Quasi-three dimensional “dot” electrode micro-array for quantifying dielectrophoretic forces on concentrated biological particles

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Induced particle motion by dielectrophoresis has been assessed on a microelectrode system consisting of two facing planes, one with circular apertures which we have termed “dots”. Dot diameters ranging between 150µm - 500µm have been used to show positive and negative dielectrophoresis, and have shown differences in particle velocities which is also influenced by the concentration of the suspended particles in the system. Simulations of the electric field morphology within the system showed a characteristic dome-shape distribution over the centre of the dot for smaller diameters which becomes distorted as the diameter increases. The design of the microelectrode geometry has effective spatial electric field penetrating capabilities over planar dielectrophoretic electrode geometries, with the potential for novel on chip processes such as particle characterisation which is described in this paper.

**Keywords:** Dielectrophoresis/ Micro-array/ Dot/ electric field/ 3D electrodes/ Particle characterisation.
1.0 Introduction

Dielectrophoresis (DEP) is the manipulation of polarisable particles in low conductive media by non-uniform electric fields and is gaining popularity for commercial use within the pharmaceutical, defence and healthcare industries. Typically used at the micrometer scale, the technique offers many potential applications to laboratory-on-a-chip systems, such as particle separation [1-3], bio-particle characterisation [4-6] and micro-fluidic pre-concentration [7, 8].

DEP is driven by electric field non-uniformity, and is therefore dependent on the electrode geometry used. Some important electrode geometries which have been used to manipulate nano- and micro- particles include pin-type electrodes [9, 10], interdigitated electrodes [11-13], castellated inter-digitated electrodes [14-17], polynomial electrodes [18, 19] and the isomotive electrode [20, 21]. A common feature amongst these geometries is the planar arrangement of the electrodes on a single substrate. In 1997, Green et al constructed a large area multi-layered electrode structure for effective dielectrophoretic fractionation of whole blood cells into specific components [22]. Suehiro and Pethig demonstrated the precise movement and positioning of biological cells using a three-dimensional grid electrode system, using positive and negative dielectrophoresis [23]. Micro-systems were soon constructed using the three dimensional aspect of electrode arrangements, for the sorting, handling and caging of cells and particles [24, 25]. The common feature of these systems is the use of a second electrode layer. With the complex and predominately asymmetrical nature of the electric fields produced by these electrode geometries, measurements of the dielectrophoretic force on a particle can be difficult, with particle velocities varying in direction.
A polarisable particle suspended in a non-uniform ac electric field will become polarised setting the particle into motion. The lateral displacement of a particle is termed dielectrophoresis ($\vec{F}_{\text{DEP}}$) which is a vector quantity described by [26]. For a homogeneous spherical particle, the force is given by

$$\vec{F}_{\text{DEP}} = 2\pi r^3 \varepsilon_0 \varepsilon_m \text{Re}[K(\omega)] \nabla E^2 \quad (1)$$

where $\varepsilon_0$ is the permittivity of free space, $r$ is the particle radius, $\varepsilon_m$ is the permittivity of the suspending medium, $\nabla$ is the gradient operator Del, $E$ is the electric field and $\text{Re}[K(\omega)]$ is the real part of the Clausius-Mossotti factor given by equation (2), where $\omega$ is the angular frequency, $\varepsilon^*$ is the complex permittivity of the particle (p) and suspending medium (m).

$$\text{Re}[K(\omega)] = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \quad (2)$$

The direction of particle motion is dependent on the relative polarisability of particle and suspending medium at an applied frequency, which for DEP is dictated by the real part of the Clausius-Mossotti factor, a frequency dependent complex value [27]. Particles are either attracted to the electrode edges where the field gradient is at its maximum or repelled from the electrode edges where the field gradient is at its minimum. Attraction to high electric fields is known as positive dielectrophoresis and repulsion from high electric fields is known as negative dielectrophoresis. When the net force on the particle is zero, a particle exhibits no movement. This situation arises
at the particles crossover frequency which has been used as a parameter to obtain the membrane capacitance and conductance of single shell particles [28, 29].

In this paper we present what we refer to as the dot micro-system, comprising parallel electrode plates facing each other with particles suspended between them. The bottom electrode creates the required inhomogeneous electric fields due to the presence of an array of circular apertures known as dots. These apertures are etched through a thin layer of conductive material deposited on a transparent dielectric substrate. A counter electrode is situated a distance away above the dot array.

Using a combination of finite element modelling and empirical studies, the electric field morphology and characteristic particle motion on the dot array is analysed for different aperture sizes. We also offer a theoretical approach which can be used to relate shifts in light intensities to the dielectrophoretic force exerted on a suspension of particles in an axisymmetric micro-system.

2.0 Materials and Methods

2.1 Electrodes

Using a photolithographic process described by Hoettges [30, 31], gold coated slides (10nm Ti, 100nm Au) were etched through revealing circular apertures (dots) of varying diameters (150µm-500µm) on a single substrate. A schematic of the dots on a glass substrate is shown in Figure 1. The patterned gold slide was epoxy mounted on to a prototype board of 2.54mm pitch with a window sawn out of the board to allow visualisation of the dots. Half a dual-in-line 10 pin connector was soldered on to the prototyping board providing connection to a function generator (Thurlby Thandar Instruments, TG120) via a coaxial cable. A high pass filter was surface mounted on to the board allowing frequencies up to 5MHz to be applied to the electrodes with
minimal attenuation via an insulated copper runner attached to the dot electrode by silver loaded epoxy (RS 186-3616, UK). An indium tin oxide (ITO) covered slide (Delta Technologies, Stillwater, MN, USA; \( R_s = 6\pm2\Omega \)) was cut to size and a flexible wire was connected to the ITO surface with silver loaded epoxy. The flexible wire was connected to the ground track of the prototype board via a gold connector. The top and bottom electrodes were separated by heat treated Parafilm, 125\( \mu \)m thick which had a 25 mm\(^2\) circular area cut out and placed around the dot array to contain suspended samples between the electrodes.

The micro-system was placed in a purpose-built slide holder, and placed on a microscope stage (Nikon Eclipse E400). Insulating clamps on the slide holder applied pressure on to the micro-system to make the system stable and keep the distance between both electrode layers constant whilst focussing the objective into the plane of the dot electrode. Using phase contrast microscopy, particle motion was captured through a mounted CCD camera (Photonic Science, Cambridge) connected to image acquisition software PhotoLite on a PC.

### 2.2 Experimental Procedure

Yeast pellets (Allison Dried Yeast, Tescos, UK) were cultured in a 10ml sterile YPD broth media (Y1375, Sigma-Aldrich, UK) and incubated at 30\(^{\circ}\)C for 18hours. The cells were centrifuged for 5 minutes and washed three times before being finally re-suspended in distilled water. Cell concentrations of \(3.1\times10^6\), \(7.8\times10^7\) and \(1.1\times10^8\) cells per ml were made up and re-suspended in 0.2mSm\(^{-1}\) distilled water which were used as stock solution for assessing the influence of concentration on electrode behaviour for different dot sizes. Aliquots of yeast suspensions (5\(\mu\)l) were pipetted on to the dot arrays and covered with the ITO counter electrode. Using a 6V\(_{p-p}\) signal,
10 kHz and 1 MHz frequencies were applied to the system and timed. Images were captured before application of the voltage signal then 1, 2 and 5 minutes after application of the voltage to the system. To assess the effects of concentrations on individual cells, the time taken for single cells to travel the radius of the aperture amongst varied cell concentrations were also tracked and recorded. Post-processing of images was carried out using MATLAB 7.1 image processing toolbox (Mathworks, Inc.).

2.3 Finite Element Modelling

The electric field distribution of the dot micro-system was modelled for single dot diameters of 150µm, 250µm, 350µm and 500µm using the electromagnetic module of Comsol Multiphysics 3.2 (Comsol, UK). Figure 2 shows a 3D model of a single 150µm dot used in the simulations, created from modelling one quarter of the dot system and revolving it about it’s axis of symmetry. The main dot electrode was assigned material properties of gold (εᵣ = 1), the top counter electrode was assigned material properties of ITO (εᵣ = 10). To allow greater processing memory and better meshing of the models, electrodes were given a thickness of 2µm. Top and bottom electrodes were separated by a 128µm gap given material properties of the suspending medium taken to be deionised water (εᵣ = 78, σ = 2×10⁻²⁴ Sm⁻¹). Material properties defined for the model were taken to be isotropic. Boundary conditions for the top and bottom electrodes were defined as -10V and +10V respectively. Surface charges equivalent to zero, were set as boundary conditions around the model and the continuity equation set at the interface of the medium in contact with the glass substrate.
3.0 Results

3.1 Experimental Observations

The characteristic displacement of live yeast cells exhibited in the dot micro-system under the influence of a non-uniform ac electric field is shown in Figure 3. Cells experienced positive dielectrophoresis at an applied frequency of 1MHz, characterised by cells being cleared from the centre of the dot aperture and collecting around the edge of the dot electrode, whilst at an applied frequency of 10 kHz the cells exhibited negative dielectrophoresis and accumulated at the centre of the aperture. Individual cells could be resolved at concentrations below $10^8$ cells per ml.

As the concentration of cells increased and the size of the dot decreased, the time taken to remove cells from the aperture region under positive dielectrophoresis was found to decrease. At a concentration of $3.1 \times 10^6$ cells per ml, 68% of cells were removed from the centre of the dot within 1 minute and 100% within 2 minutes for the 150µm diameter dot. For a concentration of $7.8 \times 10^7$, 87% of the cells were cleared within the first minute. In comparison, for the 500µm dot 77% and 87% of the cells were cleared after 2 mins of the electric field being applied for concentrations of $3.1 \times 10^6$ and $7.8 \times 10^7$ cells per ml respectively. It was observed that after 5 minutes, all dot sizes except the 150µm dot still contained some cells, moreso for the larger dots with smaller concentrations.

To assess the effect of concentration on an individual cell experiencing both negative and positive dielectrophoresis, the average particle velocity was measured for particles moving from the centre of the dots to the edge (or *vice versa* for negative dielectrophoresis) were measured along the radius of the dots and are shown in Figure 4. The results indicate that the positive DEP velocity a particle experiences in a dot is faster than the negative DEP velocity across all diameter and concentration.
combinations. Furthermore, increasing the concentration of cells caused a dramatic increase in the velocity of the particles in the smaller size dot. As the size of the dot increased the effects of concentration on particle velocity became less significant, particularly for negative dielectrophoretic forces. For the 150µm dot, particle velocities were seen to reach speeds of 2.84µms⁻¹ and 0.94µms⁻¹ for concentrations of 7.8×10⁷ and 3.1×10⁶ cells per ml under positive dielectrophoresis, representing a 3-fold increase with increase in concentration. A doubling of the dot radius to 150µm showed a 4.6-fold decrease in particle velocity for the 7.8×10⁷ cell per ml sample, with further decreases as the radius increased to 250µm. The velocities of particles experiencing negative dielectrophoresis showed a characteristic decrease as dot radius increased, though differences in velocities based on cell concentrations were found not to vary significantly with respect to dot sizes.

3.2 Simulations

Electrostatic simulations allowed the determination of the field distribution within single dot volumes. Slice plots of the radial component of \( \nabla E \) are shown in Figure 5 for the 150µm diameter dot at different heights (y-plane) within the system. The field gradient at the electrode edge was found to be of the order 1×10¹² V² m⁻³ for all dot sizes. From figure 5 we can see that as we approach the counter electrode, starting from 5µm above the dot electrode plane, in incremental steps of 30µm, the maximum magnitude of the field gradient between planes reduced initially by 2 orders of magnitude then by an order of magnitude until the counter electrode plane is reached. The circular bands indicate that the field gradient values are constant radially from the centre of the dot at their varying heights.
Figure 6 shows the 2D surface plot of $|\nabla E^2|$ for all dot sizes. In the plane of the gold electrode surface (2\(\mu\)m), at the centre of the aperture, the field gradient decreased in magnitude compared to that at the substrate surface for all dot sizes; however, above the plane of the gold electrodes, the magnitude of the field gradient gradually increased for all dot sizes. 5\(\mu\)m away from the counter electrode surface, it was seen that the magnitude of the field gradient for the 150\(\mu\)m diameter dot was 2.4 times greater than the 250\(\mu\)m diameter dot and an order of magnitude greater than the 500\(\mu\)m diameter dot.

The field gradient of the dot micro-system can be seen to adopt a dome-like geometry at the centre of the dot aperture. It is more pronounced for smaller diameter dots, but as the diameter increases the dome’s sides begin to slope at an angle as the centre is approached, contributing to more of a triangular distribution over the aperture region. The axisymmetrical nature of the field gradients about the centre of the dot aperture indicates that the dielectrophoretic force is also axisymmetrical within the dot volume, with the force being greater at the electrode edge, diminishing in magnitude as the centre of the aperture is approached radially. As the dot diameter increased the electric potential at the centre of the dot was found to change from being near the value of the bottom electrode, to being more influenced by the counter-electrode.

4.0 Discussion

4.1 Theoretical Correlation between DEP force and Light Intensities

For relatively low concentrations of cells (up to \(\sim 10^8\) cells per ml), light passing through the cell suspension obeys the Beer-Lambert law of light absorption. If we consider a medium with a cell concentration \(c_0\) illuminated by a light beam of surface
intensity $\delta^2 I_0(r, \theta)$, then the light intensity $\delta^2 I(r, \theta, t)$ collected at the top of the volume will be:

$$\delta^2 I(r, \theta, t) = \delta^2 I_0(r, \theta) \cdot \exp \left[ -\alpha_{\text{medium}} - \frac{1}{Z} \int_{z_1}^{z_2} \beta_{\text{cell}} \cdot c(r, \theta, z, t) \, dz \right]$$  \hspace{1cm} (3)

where $\alpha_{\text{medium}}$ and $\beta_{\text{cell}}$ are the light absorptions of the media and the cells respectively and $Z$ is the total geometry height. If we introduce the averaging operator $< >$, equation (3) becomes:

$$\delta^2 I(r, \theta, t) = \delta^2 I_0(r, \theta) \cdot \exp[\alpha_{\text{medium}} - \beta_{\text{cell}} \cdot < c >_z (r, \theta, t)]$$  \hspace{1cm} (4)

Inhomogeneities of the light source, usually coming from the diffusion of the aperture, can be compensated by a normalising procedure. This can be achieved by capturing the intensity value of a suspension of cells in a static state, prior to the application of the electric field. This can be used via the function $L(r, \theta, t)$ defined by:

$$\frac{\delta^2 L(r, \theta, t)}{\delta S} = \log \left( \frac{\delta I(r, \theta, t = 0)}{\delta I(r, \theta, t)} \right) = -\alpha_{\text{medium}} + \beta_{\text{cell}} \cdot < \Delta c >_z (r, \theta, t)$$  \hspace{1cm} (5)

where $\Delta c(r, \theta, t) = c(r, \theta, t) - c_0(r, \theta, t)$ is the concentration displacement. $L$ provides a measure of the concentration averaged along the $z$-axis. It can be seen here that all the inhomogeneities of the light source cancel out as long as they are constant over time.
However, a region with a lower light intensity will have a resolution less important than a bright region.

For the axisymmetrical microelectrode geometry described here it is possible to reduce the measured noise by averaging the function $L$ over circular bands:

$$\Delta L(r,t) = \left\langle \delta^2 L(r,\theta,t) \right\rangle_{\theta} = -\alpha_{\text{medium}} + \beta_{\text{cell}} < \Delta c >_{z,\theta}(r,t) \quad (6)$$

Hence, from the values of light intensity levels we can obtain a measure of the concentration displacement. This concentration is linked to the force field $\vec{F}$ by the diffusion equation under a constant force field written as:

$$\frac{\partial c(r,z,t)}{\partial t} = D \Delta c(r,z,t) - \frac{1}{6\pi\eta a} \text{div} \left( c(r,z,t) \vec{F}(r,z) \right) \quad (7)$$

where $a$ is the cell radius, $\eta$ is the dynamic viscosity of water and $D$ is the cell diffusion coefficient. This equation is quite general, and does not provide a simple analytical solution in general. However, it is possible to greatly simplify it if for instance $a = 1\mu m$ which corresponds to a diffusion factor $D \sim 4.10^{-13} m^2 s^{-1}$. If we also assume the time of an experiment does not exceed $t = 10s$, we obtain a diffusion length $l = (D t)^{\frac{1}{2}} \sim 2\mu m$. Compared with the electrode dimensions (>100\mu m), the diffusion due to Brownian motion can be neglected and the diffusion term in the equation removed. In addition, if experiments conducted are not long compared to the average collection time (time required for a cell to reach the electrode), we can use the small-time approximation which assumes that at any moment the system is far from
its equilibrium. Under these conditions the concentration can be considered to be homogeneous during the experiment. Finally, equation (7) becomes:

$$\frac{\partial c(r,z,t)}{\partial t} \bigg|_{t=0} = -\frac{1}{6\pi \eta} c_0 \cdot \text{div}(\mathbf{F}(r,z)) \quad (8)$$

It is now possible to average this equation over the z-axis and over θ so we can combine it with equation 6. After substituting the general force by the DEP force expression, we obtain the following equation:

$$\frac{\partial \mathbf{A}(r)}{\partial t} \bigg|_{t=0} (r) = -\gamma \cdot R e(K(\omega)) \langle \text{div}(\nabla \mathbf{E}^2) \rangle_{z,\theta} (r) \mathbf{\hat{r}} \quad (9)$$

where $\gamma = r^2 e_0 c_0 / 3\eta \beta_{cell}$. This last equation relates the concentration measured and the Clausius-Mossotti factor together; the left side term is given by the intensity value determined and provides a measure of the DEP force over the region observed. We know that the electric field does not vary in space from one experiment to another, so it is possible to compare several experiments at different frequencies by comparing the normalised intensity values.

$$\text{Re}(K(\omega)) = \frac{\left\langle \frac{\partial \mathbf{A}}{\partial t} \bigg|_{t=0} \right\rangle(\omega)}{\left\langle \text{div}(\nabla \mathbf{E}^2) \right\rangle_{z,\theta}} = -\frac{1}{\gamma \tilde{\psi}} \left\langle \frac{\partial \mathbf{A}}{\partial t} \bigg|_{t=0} \right\rangle_{r}(\omega) \quad (10)$$

where $\tilde{\psi}$ is the mean divergence of the electric field gradient. As the electric field morphology is constant for sub-radiofrequency values, this value is constant over the frequency for a given averaging area. Theoretically, an averaging over the total
surface could be performed, but in practice the noise level may be high in the areas of low DEP force or in the regions of high light diffusion so that a global averaging would result in poor signal over noise ratio. It is therefore practical to average over an area of low light diffusion which would correspond to the centre of the geometry. Once the region is chosen, $\bar{\psi}$ remains constant for all frequencies and the measured change in light intensity ($\delta L$) over time is found to be proportional to the Clausius-Mossotti factor. Therefore, the analysis of light intensity through the dots has potential to act as a rapid indicator of polarisability.

Equation (2) describes how the medium and particle’s complex permittivity values vary as a function of frequency. Differences in the observed negative and positive dielectrophoretic velocities of the cells are attributed the magnitudes of the Clausius-Mossotti, which is frequency dependent [32]. As the diameter increases effective field magnitude penetration diminishes, decreasing the dielectrophoretic velocities of the cells and also distorting the inherent velocity ratios between positive and negative dielectrophoresis. For a 500µm dot diameter, the negative dielectrophoretic particle velocity is faster than the positive dielectrophoretic particle velocity at both concentration levels. Reducing the dot diameter from 300µm to 150µm, the positive dielectrophoretic particle velocity becomes faster than the negative dielectrophoretic particle velocity, with the ratio between velocities increasing by a factor of 3 at the highest concentration level. From field simulations it can be seen that the field magnitude tends to zero as the centre of the dot is approached. Hence as particles experience positive dielectrophoresis in the largest size dot, the dielectrophoretic force at the centre of the dot is not sufficient to induce rapid particle motion. On the other hand, particles experiencing negative dielectrophoresis will have a force sufficient in magnitude near the electrode edge capable of rapidly repelling the
particle towards the centre of the dot. This suggests that for accurate measurements of the Clausius-Mossotti factor using this electrode geometry, smaller dot sizes would be beneficial as electric field distortions are minimised and increased suspension concentrations ($\leq 10^8$ cells per ml) would aid rapid particle motion and increase detection sensitivity through light absorption methods.

The dot array has a number of useful attributes such as the large surface area providing the electrical transmission line to the edges of the dot array, meaning signal attenuation is minimised reducing inconsistencies in voltage amplitudes across the micro-array. Also, there is the additional benefit over other electrode geometries with the well defined area which is related to the diameter of the dot. This provides the user with a definitive region where processes can be confined and easily monitored and quantified through imaging techniques, fluorescence labelling or micro-sensor systems. Based on the theoretical relationship between the dielectrophoretic force and light intensity described, the axisymmetrical nature of the electric field gradient generated by the dot electrode geometry gives it an advantage over other electrode arrays for easy force measurements. Unlike other geometries the magnitude of particle trajectories are equivalent in all directions, with little or no generation of fluid motion [33]. This valuable property means that accurate DEP force measurements (both positive and negative) of suspensions can be detected over frequency ranges to obtain the dielectrophoretic spectra, the biophysical blue-print, of the cell suspension [6, 10, 34].

5.0 Conclusion

A quasi-three dimensional microelectrode geometry has been assessed for dielectrophoretic manipulation of biological cells and shows regions of electric field
maxima and minima lying around the circumference of the dot electrode and at the
centre of the dot aperture respectively. The electric field distribution was found to be
axisymmetric around the centre of the aperture. Radial particle velocities under
positive dielectrophoresis were greater for smaller size dots, and significant increases
were seen as particle concentration increased. Reduction in particle velocities
occurred as dot size increased, with smaller increases in velocities as particle
concentration rose. With negative dielectrophoresis, negligible differences in particle
velocities were found for all dot sizes against varied particle concentration, though
there was a gradual decrease in velocity as the dot size increased.

The large area used to provide the electrical voltages means that the transmission line
does not easily attenuate the voltage signal, giving the same potential and hence force,
at the dot electrode edge for large arrays of dots. With optimal geometric dimensions
set for effective electric field penetration, this microelectrode geometry is attractive
for a host of potential processes such as immuno-assaying, particle confinement for
growth studies or particle characterisation.

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References

1. Markx, G.H., M.S. Talary, and R. Pethig, Separation of viable and non-viable

2. Becker, F.F., et al., The removal of human leukaemia cells from blood using


**Figure Legends**

Figure 1: Schematic of the micro-system consisting of the bottom dot micro-array and the ITO (indium tin oxide) counter electrode.

Figure 2: 3-D model of a single 150µm dot electrode system used in electrostatic simulations.

Figure 3: Characteristic distribution of yeast cells in the dot system for (A) no DEP signal; (B) negative DEP and (C) positive DEP.

Figure 4: Graph showing average particle velocities between electrode centre and edge, as a function of dot size and surrounding particle concentration.

Figure 5: Slice plots of the radial component of $|\nabla E|^2$ at 15µm (A); 45µm (B); 75µm (C) and 105µm (D) above the dot electrode plane.

Figure 6: Comparison of 2D surface plots of $|\nabla E|^2$ distribution for the (A) 150µm; (B) 250µm; (C) 350µm and (D) 500µm diameter dots; logarithmic scale used for visualisation.
Figures

Figure 1

Figure 2
Figure 3

Figure 4
Figure 5

Figure 6