Combined Dielectrophoretic/

Electrohydrodynamic/Evanescence based

biosensor devices

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Abstract

A common problem facing the application of dielectrophoresis for practical problems in medicine and biology is that the electric field gradient required to manipulate cells limits both the geometry of the electrodes, the volume of solution over which the device is effective, and the time taken to trap particles on the electrode surface. Furthermore, the nature of the dielectrophoretic force is such that particles generally collect on electrode edges, whereas for many surface detection methods, collection on the metal electrode surface is preferable. In this paper, we present a method of particle collection for enhancement of surface-based biosensors using evanescent light scattering for particle detection, using combined dielectrophoretic forces and electrohydrodynamic fluid flow to concentrate particles onto the central electrode area using relatively large electrode arrays. Bacterial spores of *Bacillus subtilis var niger* were observed to collect from particle concentrations as low as 5x10^3 spores/ml.
I. Introduction

There exists a number of applications where a method of rapid detection of particulate biomatter on the micron- and sub-micron scale, such as cells, bacteria, proteins and viruses, is of great importance. Such applications include the development of point-of-care diagnostic devices for the detection of rare cells; in the testing of water quality for the detection of micro-organisms; and in the monitoring of the environment for the detection of bioweapons such as anthrax. In order to detect such particles, a highly sensitive biosensor must be employed. Many biosensors technologies involve the detection of particles adsorbed on a surface. These methods, including examples such as evanescent light scattering and surface plasmon resonance [1,2] require that the particle to be detected be captured onto the surface of the sensor for detection. However, such detection methods can be limited when used to detect colloidal bioparticles, since low particles concentrations and dominance of effects such as buoyancy or Brownian motion can limit the contact between sensor surface and particle.

Dielectrophoresis is the motion of particles caused by induced polarisation effects in inhomogeneous electric fields [3-5]. Depending on the electrical properties of the medium and the particle it can be attractive or repulsive, which we term positive and negative dielectrophoresis respectively. In the case of positive dielectrophoresis, the particle moves towards the greater field inhomogeneity, in negative dielectrophoresis it moves away from the field inhomogeneity. Since electrodes induce the electric field, the field inhomogeneity is greatest at the edges of the electrodes, therefore the particles move either towards or away from the electrodes. Used for a range of biomedical settings including the manipulation of bacteria (e.g. [6-8]), viruses (e.g.
[9-11]), and cells such as cancers and algae (e.g. [12-15]), the technique has been used in conjunction with methods such as bulk light scattering [16] and evanescent light scattering [17] to detect the motion of particles. However, conventional dielectrophoretic methods also present limitations; where the particles are attracted to the surface on which microelectrodes have been patterned, the collection generally occurs along the edge of the electrodes; many sensing devices require collection to take place primarily on top of the electrode (metal) surface (e.g. [1]). Secondly, since field inhomogeneity reduces rapidly with distance from the electrode edge, the trapping of small particles (e.g. bacteria and viruses) has in the past only taken place using electrodes with very fine structures (sometimes of the order of a few µm) and very constrained volumes in which the particles can be detected (of the order of a few nl).

In addition to dielectrophoretic forces, another form of behaviour is observed in particles when exposed to lower-frequency non-uniform fields. This phenomenon occurs due to a combination of dielectrophoresis and AC-electrohydrodynamic flow (EHD); first observed by Pethig et al. [18] to cause particles to collect on electrode surfaces, it was originally referred to as “anomalous dielectrophoresis”. Later work by Green and Morgan [19] showed that the effect could be attributed to a balance between dielectrophoretic forces and fluid flow in the bulk medium, which occurs due to electro-osmotically induced motion of charge in the electrical double layer across the electrode surface. This effect appears at relatively low frequencies (<100KHz) and is perpendicular to the electrode edge, driving fluid onto the electrodes. In practice, this forms a vortex of liquid over the electrode edge [20].
In this paper we present electrode structures that combine dielectrophoretic effects with electrohydrodynamic fluid flow to concentrate particles on active sensor surfaces, using electrodes up to 1mm across. By construction of electrodes which promote combined EHD/DEP vortices at electrode edges, particles are “pulled” out of the bulk liquid in a downdraft above the electrode edge and inwards along the electrode surface, concentrating them on the electrode surface. Since the effect causes a bulk flow in the liquid the particle collection is independent of the particle size and a wide range of particles can be collected.

To optimise the collection effect on a surface a novel electrode configuration called "zipper electrodes" has been developed. "Zipper electrodes" consist of an array of interlocking, approximately circular electrode pads. Electro-hydrodynamic forces draw liquid inwards form all sides and thereby focus particle into the centre of each pad for detection. The size of the single pad can be significantly larger than conventional dielectrophoretic devices since EHD forces affect a much larger volume than dielectrophoretic force. Pads with a diameter of up to 1 mm have been demonstrated. Since the liquid vortex extends further into the medium than the electric field, a significantly larger volume can be probed than by dielectrophoresis alone. On small electrode pads, the vortex can extend over the whole surface of the pad and "collide" with the vortex from the opposite edge in the centre of the pad. In this case, a strong updraft forms over the centre of the pad lifting particles off the surface again. In an integrated sensor device, antibodies could trap the analytes as they are carried over the surface, while contamination such as dust or other bacteria would be removed from the surface by the updraft. This could be used to improve the
signal to noise ratio of an integrated device, since unwanted particles are returned faster into the bulk medium than by diffusion alone.

II. Theory

Dielectrophoresis, the phenomenon of induced motion in particles suspended in non-uniform electric fields [3-5], is well-characterised and understood. However, in addition to this phenomenon, other electrokinetic phenomena exist which exhibit themselves under similar circumstances to those usually used for dielectrophoresis – that is, using particles suspended in ionic aqueous media, and using planar electrode arrays. One such phenomenon is the induction of fluid flow across the electrode surfaces, which causes particles collecting by dielectrophoresis to move over the electrodes. This effect was first observed in 1988 and described by Price et al. [21], who noted in early experiments using planar electrodes that, at low frequencies, particles trapped by positive dielectrophoresis moved to form diamond-shaped aggregations on the upper surface of the electrode arrays. Investigations of the electric field across the electrode array surface [22] determined that these diamond-shaped areas corresponded both in location and shape to regions of low electric field strength, and it was thus attributed to being due to an unexplained form of negative dielectrophoresis. Particle collection due to combined dielectrophoretic/fluid flow phenomena were subsequently described by Pethig et al. in 1992, where cells were observed to collect on the upper edges of castellated electrode structures at low frequency [18]. This behavior was attributed to an unknown dielectrophoretic effect and dubbed “anomalous dielectrophoresis”. The effect was revisited and explained by Green and Morgan [19] as being due to the balance of dielectrophoretic force and the action of fluid flow. The source of this fluid flow was revealed by Ramos et al [23]
who described how the electric field generated by planar electrodes is such that field lines pass through the electrical double layer surrounding the electrodes tangentially; this can be considered to consist of a component orthogonal to the electrode surface, plus a second component, tangential to the surface, which acts to move the charge accumulated in the double layer, creating an electro-osmotic flow.

When particles collect on planar electrode arrays by dielectrophoresis alone, they do so at the points of highest electric field strength; that is, at the edges of the electrodes. However, as frequency is decreased, fluid flow due to electro-osmosis becomes increasingly prominent; as described above, the location where this is strongest is where the electric field intercepts the double layer at the sharpest angle, which is across the electrode surfaces where the electrodes are closest together. Therefore, those particles which have collected by positive dielectrophoresis are those that experience the greatest fluid motion, causing them to be “swept back” on to the electrode surface. As they move further from the electrode edge, the angle of the electric field becomes more orthogonal and the fluid flow diminishes; eventually a “neutral point” is reached where the two processes are in equilibrium and the particles remain at rest; this is at the center of the array and is responsible for anomalous collection behaviour. The effect was actually used by Green and Morgan [19] to demonstrate that the phenomenon could be used on micro-arrays to separate two sizes of nanoparticle.

The fluid-flow effect is frequency-dependent, being strongest at low frequencies where the double layer has time to form, diminish and reform with opposing polarity for every cycle of the electric field, but becoming limited at high frequencies where
the electrode polarity changes too fast for the double layer to form. Ramos et al. [23] and subsequently Green et al. [24] demonstrated that the velocity profile $v$ of the fluid, and hence the particles, follows a bell-shaped frequency dependence governed by the expression [23]

$$v = \frac{1}{8} \frac{\varepsilon V_o^2 \Omega^2}{\eta x (1 + \Omega^2)}$$

where $\varepsilon$ represents the permittivity of the medium, $V_o$ is the potential applied to the electrodes, $\eta$ is the viscosity of the medium, $x$ is the distance from the center of the inter-electrode gap, and $\Omega$ is a parameter given by the expression

$$\Omega = \frac{\omega \kappa \varepsilon \sigma}{2\sigma}$$

where $\omega$ is the electric field frequency, $\sigma$ represents the conductivity of the medium, and $\kappa$ is the reciprocal double layer thickness.

Since the direction of flow is dictated by the direction of the electric field vector, the fluid motion is always directed orthogonally to the electrode edges, forming a loop pattern as shown in figure 1. In order to maximise the effect of combined DEP/EHD, we have developed electrodes structures that are capable of focussing particles across a large area towards a central spot. Ideally, such a focussing structure would consist of two electrodes, an inner circular “pad” and an outer ring bordering the inner at a set distance. However, such a structure would require multi-laminate microfabrication
and may potentially be beset with capacitive losses between upper and lower electrodes; it would also be inefficient since collection over the edge of the outer electrode would not be controlled. To overcome these problems, we have devised electrode structures consisting of near-circular teardrop structures, interlocking in such a way as to form a “zipper-like” array such as that shown in figure 2. Such an array allows the concentration of particles onto a series of interlocked “pad” structures which can be patterned across an entire array, without the need for complex fabrication procedures.

III. Experimental Setup

Zipper electrodes were manufactured in a range of sizes by photolithography and wet etching, for optical observations from ITO 4-8Ωm (Delta technologies, USA) on glass with HCl 20%, 55°C; for light scattering SPR in IBIS SPR substrates, (Windsor scientific, Windsor, UK). Gold with titanium seed layer on glass with KI:I₂. Masks were fabricated form photographic film as described by Hoettges et al. [25] Electrode arrays were constructed with an approximate circle diameter of 230μm, 380μm, 575μm and 750μm and an inter-electrode gap of 100 μm . Approximately 40 μL of particle suspension was micropippetted onto the electrode array and covered with a cover slip, and observed using a Nikon Eclipse E400 microscope. Particle collection was observed and analysed using a Photonic Science Coolview HS cooled camera and
PhotoLite software (location). Electrodes were energised using a Thurlby-Thandar signal generator, providing $10V_{\text{pk-pk}}$ over a range of frequencies.

BG (*Bacillus subtilis var niger*) spores (diameter ~800 nm) were used to evaluate the performance of the electrodes for bacteria-sized bioparticles. 110nm-diameter fluorescently labelled latex beads (Molecular Probes, Oregon, USA) were used to evaluate the performance for virus particles. Yeast cells were used to evaluate the behaviour of the system for cell-sized particles. In order to evaluate the sensitivity of the system, controlled concentrations of BG spores between $5 \times 10^8$ spores/mL and $5 \times 10^3$ spores/mL were investigated in 10mSm$^{-1}$ solutions to evaluate the local increase in spore numbers in the centre of the electrode. BG spores were suspended in KCl solutions with a range of conductivities between 1 mSm$^{-1}$ and 100 mSm$^{-1}$. The distance from the edge at which spores were collected was measured at a range of frequencies to find optimum conditions for different electrode sizes and conductivities.

**IV. Results and Discussion**

On application of the electric field, vortices were observed to form along the electrode edges. These vortices extended from the median line of the inter-electrode gap for some hundreds of microns in either direction, causing a moving “front” of particles moving from their original positions, orthogonally away from the electrode edges. Where the motion was onto the bus-bar surrounding the zipper pad, the motion continued for several hundred microns; where the motion was on the pad, particles were observed to rapidly coalesce onto the centre of the pad, as shown in figure 3.
The vortices were observed to extend high into the bulk liquid, attracting particles from the volume enclosed between the pad and the top of the chamber (approximately 100μm). Particles trapped in the vortex were observed to migrate from the centre of the vortex to the outside, where they are attracted by dielectrophoretic forces to the electrode edge. After passing that edge, they move along the electrode surface and collect, either at the centre of the pad or in a line along the counter-electrode bus bar. Experiments with spores, yeast and 110nm beads all demonstrated similar behaviour and collection speed; the phenomenon is largely driven by the speed of fluid flow, rather than the properties and dimensions of the particles themselves.

The size of the vortex can be estimated by measuring the distance between the region where the particles collect and the electrode edge. However, in order to obtain reliable results this can only be performed using larger electrodes, since vortices form on all sides of the electrodes; If the diameter of the electrode is too small, the two vortices will influence each other's shape. In our experiments the bus bars were used to have a large distance between the two edges and therefore to see an undisturbed front while the collection band in the electrode was measured as well for comparison. This is illustrated in figure 5a4a, where the distance between particle front and electrode edge is significantly larger on the bus bar than on the pad, because the pattern of fluid flow over the centre of the pad is affected by the fact that the vortices are unable to extend beyond the centre point and overlap. Instead, when the diameter of the vortex at the electrode edge is approximately equal to, or larger than, the radius of the zipper pad then the vortices will "collide" in the middle of the electrode. When this occurs, the velocity of the updraft increases and becomes strong enough to lift particles of the surface. In this case the particles collect in a small spot, but are then lifted in the
vortex the vortex and recirculated as shown schematically in figure 5. After a short time an equilibrium forms between particle deposition and lifting, and a small collection spot remains on the surface. In a sensor device this might be used to increase selectivity; an antibody-coated surface binds to the spores the sensor is designed to detect, particles that do not bind to the antibody are quickly removed. However, this effect can be only used on smaller electrodes as the observed vortices under the conditions described have been insufficiently large to cause this effect on the larger electrodes used. At 10 mSm$^{-1}$ the largest vortices observed were 220 µm, and pads with a diameter of more than 500 µm will not generate vortices with sufficient radius to lift particles off the surface using the signal amplitudes and frequencies described here. When the vortices are significantly smaller than the pad radius, particles instead form a “ring” around the electrode edge, as shown in figure 4b. The size of the vortex over the electrode edge is influenced by the frequency of the applied field and the conductivity of the medium. Reaching a maximum strength at the electrode edge, the vortex effect extends a distance over the electrode surface that is independent of the electrode size. Therefore, the frequency, voltage and pad size can be optimised for a particular set of parameters. To achieve optimum collection in the centre of the pad, the electrode radius should be approximately the same size as the distance the particles are pushed by the vortex effect.

Since the technique is inducing a bulk flow into the liquid the size of the particle is less relevant. This offers an important advantage over other dielectrophoretic techniques. Since conventional dielectrophoretic force depends on the volume of the particles, it is extremely weak for small particles such as viruses. To assess the effect of EHD forces on virus-sized particles, experiments with fluorescently labelled latex
beads (110 nm diameter) were performed on zipper-electrodes. Even for the small latex beads the "zipper"-electrodes worked extremely well, and pulled a large number of particles out of the liquid. The results also suggest that the smaller particles get lifted off the surface by a much smaller updraft and therefore even small vortices might be sufficient to lift the particles off the surface and re-circulate them in the vortex. This is a significant improvement over conventional DEP trapping methods, where the electrodes required to trap nanoparticles must be of the order of a few microns across in order for trapping to take place [26]. The working volume over which the zipper electrodes are effective in attracting particles to the electrode surface are of the order of tens or hundreds of nanolitres per electrode, representing an increase per electrode array of perhaps three orders of magnitude over the quadrupole array previously used to trap 100nm particles by dielectrophoresis (e.g. [26]).

In order to quantify particle collection, controlled concentrations of BG spores in 10mSm$^{-1}$ solution were placed on 500µm electrodes and energised with a 10Vpk-pk, 1kHz signal. The number of particles counted per pad is shown in figure 6. As can be seen, the number of particles collected increases as an inverse exponential as particles from the surrounding region are depleted, though the collection number was also found to be limited by the total size of the trap before becoming saturated (as can be seen for the trace for 5x10$^6$ particles/ml). In all cases, the increase in the number of particles observed on the electrodes is in excess of an order of magnitude. Collection was still possible for concentrations of 5x10$^3$ spores/ml, where 3-4 particles were found per pad; optimisation of geometry and power characteristics should potentially allow the detection limit of 10$^3$ particles/ml.
The pads also showed excellent recovery of particles from the liquid. The recovery can be evaluated by calculating the amount of particles the liquid directly above the electrode pad contains and comparing this to the number of particles collected by that pad. The electrodes show recovery rates between approximately 90% and 120% after trapping for 10 minutes. Recovery rates in excess of 100% can be explained by the fact that the volume from which an electrode can trap particles is actually greater than the volume directly above the pad. A single circular pad with a ring electrode has vortices not only on the inner circle but also on the outer ring. These vortices on the ring also pull liquid down over the inter-electrode gap. In this stream the inner and outer vortices merge and particles that were in the outer vortex can diffuse into the inner one and vice versa. However, the vortex over the outer ring covers a larger area/volume and can therefore collect a larger number of particles. By random diffusion this may account for a slightly larger number of particles from the outer vortex diffusing into the inner one, and thereby explain the high recovery rates. On zipper electrodes there is no outer ring, but the bus bar on one side can fulfill a similar function. This is also supported by the fact that the pads at the corners of an array (where the bus bar surrounds them form two sides) have a slightly higher collection than the pads in the centre of the array.

V. Combined DEP/EHD devices with evanescent light scattering detection
Aside from the increased capture volume over which particles can be captured, the prime advantage of the zipper electrode array is that the particles are deposited at the centre of a large metal electrode, which makes the system ideal for detection methods such as SPR or evanescent light scattering. Furthermore, the large pad size of the zipper electrodes addresses a second problem with etched electrodes in light-scattering systems, that of scatter from the electrode edges. Previous studies using evanescent light scattering techniques to observe dielectrophoresis [17] were impeded by the fact that scatter from the electrode edge (where the particles collect) reduced the sensitivity of the system for the detection of small numbers of particles. In the case of the zipper electrodes, the distance between the collection point and the electrode edge (made possible by the larger electrode size) means that optical detection equipment (e.g. camera or photomultiplier) can focus on a detection region whilst the electrode edges are outside the region of observation.

In order to test this, IBIS SPR chips (Windsor scientific, Windsor, UK) were modified by etching the electrode pattern into the slides so as to cover an area in the centre of the slide approximately 7mm x 7mm. The chips were fitted into an adapted flow cell to allow electrical connections to be made and this was then illuminated using a laser, using an adapted version of instrumentation previously developed for combined light scattering/SPR detection [1]. A schematic of the combined DEP/EHD/light scattering system is illustrated in figure 7, and an example of an image of BG spores collected from a 1x10^5 solution using this equipment is shown in figure 8. As can be seen, the system shows a high degree of light scattering due to particles focussed at the centre of the electrode and darkness from the centre of the electrode to the edge. There are two sources of artefacts in the image; the electrode edge remains visible using light-
scattering techniques, and there is additional scatter from particle illuminated by the laser passing through the inter-electrode gaps (this appears as a horseshoe-shaped region in the bottom half of the image), though this can be eliminated using a greater magnification on the camera, or by optical masking. Simple enhancement of the image can be performed by using black-white thresholding, as shown in figure 8c.

VI. Conclusion

In order to address the shortcomings of conventional dielectrophoretic systems for enhancing biosensors – including small working volume and collection on metal edges rather than surfaces – we have devised the new zipper-electrode structures. These have shown to be highly effective in collecting particles from suspension and depositing them on a small surface. The local enrichment effect of these electrodes is such that particles at local concentration of $5 \times 10^3$ spores/mL and can be collected using a single electrode pad. The fluid flow induced in the bulk flow is an efficient mechanism especially for very small particles, since the dielectrophoretic forces working on these particles are normally very small and do not penetrate the liquid as far as the vortex induced by combined dielectrophoresis/fluid flow does. The bulk flow makes it also a very versatile method that can extract a wide range of particles out of the liquid. The mechanism of removing the particles off the surface again with a strong updraft in the middle of the electrode may be useful to wash clear away unwanted particles while analytes are held down by specific antibodies.
VII Acknowledgements

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BIOGRAPHIES

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Figure Legends

**Figure 1**
The particle collection phenomenon originally referred to as “anomalous dielectrophoresis” is principally driven by bulk flow of liquid at the electrodes. The fluid flow is driven by the liquid in the electrical double layer (contained in the first few nanometers above the electrode surface) being swept away from the electrode edge. Liquid flows inwards, perpendicular to the electrode edges, to replace that pumped away by EHD. The liquid flows upwards further away from the electrode edge and forms a vortex above the electrode edge that pulls particles out of the bulk liquid; dielectrophoresis then pulls them from the liquid, where they move across the electrode surface. Since the updraft over the electrode surface is much weaker than the downdraft over the edge, most particles do not lift off the surface but collect in a band parallel to the electrode edge.

**Figure 2**
A schematic of the “zipper” electrode structures used in this report. The electrodes consist of interlocking circles of alternating polarities. Each pad is focussing particles in its centre by DEP/EHD fluid flow; particles also collect on the bus-bars which form the outer part of each electrode “ring”.

**Figure 3**
A suspension of BG spores (~$10^8$ spores/mL) in 30 mS/m KCl Solution at 1 kHz, 10V on 575μm electrodes. (a) Before the field is applied the spores are randomly distributed.( b) 90 sec. after the field is applied, a large number of spores are already
focused into the middle of the electrode. c) 180 sec. after the field was switched on a
large number of spores are focussed into a small spot on the middle of the electrode.

**Figure 4**

The size of the vortex determines how far from the electrode edge the particles start to
collect. However, since there is a synergistic effect from the vortices around the pad,
it is impossible to measure the vortex size at low frequencies due to the effects
illustrated in figure 4. However, since the bus-bars have only one electrode edge, it is
possible to measure the vortex size directly for a given set of conditions. (a) Where
the vortices overlap in the pad, it is possible to measure the front on the bus bar,
which can be seen here to be larger than the edge-front distance on the electrode. (b)
If the vortex is much smaller then the radius of the electrodes the particles collect not
in the middle but in a ring along the electrode edge.

**Figure 5**

A schematic showing the effect of vortex intensity on particle collection. (a) Where
the vortex extends less than half the pad radius over the electrode surface, particles
are deposited in a small space at the centre of the electrode. (b) Where the vortices are
larger then the radius of the electrode, the vortices from opposite sides "collide" and
form a strong updraft in the middle that lifts the particles off the surface back into the
vortex.

**Figure 6**

Number of particles collected on a 500μm-diameter electrode pad energised with a
10V_{pk-pk}, 1kHz signal, for a range of particle concentrations. The number of particles
collected increases as an inverse exponential as particles from the surrounding region are depleted, though the collection number was also found to be limited by the total size of the trap before becoming saturated (as can be seen for the trace for $5 \times 10^6$ particles/ml). In all cases, the increase in the number of particles observed on the electrodes for time=0 sec is between in excess of an order of magnitude.

**Figure 7**

A schematic diagram of the combined DEP/EHD zipper electrode system combined with a flow cell and evanescent light scattering detection.

**Figure 8**

Figure 4

Figure 5
Figure 6

Spore collection on 500 μm electrodes

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Number of spores vs Time [min]
Figure 7

Figure 8