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A WIRE LOOP DESIGN FOR CONVECTION-ENHANCED DIELECTROPHORETIC BIOPARTICLE TRAPPING

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ABSTRACT

Tailor-designed AC electro-osmotic (AC-EO) stagnation flows are used to convect bioparticles globally from a bulk solution to localized dielectrophoretic (DEP) traps that are aligned at flow stagnation points. The multi-scale trap, with a typical trapping time of seconds for a one cc sample, is several orders of magnitude faster than conventional DEP traps and earlier AC-EO traps with disjoint electrodes. A novel serpentine wire resistor loop capable of sustaining a high field, up to 20,000 V/cm, is fabricated to produce strong AC electro-osmotic flow with two separated stagnation lines, one aligned with the field minimum and one with the field maximum. The continuous loop design allows a large applied voltage without inducing Faradaic electrode reactions. Particles are trapped within seconds at one of the traps depending on whether they suffer negative or positive DEP (n-DEP, p-DEP). The particles can also be rapidly released from their respective traps (and recaptured in the opposite traps) by varying the frequency of the applied AC field below particle-distinct cross-over frequencies. Zwitter ion addition to the buffer allows further geometric and frequency alignments of the AC-EO and DEP motions. The same device hence allows fast trapping, detection sorting and characterization of a sample with realistic conductivity, volume and bacteria count.

INTRODUCTION

In the last decade, the advent of micro-fluidic research has spawned a new array of lab-on-a-chip technologies operating at length scales typically on the order of tens of microns. However, there has been relatively little success in developing portable technologies that can rapidly detect, distinguish, and analyze dilute solutions of bioparticles such as bacteria or viruses.

Current bioparticle analysis techniques typically rely on labor intensive culturing or PCR amplification to first increase the concentration of a dilute sample. Culturing is then routinely followed by a relatively fast and accurate assay involving fluorescent antibodies, magnetic beads, or fluorescent nanoprobes. While such tests are usually quick, taking only several minutes, they are still limited by the time and effort required, usually 1-7 days, to culture or amplify a dilute sample [9].

One possible means of amplifying the bacteria or virus signal from a dilute sample is to concentrate the bioparticles at a specific location in order to magnify the fluorescent intensity or electrochemical signal of the sample. Over the past ten years there has been significant interest in utilizing DEP forces generated by microelectrodes in order to capture and concentrate charged bioparticles in suspension. DEP has been successfully used to capture a range of bioparticles such as viruses, DNA, and proteins [4,6,8,13,14,16].

However, the DEP velocity of a particle is small, and can be shown to scale quadratically with particle radius and linearly with the applied voltage. The quadratic dependence on the particle radius and practical limitations on the applied voltage render the particle velocity produced by a DEP force miniscule, typically on the order of 10 $\mu m\text{-}sec^{-1}$ for bacteria and 1 $\mu m\text{-}sec^{-1}$ for viruses and thus lead to a concentration time on the order of hours. Also, the field gradient necessary to drive DEP motion can only be achieved with relatively narrow inter-digitated electrodes whose field penetration depth is also limited by the electrode width. As a result, DEP channels are usually less than 50 microns in transverse dimension. The slow capture and the small transverse dimension produce extremely low throughputs for continuous flow kits.

Due to the above throughput limitations, there is considerable interest in replacing or augmenting DEP traps with AC-EO flows. Such flows are due to field-induced polarization on the AC electrodes and are not dependent on the particle dimension. They convect the particles by viscous drag and hence can endow the particle with the flow velocity. As such, they are, at least in theory, longer range and can impart a higher particle velocity than DEP designs.

However, convection by AC-EO alone is not sufficient to trap bioparticles. The particle trajectory would be identical to the AC-EO streamline and due to the volume-conservation property of incompressible flow, none of the stagnation points of the flow field would be attracting-they are either hyperbolic saddle points or elliptic centers. The electrode surface itself is also a slip plane due

to the AC-EO slip velocity, although saddle-point stagnation lines or points could appear. The optimal design would be to impose yet another field on the particles at the electrode stagnation points or lines, where the viscous drag is weakest, such that the stagnation points or lines become attracting and can perform as a multi-scale bacteria/virus trap. Such a local field near the stagnation point/line can be a DEP field, as is the case for the above references [4,6,8,13,14,16], or other short-range local particle forces like gravity or magnetic force.

The purpose of this work is to introduce a new wire trapping design and buffer solution selection to enhance the AC-EO stagnation flow by orders of magnitude. These new designs allow for rapid concentration, detection, and manipulation of dilute solutions of bioparticles within a one cc sample of strong electrolyte within seconds. Bioparticles are not trapped on disjoint microelectrodes, but rather they are convected across a coplanar serpentine wire, with a large voltage drop and surface polarization, to local DEP traps on the substrate where the local particle force fields can be tangentially attracting. In this manner, both diverging and converging stagnation flows are rendered effective and rapid traps. The challenge is to align the substrate and wire stagnation lines of an AC-EO flow with that of a local electric field minima or maxima such that bioparticles are now rapidly convected from the bulk solution to the substrate surface where they are trapped in one of the traps by a local negative or positive DEP force. Rapid particle sorting of live and dead cell by DEP direction is hence achieved. Zwitter ion addition to the buffer allows one to enhance the AC-EO stagnation flow by changing the dielectric and surface conducting properties of the strong electrolyte.

BACKGROUND

When a dilute aqueous suspension of polymer microspheres, or biological cells such as bacteria, is exposed to an alternating current (AC) electric field, electrical forces can act both on the particles and the surrounding fluid. For AC fields, the governing electrical force acting on a particle suspended in a fluid is dielectrophoresis. The electrical force acting on the suspending fluid leads to what is commonly referred to as AC electro-osmosis.

The classical DEP theory [14,15], produces a DEP velocity of the form

$$u_{DEP} = \frac{1}{3\mu}\varepsilon_m r^2 \,\mathrm{Re}[K(\omega)]\nabla\left|E\right|^2, \qquad (1)$$

where, for a homogenous spherical particle, the Clausius-Mossotti (CM) factor, $K(\omega) = (\varepsilon^*_p - \varepsilon^*_m)/(\varepsilon^*_p + 2\varepsilon^*_m)$, and $\varepsilon^* = \varepsilon - i\sigma/\omega$ is the complex permittivity which is dependent on σ, the conductivity, and ω, the applied field frequency. The imaginary part is out of phase with the applied field and, to first order, can be experimentally determined by measuring the torque on a particle in electrorotation experiments [2,14]. However, the real part of the CM factor is in phase with the applied field and describes the particles polarizability and field induced dipole moment [16].

The particle polarizability, and hence the direction of the DEP force, is dependent on the frequency of the AC field. If $\mathrm{Re}\{K(\omega)\}$ is positive, the particle will experience p-DEP and be attracted to regions of high field. If $\mathrm{Re}\{K(\omega)\}$ is negative, the particle will experience n-DEP and be attracted to regions of low field. The frequency at which the effective polarizability equals that of the surrounding medium, or when $\mathrm{Re}\{K(\omega)\}$ is zero, is known as the cross-over frequency. Because different types of cells or bacteria have widely varying polarizability, some experience p-DEP under the same conditions that others experience n-DEP. Particle separations can therefore be achieved using DEP forces in selective frequency ranges, where one particle migrates to a high field, and one a low field.

Electro-osmotic flow is typically produced by placing two planar parallel microelectrodes in contact with an electrolyte solution. When an AC voltage is placed between these two electrodes, an electric field is produced, which interacts with the electrolyte ions, and in the absence of charge injection, or Faradaic reactions [11], the electrode surface is polarized by counter-ions in the electrolyte and form a field induced electrical double layer. Because the double layer is essentially charged like a capacitor, this particular charging mechanism is typically referred to as capacitive charging.

Based on the work by Gonzalez and Ramos [1], the time-averaged slip velocity on the electrode is

$$u_{AC-EO} = -\frac{\varepsilon_m}{4\mu}\frac{\partial}{\partial x}\left|\phi - V_0\right|^2, \qquad (2)$$

where Φ is the value of the potential at any given location above the electrode surface and V_0 is the potential applied to the electrode. The equation represents the effective slip velocity on the electrode surface. If values of the electric field are known, the bulk fluid velocity can now be solved using the Navier-Stokes equation with equation 2 as a boundary condition. The electric field on the electrode surface is frequency dependent and can be shown to be

$$\sigma\frac{\partial\phi}{\partial y} = i\omega C_{DL}(\phi - V_o) \qquad (3)$$

which is nothing more than a charge balance in the normal direction across the double layer, λ, where $C_{DL} \sim \varepsilon_m/\lambda$, is the capacitance per unit area of the total double layer. The time averaged electric field can now be solved using the Laplace with equation 3 as a boundary condition and using equation 2 together with the Navier-Stokes equation, the complete hydrodynamic-electrical problem can be solved.

As shown in figure 1, it is evident that theory predicts an optimum frequency where a maximum in slip velocity exists. This can be argued from physical reasoning. Because ion migration to the surface of each electrode requires a finite amount of time, the electrode charging dynamics will have some dependence on the applied AC signal frequency. This dependence can be estimated by simple scaling arguments. The circuit equivalent to the electrode system can be approximated as a double layer capacitor, with charge separation over a length λ, in series with a bulk fluid resistor. The resulting RC charging time for this equivalent resistor-capacitor in series is simply $\lambda L/D$, where L the electrode separation and D is the ion diffusivity ($\sim 10^{-5}$ cm^2/sec). Hence, there

is an optimum frequency at which one observes a maximum electro-osmotic flow.

At frequencies below D/λL, the half-cycle is long enough such that counter-ions have enough time to completely saturate the double layer, effectively shielding the electric field from the bulk solution. Additionally, at frequencies above D/λL, the counter-ions do not have enough time to migrate to the electrode surface and form a double layer. Since the time-averaged electrokinetic flow requires both double layer polarization and external field, it must vanish at these two extremes and a maximum AC electrokinetic velocity should occur at a frequency of D/Lλ.

DEP/AC-EO TRAPPING

It is clear from equation 2, that a stagnation line exists in the flow field when the tangential electric field vanishes. Ben and Chang [5] have shown that, for capacitive charging, this stagnation line occurs at the center of the electrode at frequencies lower than D/Lλ. For frequencies near or higher than D/Lλ, the stagnation line shifts towards $1/\sqrt{2}$ of the width as measured from the inner electrode edge. Therefore, for capacitive charging there is a converging stagnation line on the electrode surface, and by flow continuity, a diverging stagnation line in the middle of the electrode gap.

It is evident that the AC-EO slip velocity is larger than the DEP velocity by a factor of $(L/a)^2$. Since most particles are of low permittivity compared to a surrounding aqueous solution they exhibit a p-DEP force at frequencies near D/Lλ, when the capacitive charging electrokinetic flow is most robust. Therefore, because strong electro-osmotic convection forces exist under conditions in which particles exhibit p-DEP, and the flow stagnation lines are aligned with that of a high field region, one would expect particles to be convected from the bulk electrolyte and trapped at the converging stagnation line on the high field electrode surface. Additionally, the field between the two electrodes is generally weak and hence represents a n-DEP trap. Hence, if operating at frequencies above the cross-over frequency of the particle, and assuming strong electro-osmotic flow still existed, one would also expect the particles to be trapped in the gap at high frequencies when the particles suffer from n-DEP.

CONCENTRATION/SEPARATION REQUIREMENTS

It is important to note that DEP and AC-EO typically suffer from widely differing optimum frequencies, and it is for this reason that rapid particle separation or rapid n-DEP trapping, on the order of seconds, has not been feasible. The DEP cross-over frequency of a particle is directly related to the charge relaxation time of the particle, which is usually much shorter than the charge relaxation time for AC-EO flow. In fact, the typical cross-over frequencies for common types of particles and bacteria exist where AC-EO has tended toward zero [7,13]. Rapid particle concentration requires both strong AC convection and particles operating under p-DEP or n-DEP. Because most particles exhibit n-DEP at high frequencies where electro-osmotic flow is weak, convection enhanced particle trapping is typically not possible when particles are operating under n-DEP. This detail places limits on current DEP traps. The majority of biological samples used today are not pure. In reality, they contain multiple species of bacteria, cells and waste products. Rapid particle concentration is not practical if the collected sample requires hours of careful preparation, or if the collection device simply collects everything in the sample on an electrode. Eventually, some sort of particle separation would be required either before, during, or after particle trapping had occurred. The current problem is that the rapid separation of a multi-particle sample requires strong electro-osmotic convection at frequencies where some particles are operating under p-DEP, and other exhibit n-DEP, and as explained before, the difference in charge relaxation times between DEP and electro-osmotic phenomena in typical electrolyte solutions prevent this.

One possible solution is to modify the Clausius-Mossotti factor such that the cross-over frequency of a bioparticle is reduced and exists at a frequency where AC electro-osmosis is strong. One possible way to reduce bioparticle cross-over frequency is to increase the permittivity of the electrolyte solution.

The relaxation time for AC electro-osmosis is also dependent upon the permittivity of the medium in that λL/D can be shown to scale as ε/σ, where σ is the conductivity of the medium. It is clear that an increase in permittivity will shift the optimum electro-osmotic frequency further away from the DEP cross-over frequency. However, while the magnitude of the DEP force acting on a particle is dependent on electrolyte conductivity, the polarizability of the medium depends solely on a solutions permittivity. Therefore, in order to achieve an optimum balance of both DEP and convection forces, one simply needs to increase the electrolyte permittivity, which will decrease a suspending particles cross-over frequency, and increase the electrolyte conductivity to counteract the increase in electro-osmotic relaxation time. As shown in figure 1, a desired separation can most rapidly take place when the cross-over frequency exists at or near the optimum electro-osmotic frequency.

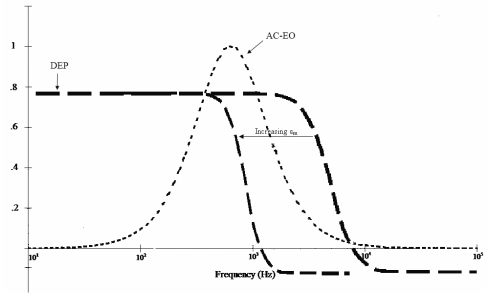

Figure 1. Real part of the CM factor superimposed on AC-EO slip velocity. Electrolyte permittivity increase shifts Re[K(ω)] to the left into a region of stronger AC-EO flow.

Increasing electrolyte polarizability is quite straightforward. It has been shown that the electrical permittivity of a suspending fluid can be increased by adding ionic molecules, or Zwitter ions, of high polarizability to an aqueous solution. For example, Arnold and Zimmerman [3] have shown that the addition of two moles of a glycine peptide to one liter of water increases it relative permittivity by ~252, reducing the Maxwell-Wagner relaxation frequency by ~25%.

DEVICE DESIGN

It is clear from the above discussion that, while AC-EO traps should function better than DEP traps, the limitation to low

voltages and low-conductivity fluids renders them quite ineffective. The low voltage requirement stems from the fact that, for disjoint electrodes, Faradaic reactions will eventually occur at sufficiently high voltages and low frequencies. This is because the field for disjoint voltages must cross the electrode/electrolyte interface and with sufficient voltage drop and sufficient reaction time will induce an electrode reaction. As thin as the double layer is for strong electrolytes, a sufficiently large electric field should still produce a healthy AC-EO flow. Hence, limitation to weak electrolytes can be alleviated if Faradaic reaction can be avoided such that a high rms voltage can be applied.

In this work, we remove both limitations by discarding the disjoint electrode design. Instead, a continuous serpentine wire is used such that most of the AC current passes through the wire and not the electrolyte. Large voltage drop exists along the wire to produce enormous capacitive charging on the wire surface but without a significant field across the electrode-electrolyte surface. With this design, capacitive charging up to 2500 V can be achieved to produce AC-EO flows that are orders of magnitude higher than earlier disjoint electrode designs. Bioparticle concentration, separation, and manipulation can now be achieved in large (cc) volume of strong electrolytes.

As shown from a top view in figure 2, the micro device consists of a thin 10/200 nm Ti/Pt wire on an insulated silicon substrate in contact with an aqueous suspension of bioparticles.

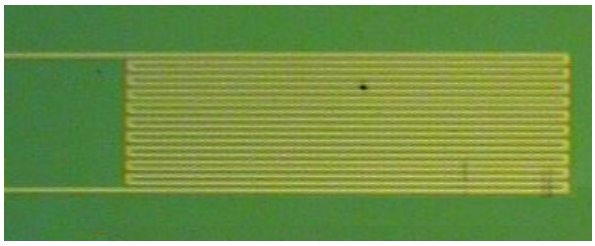

Figure 2. Fabricated DEP/AC-EO Trap

Due to the large difference in electrical conductivity between the wire and the electrolyte, the applied current will be largely confined to the wire, thereby eliminating any noticeable electrochemical reactions, pH gradients, and Joule heating effects in the electrolyte, even at high voltages (~2500V AC). This new configuration allows one to achieve much higher fluid velocities and field strengths than existing electrode geometries. Moreover, because the surface of the wire is of the same sign for each half cycle, the device is anti-symmetric and produces a net flow across the entire serpentine wire.

Figure 3. Side view of the theoretical electric field magnitude of the device showing the highest values in field are predicted on the outer wires

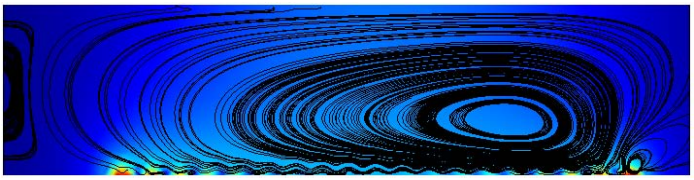

Figure 4. Theoretical fluid streamlines illustrating two converging stagnation lines, one on right edge of the device and the other on the outer left.

Following earlier work by Gonzalez and Ramos, one can solve for the electric field and electro-osmotic velocity profile numerically for a cross-section (cut across wires) of the serpentine wire using the model discussed previously. The magnitude of the electric field and the resulting velocity field are shown in figures 3 and 4 respectively.

From a side view of the device shown in figure 3 it is clear that the wires on the outer region of the serpentine have the largest field. Additionally, the velocity profile is anti-symmetric with a stagnation point near the two outer wires. The important thing to note, superimposing these two figures, is that a converging stagnation line exists directly above a local field minimum on the right hand side of the device, as shown in figure 5.

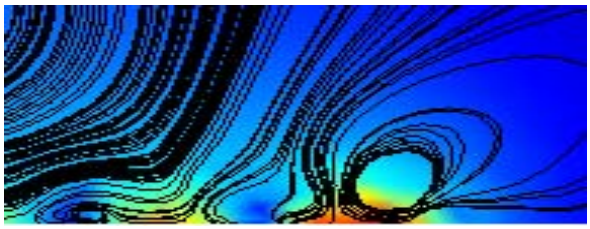

Figure 5. Fluid streamlines superimposed atop electric field magnitude for the last two wires on the right side of the device illustrating a stagnation line aligned directly atop a field minimum

The left hand side of the device has a small flow stagnation line, however it not aligned with a local field minimum, but with a field maximum. Therefore, if operating under the correct electrolyte and frequency conditions, one would expect bioparticles in the bulk solution to be convected directly to a local n-DEP trap on the right hand side, collecting much faster than the left hand side where the particles are brought only in the vicinity if a field minimum (~ 40 μm), but not directly upon it. If operating under p-DEP, the particles should be attracted to the wires with the highest field, as they will follow the streamlines and continue to sample the entire device surface. Particle separation can be achieved by noting that the particles suffering n-DEP will be rapidly attracted to the field minimum regions between the wires, while the p-DEP particles will be attracted to the high field wires.

MATERIALS AND METHODS

Thin film serpentine platinum wires were fabricated using conventional semiconductor techniques. Briefly, serpentine wire geometries were photo patterned onto a dielectric (SiO_2) coated silicon wafer. 50nm titanium/200 nm platinum was then deposited using electron beam evaporation, and the photoresist was then

lifted off in an organic solvent to yield thin film wire patterns. The resulting structure consisted of a serpentine structure with wires 35 um wide, 2500 µm long, arranged in a serpentine structure with a periodicity of 5040 µm, and consisted of 10 periods, or 20 parallel wires interconnected at every other end, as shown in figure 2.

Experiments were conducted in polymer micro-channels which were produced using conventional soft-lithographic techniques [17]. Briefly, micro-channel master molds were fabricated using thick SU-8 photo resist (SU8-2075, Microchem). Uncured Polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning), was then poured and cured atop the molds, peeled off, and carefully aligned atop the serpentine wire structure. This construction formed a channel that was 300 µm deep, 1000 µm wide, and 3 cm long aligned lengthwise above the wire structure. Fluid entrance and exit ports were made in the polymer channel using a 22 gauge syringe needle. Finally, a 300 µm glass capillary was inserted at the entrance port and attached to a syringe. The port and syringe connections were sealed using a quick dry epoxy resin and the syringe placed in a digitally controlled syringe pump. A new polymer channel was used for each experiment. Particle suspensions were then injected into the microchannel via a syringe pump. An AC potential was dropped across the wire structure by a lock-in amplifier and the final assembly, as shown in figure 6, was then viewed under Fluorescent Microscope for visualization.

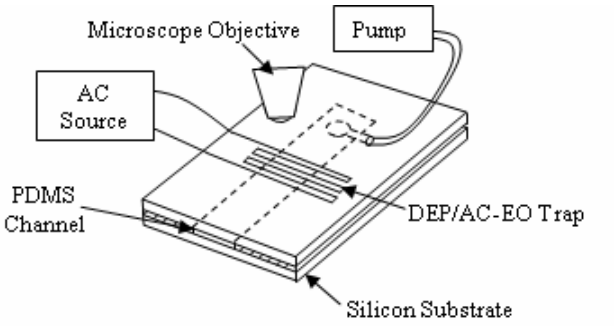

Figure 6. Experimental Setup

Dried bakers yeast cells were obtained and reconstituted in deionized water, 1 µm fluorescent polystyrene microparticles and 5 µm fluorescent microparticles were obtained from Polysciences and diluted to approximately 10^3 particles per mL in deionized water. Electrolyte permittivity was adjusted by adding varying amounts of the zwitterion 6-aminohexanoic acid (AHA) (Sigma-Aldrich) to a prepared polymer suspension.

RESULTS AND DISCUSSION

The p-DEP behavior, in the absence of convection forces, of 1 micron polystyrene fluorescent microparticles suspended in pure DI water was first investigated. The suspension solution was injected into the microchannel and an AC potential (5 V RMS) was dropped across the wire at a frequency of 40 kHz. Prior to this experiment, the AC-EO fluid velocity was measured as a function of voltage and applied frequency for pure DI water using previously described techniques [7]. As shown in figure 7, when 5 V RMS is dropped across the wire at 40 kHz, the fluid velocity is weak at approximately 7 µm-sec^{-1}.

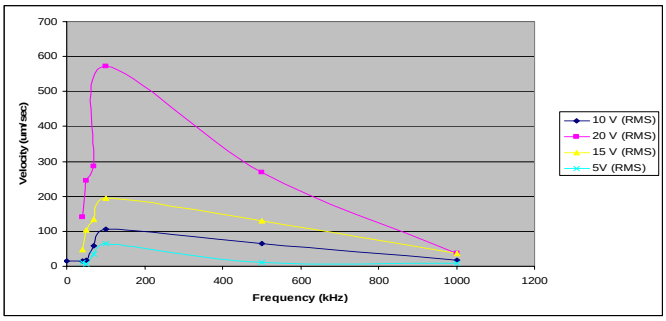

Figure 7. Measured fluid velocity for DI water as a function of AC frequency for four different applied voltages: 5V, 10V, 15V, and 20 V.

Figure 8 shows the optical fluorescent microscopy images of the microparticles patterned by p-DEP. This image took approximately 22 minutes to form, which is consistent with the trapping time of earlier DEP traps that operate at roughly this voltage. It is obvious that the particles are experiencing a p-DEP force in that they are attracted to the high field regions of the serpentine wire. More importantly, they are highly concentrated on the outer high field wires of the serpentine pattern, thus verifying the electric field calculations as shown earlier in figure 3.

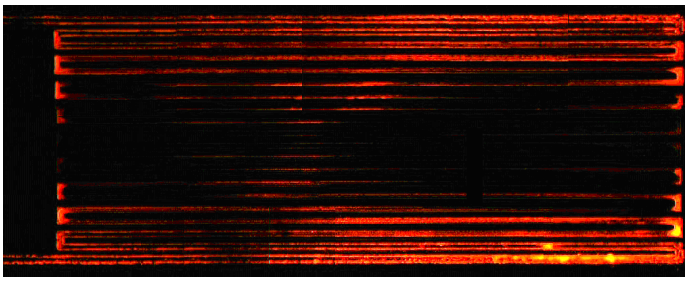

Figure 8. Image of p-DEP trapping of 1 µm fluorescent particles taken 22 minutes after device activation

The n-DEP characteristics of the device were also investigated. As mentioned earlier, n-DEP particle collection on the right side of the device should be much faster than conventional n-DEP trapping, without convection, due to the stagnation line that is directly aligned above a local field minimum. A solution of 5 micron latex particles were suspended in DI water and injected into the device. An AC voltage of 20V p-p was applied across the wire at a frequency of 1.2 MHz, and particle motion was observed. However, particle accumulation in the DEP trap was slow, as there were small amounts of particles collected in n-DEP traps on the device however these patterns took approximately 30 minutes to form. From figure 7, one can see that at 20V, the fluid velocity at 1.2 MHz is approximately 40 µm-sec^{-1}, while the calculated DEP velocity can be shown to be approximately 10 µm-sec^{-1}. It is obvious that in order to decrease the time required for collecting particles in the n-DEP traps, one needs to increase the electro-osmotic fluid velocity or the local field at the stagnation line. We shall do both below by the addition of zwitter ions.

A new suspension of 5 micron particles was created using a 1.5M solution of AHA. The solutions relative permittivity was

measured to be ~200, approximately a factor of two higher than that of DI water (~80) [3]. However, the conductivity of the DI water solution is found to be unaffected by the zwitterions, whose diffusivity is low due to their large size. Hence, the conductivity of the solution remained low (measured at 18.2 μS/cm). The AC electro-osmotic velocity was measured as a function of applied frequency prior to the experiment and is shown in Figure 9.

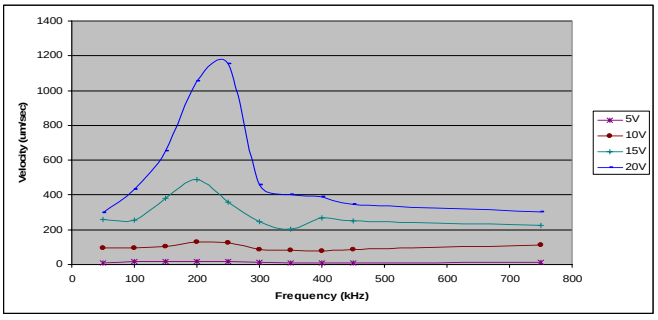

Figure 9. Measured fluid velocity for .5M AHA solution as a function of AC frequency for four different applied voltages: 5V, 10V, 15V, and 20 V

As shown, the optimal frequency is approximately 200 kHz, a factor of two higher than that of DI water, while that of the measured AC-EO velocity is also a factor of two greater. The solution was injected into the device, an AC voltage (20 V RMS) was applied and the frequency was slowly increased until a n-DEP particle collection was observed. At a frequency of 500 kHz, unlike the slow particle trapping in DI water, rapid n-DEP particle trapping in the stagnation aligned negative field region on the right hand side of the device was observed. As shown in figure 10, trapping time was reduced by two orders of magnitude, taking only 32 seconds for 5-micron particles to arrange in a highly ordered cubic array of approximately 2500 microns long. The left hand side of the device also trapped particles, however the time required to form the complete 2500 micron line took approximately 4 minutes. The 20 V_{rms} is beyond most earlier AC-EO traps with disjoint electrodes due to the appearance of Faradaic reactions. With the addition of zwitter ion, the convection flow is further enhanced as the optimal frequency for AC-EO can now be employed and still allow n-DEP trapping. Moreover, with any significant AC-EO convection, the rate-limiting step is probably the trapping speed at the local field and the addition of zwitter ion also amplifies the local n-DEP force.

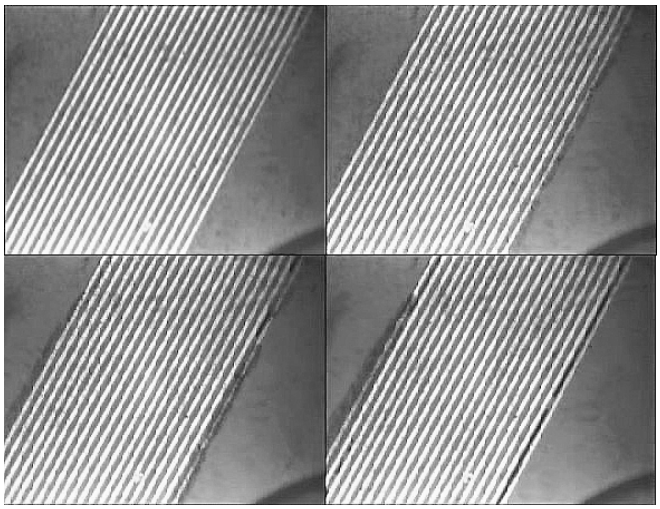

Figure 10. Images of 5 micron particle collection over a period of 35 seconds: top left image - 0 seconds, top right - 10 seconds, bottom left - 20 seconds, bottom right – 32 seconds

The observable decrease in particle collection time and increase in fluid velocity is also affected by the permittivity adjustment of the electrolyte. By adding the zwitterion to solution, the electrolytes permittivity was increased and therefore led to a decrease in the particle cross-over frequency and placed the n-DEP frequency range of the particle in a range where strong electro-osmotic flow existed. Additionally, as shown in equation 2, the electro-osmotic slip velocity is also proportional to the electrolyte permittivity, and the addition of the zwitterion also led to a factor of two increase in the observable electro-osmotic velocity. Therefore, by simply increasing the permittivity of the electrolyte solution, the frequency at which a particle experienced a n-DEP forced was reduced, driving the particle into an operating range where increased convection forces existed which in turn convected the particle across the channel gap and directly into a n-DEP trap, reducing the required collection time. This concept is further supported by the fact that the aligned stagnation trap on the right side of the device required only seconds to trap particles, while that of the trap on the left side, 100 microns away from a stagnation line, required a much longer time, taking on the order of several minutes.

Bakers yeast cells were also used to test the trapping concept. As shown in figure 15 (right) cells in pure DI exhibited a very weak response to the applied voltage (20 V RMS, 500 kHz), and after 15 minutes, there was no observable collection. However, as illustrated in figure 15 (left), the addition of a 1.5M solution of zwitterion to the suspension under identical conditions led to a collection of yeast cells in the stagnation aligned field minimum in approximately 12 seconds.

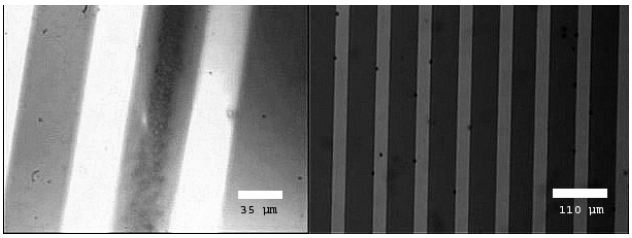

Figure 11. n-DEP behavior of yeast cells with the addition of zwitterions (left) and no zwitterions added (right)

Bakers yeast cells were also rendered inactive by starving them for 14 days and then mixed into a dilute suspension of live cells and 1.5M AHA. When activated over the device, preliminary results appear to indicate that the dead yeast cells exhibit p-DEP and are attracted to the wire, while that of live yeast cells are attracted to the local field minimum, as they did in the previous experiment. As shown in figure 12, a concentrated 2500 micron line of live-dead separated of yeast cells took approximately 7-12 seconds to form.

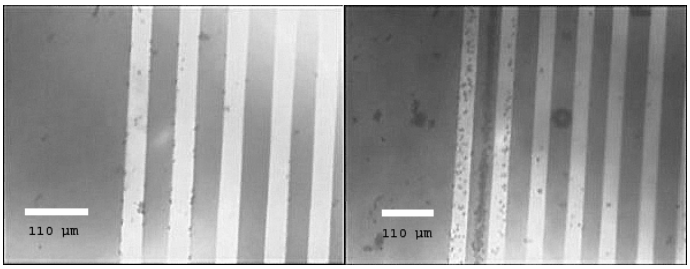

Figure 12. Suspension of dead yeast cells (left), suspension of dead and live yeast cells (right). Both suspensions under identical conditions.

A second separation experiment was also conducted using a dilute suspension of 1 and 5 micron microspheres in a .5M AHA solution. Shown in figure 13, the 1 micron polystyrene particles were attracted to the high field wire corners, while the 5 micron latex particles were attracted to the low field gap between the two wires.

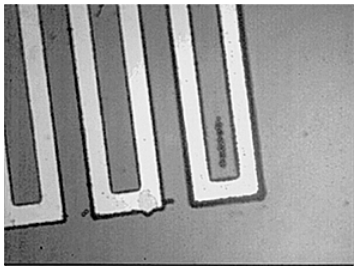

Figure 13. 1 micron particles experiencing p-DEP and attracted to wire edges, 5 micron particles experience n-DEP, attracted to wire gap. Image taken after 15 seconds.

This separation appears to be much cleaner than that observed with live-dead yeast cells, as the yeast appear much more scattered both between and on top of the wire. This is most likely due to the fact that not all yeast cells are alike, in that there is a distribution in cell diameter. More importantly, the cells are not homogenous and their polarizability is much more complex and cannot be described by the CM factor. In fact, it has been shown that biological cells actually have multiple cross over frequencies [12]. The observed scattering is most likely a result of the widely varying characteristics, such as polarizability and cell shape and size, within one particular sample of yeast cells.

The device was also used for particle manipulation. A dilute solution of 5 micron latex particles in 1.5 AHA was placed in the microchannel and a voltage was applied (20V RMS 500 kHz) for 4 minutes, which provided ample time, for the microparticles to collect on both the stagnation aligned field minimum and the field minimum on the other side of the device. Following the collection, the applied signal frequency was adjusted in a step-change manner from 500 kHz to 50 kHz and the particles were observed to lift off from the substrate and become reintroduced into the velocity field. As shown in figure 14 the particles are seen to lift off and two plugs of particles appear to approach each other. The plug on the left then overshoots the plug on the right, which gets trapped on the serpentine wires.

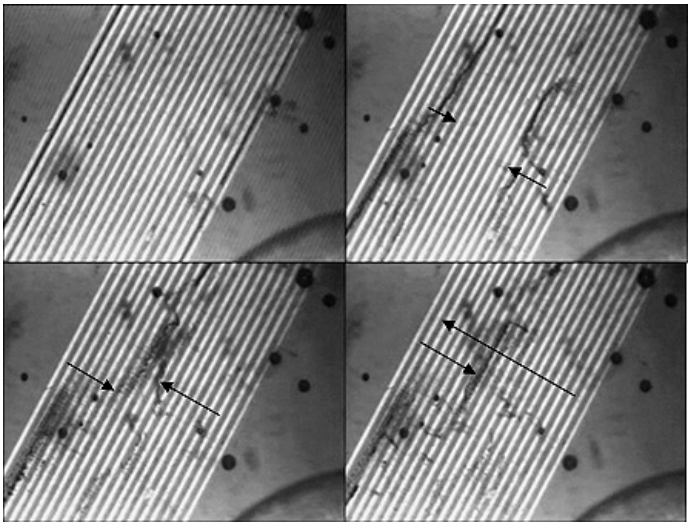

Figure 14. Rapid particle release: Top left – 0 sec., top right – 0.25 sec, bottom left – 0.50 sec., bottom right – 1 sec. Careful look at bottom right image shows the right particle slug overshooting the left particle slug

The step change in frequency from 500 kHz to 50 kHz causes the trapped particles to cross-over from n-DEP to p-DEP. At 50 kHz, the once highly attracting field minimum is a highly repulsive region, which forces the particles outward into the velocity field. In looking at the calculated velocity profile, shown in figure 4, it is clear that if trapped particles are repelled from the field minimum, they will follow the fluid streamlines until they reach a more favorable location (p-DEP trap). The particles on the right side of the device will be forced upward and to the left, while the particles on the right will be convected across the surface of the device. In looking down on the device, it would appear than the particles move towards each other, however careful imaging, shown in

figure 14, shows that the particles originally trapped on the right hand side of the device clearly flow above the particle slug arriving from the left. Additionally, because the particles on the left are convected across the device surface, and are acting under p-DEP, they are immediately trapped on the high field wires, while that of the right hand slug continues to flow over the device. These images clearly support the calculated velocity profile over the device and that particles can be manipulated to different field regions by exploiting their cross-over frequencies.

CONCLUSIONS

This paper proposes an improved method over conventional DEP techniques and earlier AC-EO traps for collecting, separating, and manipulating various types of microparticles in suspension. By designing a wire device that allows a high voltage to be applied and by tuning the permittivity of a particle suspension using ionic molecules, optimum conditions can be achieved where there exists strong electro-osmotic convection ($\sim$ 1 mm-sec^{-1}) within a frequency range where particles can exhibit both n-DEP and p-DEP. By aligning fluid stagnation lines directly on local field minima and maxima and by aligning their frequencies with zwitter ions, the required time to trap particles was reduced by two orders of magnitude. The concept of an enhanced separation technique was also demonstrated and preliminary results indicate that the live-dead separation of a large volume of yeast cells, within several seconds, is feasible. The critical factor for aligning the frequencies of AC-EO and DEP for convection-enhanced trapping is the electrolyte permittivity and double layer conductivity, and this work has produced several unanswered questions that need future investigation. For example, the permittivity of the electrolyte is assumed to be affected by AHA, however it is unknown as to how this ionic molecule affects the permittivity of the particle surface, or the membrane of a cell or bacterium. The observed decrease in particle cross-over frequency could have been caused by a modification of both electrolyte and particle permittivity brought on by the zwitterions. These ions could also change the double layer conductivity of the particles and hence produce a tangential conduction effect not related to the permittivity and not included in the classical DEP theory discussed in this work. Such detailed considerations will be analyzed with a parallel theoretical study.

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