Use of combined dielectrophoretic/electrohydrodynamic forces for biosensor enhancement

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Abstract

Dielectrophoretic and electrohydrodynamic forces have been demonstrated in the literature to cause the movement of particles across the surface of planar electrodes when exposed to low-frequency (approx. 1kHz) electric fields. In this paper we describe the development of this phenomenon for the collection of particles, covering a range of sizes, out of a liquid and focusing them at the ~~center~~centre of a novel electrode consisting of large interlocking circles. The volume of analyte across which this effect is observed is significantly larger than has been reported for conventional dielectrophoretic arrays. By altering the experimental conditions, particles can either be collected or cycled across the surface and then removed. This technique offers great scope for the enhancement of surface-based detection methods.

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Introduction

A dielectric particle suspended in a non-uniform electric field will polarize; the interaction between the dipole and the field is such that the particle experiences a force. This effect is called dielectrophoresis (DEP) [1-3]. The force can move the particle in either the direction of increasing electric field gradient (positive DEP) or decreasing field gradient (negative DEP) depending on whether the polarisability of the particle is positive or negative. The polarisability is a complex function of the electrical properties of the particle and medium and the frequency of the applied electric field, and in an appropriate medium a particle may experience positive and negative DEP at different frequencies. At low frequencies ($< 100\text{kHz}$) a third effect is observed. This was first reported by Pethig *et al.* [4] as a form of particle collection on the upper surfaces of electrode structures, which they referred to as an anomalous DEP effect. This phenomenon was later explained by Green and Morgan [5] as being caused by the interaction of the DEP force with an AC-electrohydrodynamic (EHD) flow caused by the electro-osmotic flow of layer charge in the electrical double-layer across the electrode surface [6]. EHD flow occurs on top of electrodes in high electric fields and always leads from the electrode edge inwards and is strongest at the electrode edge. Since the liquid has to be replaced, liquid is drawn downwards above the electrode edge. With a weaker EHD force further inwards from the electrode edge, the flow speed decreases, and a slow upward draft feeds back into the downdraft over the electrode edge to form a vortex. A particle suspended in the liquid will experience both EHD and DEP forces. In proximity to the electrode edge, where the field gradient (and hence DEP force) is greatest, the DEP force draws the particle towards the electrode edge whilst the EHD force pulls the particle inwards on top of the electrode edge.

Until now, this phenomenon has been demonstrated on castellated interdigitated electrodes for yeast cells [4] and latex beads [5], and in parallel strip electrodes used for studying the interactions of EHD and DEP [6-9]. Thus far, no electrode array has been demonstrated specifically to exploit this phenomenon for the collection of particles from a medium. However, there exists a wide range of applications where there is a need for particles suspended in a medium to be pulled to an electrode surface for detection. Many biosensors for the detection of bacteria and viruses rely on surface detection methods such as surface plasmon resonance or evanescent light scattering [10]. Since these techniques only detect particles on their detection surface, particles in the bulk liquid volume above the detection surface cannot be detected, and particles collected in non-conducting gaps between electrodes (such as by conventional DEP) may also not be detected.

In this paper, we present a new electrode configuration which exploits combined DEP and EHD effects to trap particles from solution onto the top of electrode surfaces. The electrodes consist of rows of interlocking circles of alternating polarity. This configuration makes optimal use of the surface and can be easily fabricated as a planar array with a wide range of pad sizes. The device can be used for the collection of several different species at once. The particles collect away from the electrode edges, which reduces interference with surface bound detection techniques.

Experimental

The electrode array used here consisted of a series of interlocking tear-drop shapes with separating gap, and with interdigitations having electric potentials of opposing

polarity applied (as shown in figure 1). We have termed these structures “zipper electrodes”. Electrodes were produced in a range of sizes (pad diameters 230 μm , 380 μm , 575 μm and 750 μm) by photolithography and wet etching with HCL 18%, from ITO on glass (sheet resistance 4-8 Ω , Delta Technology, Stillwater, USA). *Bacillus subtilis var niger* (BG) spores (diameter ~800 nm) were used to evaluate the performance of the system for bacterial particles. Fluorescently labelled latex beads (diameter 110nm) were obtained from Molecular Probes (Oregon, USA) and used to evaluate the performance for virus-sized particles. Yeast cells (*S. cerevisiae*) were used as a model of cell-sized particles. The test particles were suspended in media with a range of conductivities between 1 mS m⁻¹ and 100 mS m⁻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. Electric potentials were applied using a function generator (Thurlby-Thandar, Huntingdon, UK) and observed using a Nikon Eclipse E400 fluorescence microscope and Photonic Science Coolview HS camera. Images were grabbed at regular intervals using a computer-based system.

Results and Discussion

When an electric potential was applied across the electrode array, the particles were immediately observed to be pulled from the solution at the electrode edges, and move across the electrode surfaces to collect at the centre as shown in figure 2. Particles above the outer ring of the zipper structure (which formed bus bars) were moved over the electrode surface as a receding “front” but did not collect in well-defined regions; however, particles in the electrode “zippers” moved orthogonally to the electrode edge and moved towards the centre of the electrode “pad”, first forming a ring of

particles and subsequently focusing to a point at the centre. This is shown schematically in figure 3. The motivating force for this movement was the presence of vortices in the medium, similar to those described by Green et al [9,11]. Whilst particles in the vortex might be expected to remain there rather than be deposited on the electrode surface, we suggest that in the experimental work presented here, the DEP force acts to pull the particles from the vortex as they pass adjacent to the electrode edge, placing them in a boundary-layer laminar flow across the electrode surface to collect in the centre of the electrode pad. The vortex effect was observed to extend to the top of the chamber (approx. 100 μ m), and almost all particles within the volume extending from the electrodes to the top of the chamber were pulled onto the electrode surfaces. The particles collecting on the zipper surfaces were not seen to be adsorbed to the surface, and were released by diffusion after the electric field was removed.

The rate at which the particles moved from edge to centre of the pads was observed to be approximately equal to the rate at which particles moved from the electrode edge across the bus bars. The distance the particles were moved before reaching a stationary position was found to vary with frequency and medium conductivity, in line with the observations by Green et al [7]. The particles that swept across the electrode edge were generally observed to collect in a line or front, which reached a stable position that varied with experimental conditions. Whilst the distance moved by the particles before they collected was found to increase with increasing vortex velocity across the upper surfaces of the bus bars, particles moving across the zipper pads were constrained in that the distance moved (which we term the *effective radius* of the vortex) cannot not exceed the radius of the pad. In our experiments, the bus bars connecting the pads to the signal generator were used to determine the distance

that particles are propelled by a single-sided, unconstrained vortex pattern for comparison with the distance of particles moved from the pad edge. This is shown in figure 4, where the distance between the collection front and the electrode edge is shown for both pad and bus bar. Where the front moves further inwards across the bus bar for decreasing frequency, for example, the front on the pad can only move as far as the centre. When the effective vortex radius exceeds the radius of the zipper pads, the vortices from all sides of the zipper converge and superimpose, creating an increased updraft effect which causes those particles that collect at the electrode centre to be pulled upwards and recirculated into the medium.

For a given set of conditions, the electrode size has to be adjusted to the size of the vortex. To achieve optimum collection, the effective radius of the vortex should be approximately the same size as the electrode radius. An optimal sized vortex will deposit the particle in a defined spot in the middle of the electrode, while too small a vortex will mean that the particles do not reach the middle of the electrode and collect in a ring. For example. In solutions of conductivity 10mS m^{-1} the largest vortices observed were $220\text{ }\mu\text{m}$, and therefore pads with a diameter of more than $500\text{ }\mu\text{m}$ do not generate vortices large enough to lift particles off the surface. This suggests that there is an optimum energising signal (frequency and magnitude) for a given electrode geometry and medium conductivity, an observation which was borne out empirically in these studies since lifting of the particles was observed at frequencies where the free vortex size was larger than the electrode radius.

When different sizes of particles were tested (with diameters spanning two orders of magnitude, from 110nm to approximately $8\mu\text{m}$) the rate of movement across the electrode surface was found to be similar for a given set of conditions, indicating that the speed of particle movement was entirely dictated by fluid flow and not by the

DEP force on the particle. The speed of the focusing is dependent on the frequency and conductivity of the medium, and has a peak that shifts to higher frequencies with increasing conductivity in agreement with the observations by Green *et al.* [7]. Focusing was observed in media of conductivity up to 90 mS m⁻¹, beyond which the rate of flow diminished to the point of being unable to properly focus particles. To assess how much the electrode structures can be used to improve the local number of spores a solution of $\sim 10^5$ spores ml⁻¹ was placed on electrodes with diameter of 575 μ m and excited with a 1kHz, 10V_{pk-pk} signal. A snapshot of the centre-region was taken every 15 sec for 7 minutes. The number of cells in the centre of the electrode was counted for each frame, as shown in figure 5. As can be seen, the increase in particle numbers at the surface increased by over an order of magnitude; empirical studies (not shown) indicate that the maximum concentration effect may be approximately two orders of magnitude.

The size of the inter-electrode gap was found to have an important influence on the effectiveness of the electrodes for trapping. The two dominant forces— DEP and EHD — are generated by the electric field. Both forces depend on the gap size. Theory predicts that, at the frequencies used, the dielectrophoretic force draws particles to the electrode edge and is depended on the square of the field gradient. The electro-hydrodynamic force depends only on the electric field. This means that the dielectrophoretic force diminishes faster than the electro-hydrodynamic force with increasing gap size. Since the trapping of particles at the electrode edge is not desirable, the gap size has to be optimised to limit trapping of particles at the edge, but still forming a strong enough vortex to collect particles on the electrode surface. The narrow gaps show a significant accumulation of particles at the electrode edge due to DEP, to the extent that no clear band of particles formed on the electrode

surface. With increasing gap size, the number of particles at the edge drops rapidly and a clear particle front forms on the electrode surface. At a gap of 300 μm no collection is observed at the edge but still a clear band of particles on the electrode surface. However, the gap size has only a small influence on the distance from the electrode edge at which the particles collect by EHD effects.

Conclusion

The phenomenon of particle movement by a combination of DEP and EHD has been well-studied in the literature, both experimentally and theoretically. However, whilst the phenomenon of particle collection has been described in castellated electrodes designed for general DEP use, no design has previously been put forward for focusing of particles over relatively large volumes (by conventional DEP standards) using this method. We suggest that there are a number of advantages to using this type of electrode geometry for enhancing the effectiveness of surface-based particle detection techniques such as surface plasmon resonance. The vortex caused by a movement in the bulk liquid extends far deeper into the liquid volume than might be achieved by DEP force alone. This allows probing of a much larger liquid volume. Secondly, the particles are concentrated on a small area on the surface of the electrode, from an electrode that can be several hundred micrometers inwards from the electrode edge. When circular electrodes are used, the particles will be pushed inwards from all sides. Since a large vortex forms over the surrounding electrode edges, particles in the volume several hundred micrometers above the electrode surface will be pulled to the electrode surface and focused onto a relatively small spot in the centre of the electrode. This increases the concentration of particles in the centre of the pad considerably and therefore increases the sensitivity of the detector.

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Figure captions

FIG 1 A schematic of the “zipper” electrode array. The array uses interlocking circles with alternating polarities, and zipper “pads” act to focus particles in the centre using combined DEP/EHD effects.

FIG 2 A fluorescence micrograph of 110 nm latex beads on ITO zipper electrodes.

Latex beads were suspended in 1mSm^{-1} KCl solution on $575\mu\text{m}$ -diameter electrodes. (a) Shows a transmitted light picture of the electrodes before the experiment. In (b) and (c) the transmitted light is switched off, so only the fluorescent latex beads are visible 60s (b) and 120s (c) after the application of a 1kHz , $10\text{V}_{\text{pk-pk}}$ signal, showing clearly how the particles are concentrated on the middle of the electrode pads, the bright colour in the middle shows high concentration.

FIG 3. A schematic showing the effect of the vortex on the collection of particles.

Vortices are driven by fluid motion across the electrode surfaces, particularly at the electrode edges. Where the electrode only has one edge facing a counterelectrode (shown at left), the vortex is one-sided and particles are pushed back but do not collect in defined regions. Where the electrode is enclosed by a counterelectrode (shown at right), the vortices act together to force particles into the center of the electrode. If the vortex is smaller than the radius of the pad, then particles collect in a ring or a dot on the electrode surface as shown. If the vortices extend sufficiently across the pad to meet, the combined updraft prevents particles from collecting on the electrode surface, recirculating them into the medium instead (not shown).

FIG 4. The distance of the particle collection front from the electrode edge, on both the bus-bar and the electrode. BG spores in 10 msm^{-1} KCl solution were subjected to different frequencies in $230\mu\text{m}$ electrodes. At lower frequencies the vortex effect is larger than the electrode radius, resulting in an updraft in the middle of the electrodes which lifts the spores off the surface and recirculates through the medium.

FIG 5. Increase in spore numbers in the centre of an electrode pad. A microscope was focused on the centre of the electrode surface of a 575 μm electrode with $\sim 10^5$ spores ml^{-1} suspended in 10 mS m^{-1} KCl solution. The number of spores in the electrode centre was counted in each frame. The number of spores increased continuously for the duration of the experiment.