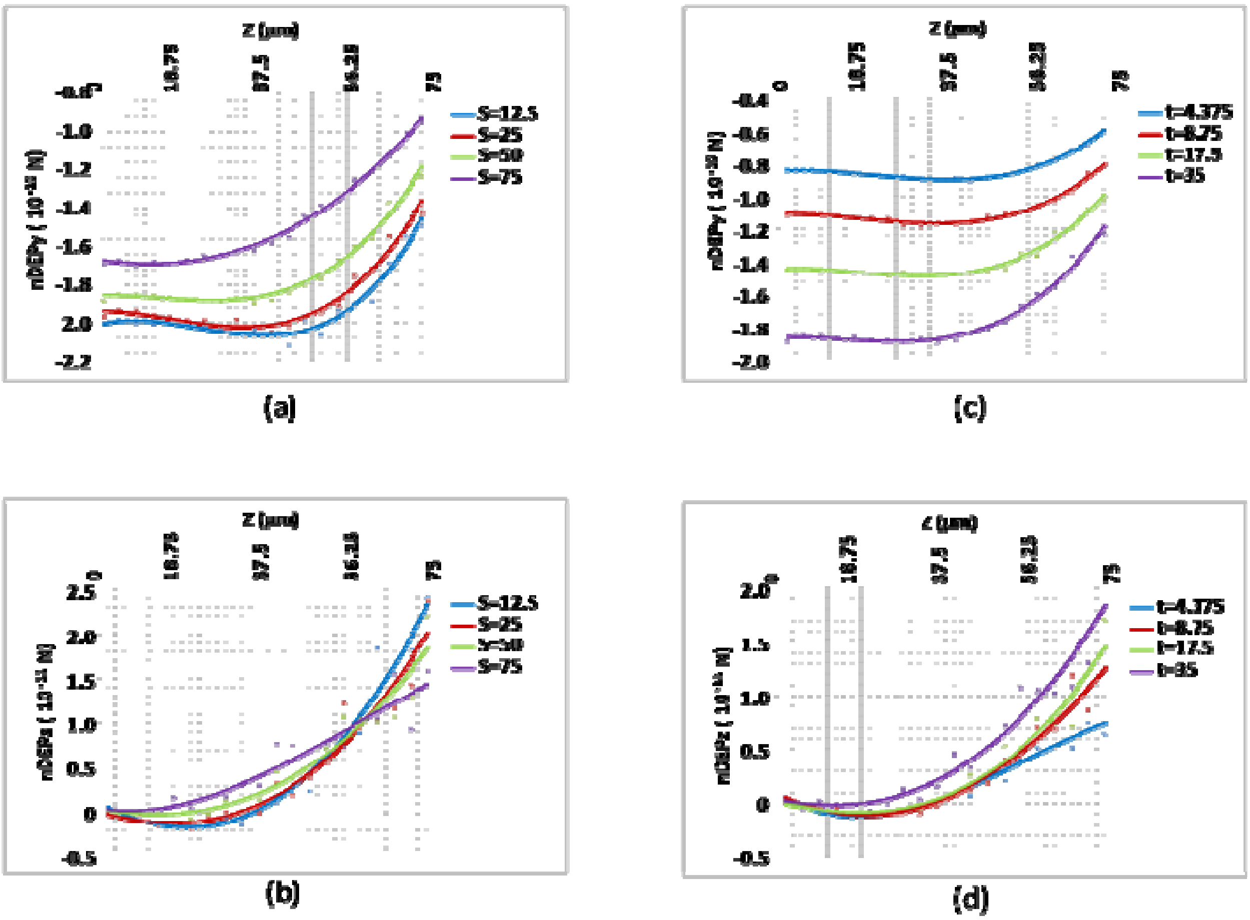

Fig. S5: Simulation of the positive octave of the 3D structure of a well with d=h=150 µm, w=200 µm. (a) DEPy and (c) DEPz along the z axis at x=0 µm and y=10 µm; t is fixed at 35 µm and s is varied between 12.5 µm and 75 µm. (b) DEPy and (d) DEPz along the z axis at x= 0 µm and y= 10 µm; s is fixed at 50 µm and t is varied between 4.375 µm and 35 µm.

Scaling the structure

Scaling the geometrical parameters of the microwell has an impact on the electric field cage. In order to maintain the same physical conditions in scaled structures, an appropriate scaling factor (α) should be applied to the electrical parameters and in particular to the voltages V applied. The buoyancy force on the particle suspended in a buffer F_B is proportional to the third power of the particle radius R:

$$F_B \propto R^3 \qquad (5)$$

F_{DEP} is proportional to R^3 as well as the gradient of the square modulus of the electric field ($\vec{\nabla}|E|^2$):

$$F_{DEP} \propto \frac{\Delta E^2}{\Delta x} R^3 \qquad (6)$$

To achieve particle trapping the vertical nDEP force (F_{DEP}) needs to equal F_B:

$$F_{DEP} = F_B \qquad (7)$$

Hence to maintain F_{DEP} proportional to R^3 when scaling the structure by a factor α it is necessary to scale V with a scaling factor of $\alpha\sqrt{\alpha}$:

$$F_{DEP} \propto \frac{\Delta E^2}{\Delta l} R^3 \propto \frac{V^2}{l^3} R^3 \propto \frac{(\alpha\sqrt{\alpha})^2}{\alpha^3} R^3 \propto R^3 \qquad (8)$$

Following this scaling rule we ensure that the particle's stable position along the BB' axis (y) has a relative distance (y_s=y/d) from the DD' axis that remains constant when scaling the structure by α. In other words, the maximum DEPy force remains constant when scaling the geometries by α and the DEP cage scales its size proportionally to α.
Table 1 contains a list of geometrical parameters and typical voltages applied. These values were obtained through physical simulations and were used as reference parameters in designing various scaled structures.
The signal amplitude was chosen for the basic prototype as the value that most firmly traps ø25µm polystyrene beads suspended in water. Hence the absolute vertical position y is derived as the coordinate where $F_{DEP} = F_B$. Table 1 shows how y is scaled proportionally to α, while the relative vertical position y_s is maintained constant.

Table 1: Geometrical parameters and voltages applied for prototypes with different scaling factors

Prototype	Symbol	Basic Prototype α =1	Scaled Prototype α =2	Scaled Prototype α =1/2	Scaling factor
Diameter	d	150 µm	300 µm	75 µm	α
Dielectric Thickness	h	150 µm	300 µm	75 µm	α
Electrodes width	w	230 µm	460 µm	115 µm	α
Electrodes Spacing	s	50 µm	100 µm	25 µm	α
Electrodes Thickness	t	35 µm	70 µm	17.5 µm	α
Signal Amplitude	V	8 V	38 V	38 V	$\alpha\sqrt{\alpha}$
Absolute vertical position	y	-2,2 µm	-4,4 µm	-1,1 µm	α
Relative vertical position	ys	14 %	14 %	14 %	1

The effect of misalignment between electrodes and the center of the cavity generated during the fabrication process.

During the fabrication process the drilling of the hole may be affected by a shift in the x and z directions.
The structure is immune to changes along the z direction caused by a misalignment mis_z if the w parameter is large enough to permit electrodes to surround the wall of the well:

$$w > d + 2mis_z \qquad (9)$$

For example for a radius d= 150 µm, if we choose w= 230 µm, the structure is immune to z-misalignments within ±40 µm.
By contrast, misalignment mis_x along x must be guarded against so as to avoid undesired behavior by the structure. The maximum mis_x that we consider as an admissible error during the fabrication process is:

$$mis_x < (d - s)/2 \qquad (10)$$

Accordingly, we simulated the structure while shifting the DD' axis compared to the AA' axis along the x direction. The results for mis_x=35µm are shown in Fig. S6a where one notes that particles will not be aligned along the z axis but along a curved line. Fig. S6b shows how the curvature of the alignment line increases with mis_x. The higher the voltage, the more pronounced the bending of the alignment line. In Fig. S6c we observe four particles aligned along a curved axis as predicted by physical simulations.

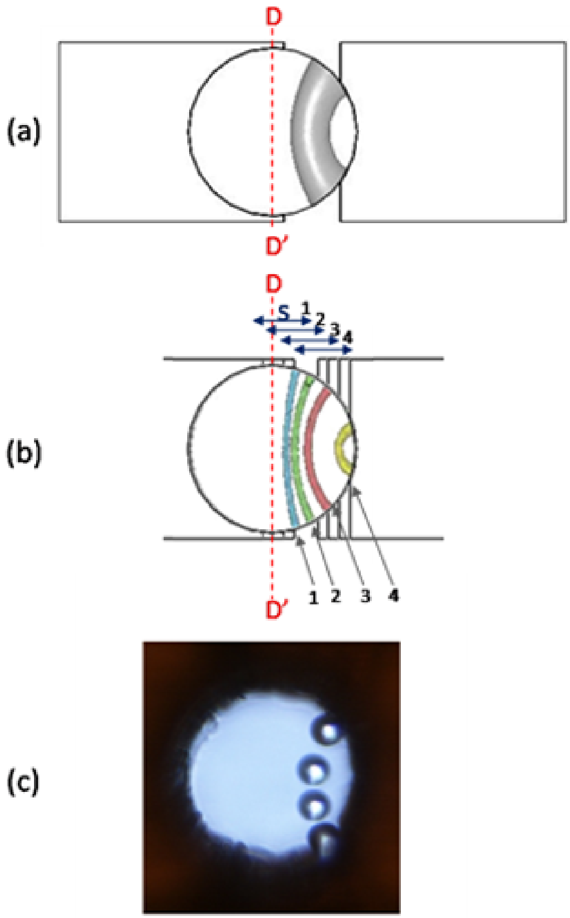

Fig. S6: (a) Cage shape in a well (ø150 µm) affected by x misalignment. Top view while considering a 35 µm misalignment (b) Different shapes of the cage considering different values of misalignments. In case 1 mis=15 µm; in case 2 mis= 25 µm; in case 3 mis= 35 µm; in case 4 mis= 45 µm. (c) Results of an experiment where the fabrication process generated a misalignment of 35 µm in a well with ø150 µm. Polystyrene beads (ø25 µm) were used in water.

Reference

25 Y. Huang, x.B. Wang, F. Becker and P. Gascoyne, *Biophysical Journal*, 1997,**73**,1118-1129.