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RAPID COMMUNICATION

Dielectrophoretic separation of nano-particles

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Abstract. We show for the first time that it is possible to separate a population of nanoparticles into two subpopulations solely on the basis of their dielectric properties. Using nanofabricated electrode arrays it has been shown that a solution of 93 nm diameter latex beads with a distribution of surface charge can be separated by the application of non-uniform AC electric fields (dielectrophoresis). The mixture separated into two populations, one experiencing positive dielectrophoresis and the other negative dielectrophoresis.

The movement of polarizable particles in non-uniform fields, termed dielectrophoresis by Pohl [1], has been exploited as a rapid non-invasive tool for the diagnosis and separation of mammalian cells and micro-organisms [2]. It has found practical applications in medicine for separating cancer cells from blood [3], enriching CD34+ cells in bone marrow samples [4] and for detecting the viability of pathogenic organisms in water [5].

Separation of particles is based on the fact that when placed in a non-uniform AC electric field, polarized particles experience a variable translational force, depending on the applied field frequency. For particles whose polarizability is greater than the medium the net movement is to regions of highest field strength, whereas particles whose polarizability is less than the medium move to the region of lowest field gradient. Because the particle's polarization is frequency dependent, the net force is also frequency dependent. Therefore, by judicious choice of suspending medium conductivity and applied frequency, even particles with very similar dielectric properties can be efficiently separated.

The sum total of the forces on a given particle is: $F_{total} = F_{deterministic} + F_{random}$. The deterministic force is the sum of the sedimentation, hydrodynamic and dielectrophoretic forces. The random force is due to Brownian motion and for particles with a diameter of the order of 100 nm this force is considerable. The mean free path of the movement is inversely dependent on mass, implying that decreases in the particle diameter require significant increases in the applied electrostatic energy (or field strength). The stable trapping of viruses and beads has been demonstrated previously [6–9]. Here we show that nanometre sized latex spheres can be both stably trapped in nanofabricated electrode arrays and, *more importantly*, that a heterogeneous mixture can be separated into two populations using dielectrophoresis.

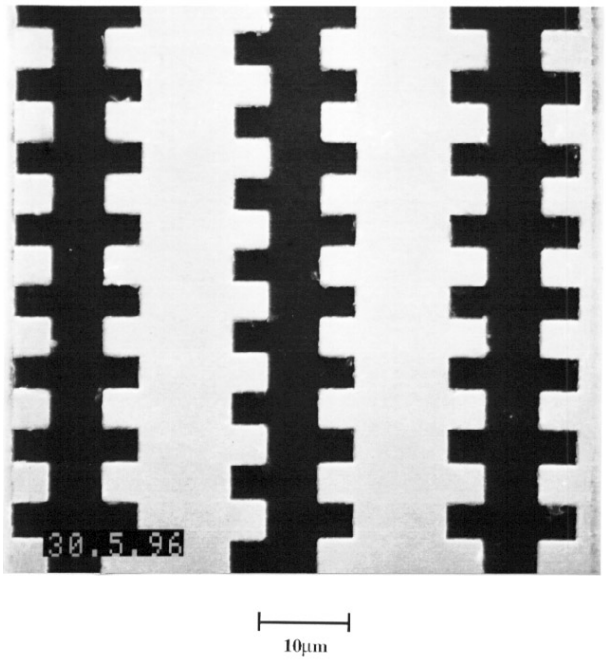

Figure 1. Scanning electron micrograph of a 4 μm castellated dielectrophoresis electrode. The electrode was manufactured using direct-write electron-beam lithography.

Experiments were conducted on charged latex spheres of 93 nm diameter, using planar electrodes manufactured by electron-beam lithography. The applied electric field strength was 2.5×10^5 V m^{-1} and the suspending medium conductivity was 0.018 S m^{-1}. Large electric field strengths cause localized heating of electrode structures which in turn gives rise to discontinuities in conductivity, permittivity and viscosity of the medium. The result is the inception

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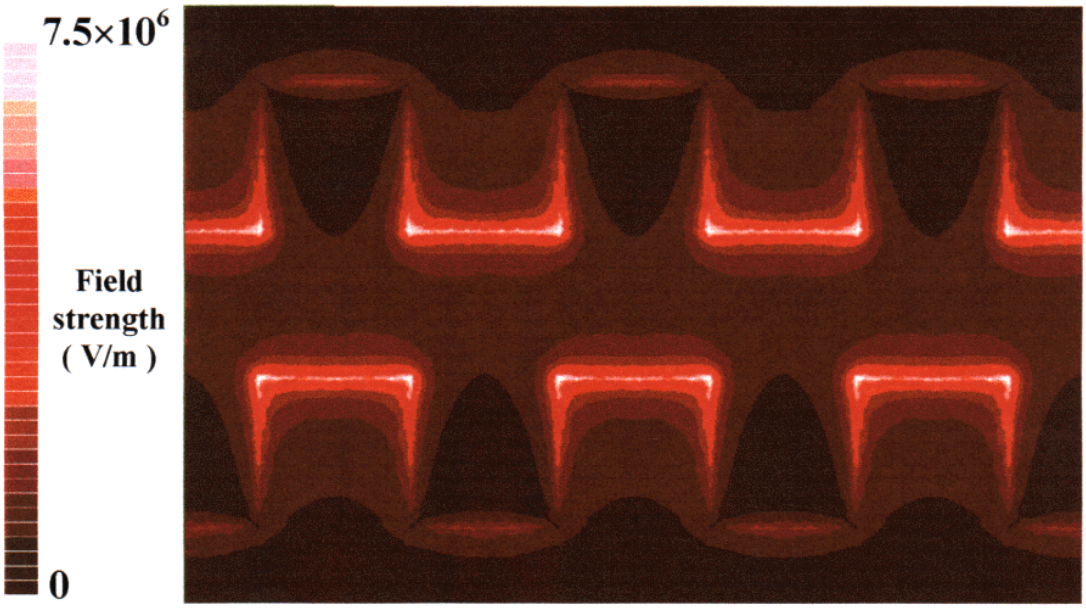

Figure 2. A grey-scale coded three-dimensional plot of the electric field distibution for a castellated electrode array showing regions of maximum and minimum field gradient. This plot corresponds to a plane immediately on top of the electrode array.

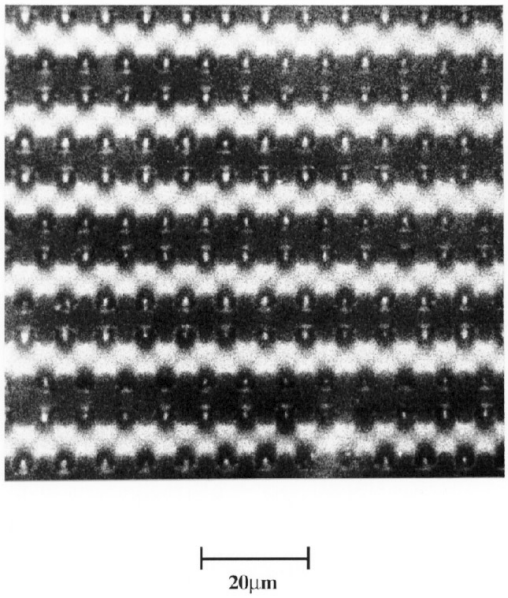

Figure 3. A photograph showing separation of a heterogeneous mixture of 93 nm diameter fluorescently loaded latex beads into subpopulations. Beads with lower surface charge move to the low-field regions between the electrode fingers, whilst the beads with the higher surface charge are trapped at the electrode tips. The photograph was taken with 2500 ASA black and white film and a Nikon Microphot fluorescence microscope. Electrode gap is 4 μm and the applied field is 2.5×10^5 V m^{-1}.

of convective currents around electrodes which can disrupt the experiments. In order to reduce these effects as much as possible, the size of the electrode structure must be minimized to maintain a low ratio of volume to surface area. Therefore, sub-micron electrode structures were used and the field strength was kept low enough so that, while there was inevitable power dissipation in the medium, no convective currents were observed.

A scanning electron microscope image of the nanofabricated planar electrode arrays is shown in figure 1. This array had a feature periodicity of 6 μm and an electrode gap of 4 μm. This electrode design was chosen because of the high electric field gradient regions produced between adjacent tips together with well defined low field gradient regions in the bays [10].

The electrodes were fabricated using direct-write electron-beam lithography (Leica EBPG-HR5 beamwriter) onto chromium plated mask plates (Hoya Corporation) followed by lift off in acetone. The electrode arrays were constructed on substrates 35 mm by 25 mm in size, with active areas covering a few hundred mm^2. Although the electrodes are not nanometres in size, the use of nanofabrication methods was critical in maintaining reproducible and well defined features in order to generate the required field gradients. Figure 2 is a grey-scale coded three-dimensional electric field plot for a small region of the electrode array taken in a plane parallel to, and 100 nm above, the plane of the electrodes. The plot was obtained using a finite element method (Ansoft Corporation, Pittsburgh, PA) and clearly shows that regions of maximum and minimum field strength occur at the tips of electrodes and in the bays respectively, in agreement with earlier 2D calculations [11].

A potential of 1 V peak–peak was applied between adjacent electrodes, equivalent to a field strength of 2.5×10^5 V m^{-1}. The frequency of the applied field could

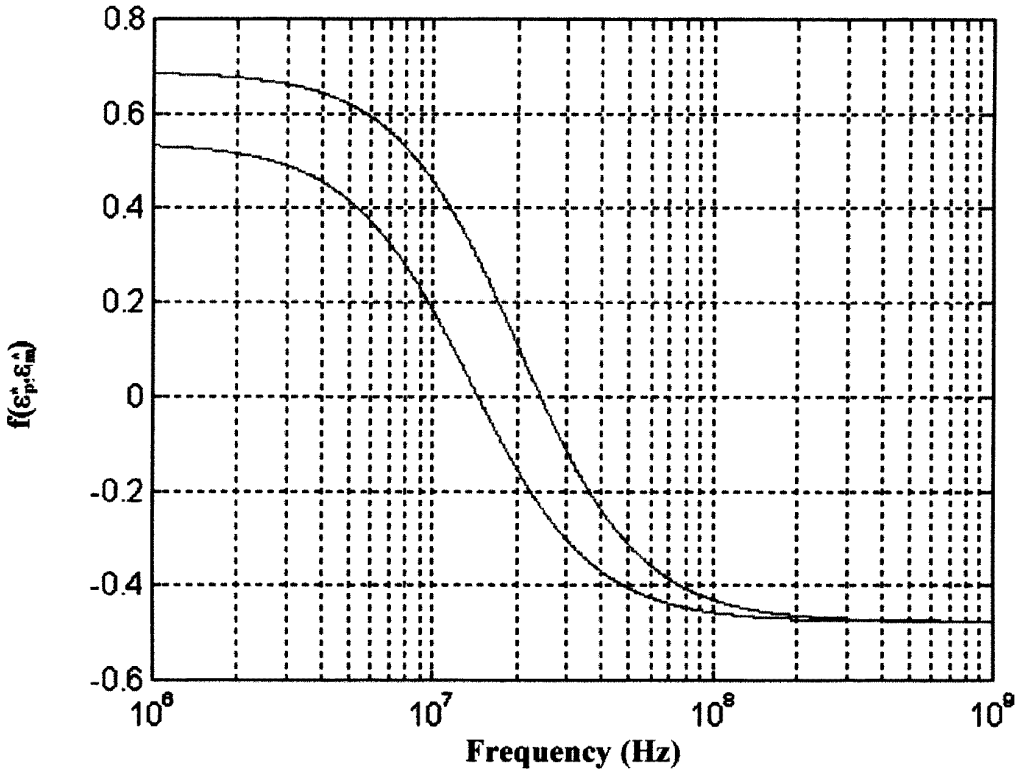

Figure 4. A theoretical plot of the variation in $f(\varepsilon_p^*, \varepsilon_m^*)$ which is directly proportional to the dielectrophoretic force for a given size of particle as a function of frequency for 93 nm latex beads. The two curves correspond to force plots calculated with values of surface charge density at the half widths of the Gaussian spread as measured directly with a Coulter particle zeta potential analyser.

be varied from 100 Hz to 250 MHz. The beads were carboxylate modified latex (Molecular Probes, Eugene, Oregon), loaded with fluorescent dye, and the suspending medium was 1 mM potassium phosphate buffer, pH 7.1. Images of the beads in the electrode arrays were collected using fluorescence microscopy (Nikon Microphot) and high-speed black and white film.

At frequencies well below 20 MHz (1 kHz–1 MHz) all the beads moved to regions of maximum field gradient at the tips of the electrodes, consistent with the polarizability of the particle exceeding the medium. Similarly at frequencies >20 MHz (20 MHz–250 MHz) all the beads collected at low field gradient regions in the electrode bays. Such an observation is consistent with that expected from dielectric studies of latex particles [12]. However, at frequencies in the region of 20 MHz a clear separation of the beads into two distinct populations was seen, as shown in figure 3. Collection of the beads occurred within a few seconds and beads remained stably trapped on the electrode array for an indefinite period of time. Removal of the field caused the beads to instantly disperse into the medium.

The instantaneous dielectrophoretic force on a particle, $\boldsymbol{F}(\omega)$, is given by [13]:

$$\boldsymbol{F}(\omega) = \mathrm{Re}\,\{(\boldsymbol{m}(\omega)\cdot\nabla)\boldsymbol{E}\} \tag{1}$$

where $\boldsymbol{m}$ is the (field) induced dipole moment of the particle, $\boldsymbol{E}$ the RMS electric field and Re indicates only the real part. For a sphere the induced dipole moment is given by the following well known equation:

$$\boldsymbol{m} = 4\pi\varepsilon_m f\left(\varepsilon_p^*, \varepsilon_m^*\right)a^3\boldsymbol{E} \tag{2}$$

where

$$f\left(\varepsilon_p^*, \varepsilon_m^*\right) = \left(\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right) \tag{3}$$

ε_m^* and ε_p^* are the complex permittivities of the medium and the particle of radius a, respectively. A general complex permittivity is given by $\varepsilon^* = \varepsilon - \mathrm{j}(\sigma/\omega)$ where ε is the real permittivity, σ is the conductivity, $\mathrm{j} = \sqrt{-1}$ and ω is the angular frequency. Thus for particles of identical size exposed to a constant field gradient the induced dipole moment, and thus the magnitude and sign of the dielectrophoretic force, depends on the difference between the polarizability of the particle and of the medium. The dielectrophoretic force is governed by the real part of this expression and for latex particles is dominated by conductivity effects. The conductivity of a colloidal particle consists of two components [14, 15] a bulk and surface component given by $\sigma_p = \sigma_s + \frac{2K_s}{a}$, where σ_s is the bulk conductivity of the particle of radius a, which can be assumed to be approximately zero for latex spheres. The surface conductance, K_s, arises from the movement of ions in the electrical double layer and is directly proportional to the surface charge density.

Thus for the latex beads the sign and magnitude of the dielectrophoretic force is governed almost exclusively by surface conductivity effects. Independent measurements of the electrophoretic mobility of the beads were made with a Coulter particle analyser. A Gaussian spread in mobilities with a mean of -3.8 ± 0.6 μm–cm V^{-1} s^{-1} was observed. Using the Helmholtz–Smoluchowski equation [16] and the Gouy–Chapman and Grahame theory of the double layer [17] this spread in mobility was translated into a spread in surface charge density around a mean of $-3.3(+1, -0.7)$ μC cm^{-2}. The variation in $f(\varepsilon_p^*, \varepsilon_m^*)$ and therefore the relative magnitude of the dielectrophoretic force was calculated as a function of frequency using the mean and the half point in the Gaussian spread in electrophoretic mobility. The results are shown in figure 4. In the calculation of this data the diameter of the beads was taken as 93 nm, a value confirmed by electron microscopy. It can be seen that at a frequency of 20 MHz the force on the beads is either positive or negative depending on the absolute value of surface charge density. This figure shows that, at this frequency, the beads divide into two populations with one moving to the high-field region (positive DEP) and the other to the low-field region (negative DEP) as seen experimentally. Thus for a mixture of beads with slightly different values of surface charge density $\pm 20\%$ around the mean, the mixture rapidly separates into two sub-populations as seen in figure 3.

It is conceivable that separation of the beads may occur due to the formation of aggregates with different dielectric properties because of dipole–dipole interactions. This phenomenon is likely to depend on the concentration of the beads on the electrode array. However, we did not observe the formation of aggregates before the beads separated. Separation was extremely rapid, within seconds of applying the field, and furthermore all of the beads dispersed into single entities upon removal of the electric field.

The beads trapped at field gradient maxima, by positive DEP, are held by a stronger force than those experiencing negative DEP [10]. As demonstrated by Markx *et al* [18], physical separation of a mixture of particles into two populations can be achieved by subjecting the electrode array to a flow of liquid of sufficient pressure to remove particles trapped at field minima leaving the other particles trapped at the electrode tips. The remaining beads can then be removed by switching off the field and flushing fresh liquid across the electrodes.

Our experiments clearly show that it is indeed possible to separate nanometre scale particles according to their dielectric properties alone. Since it is virtually impossible to separate small particles of similar size but with different biological properties nanoscale dielectrophoresis may find new applications in separating a range of structures such as chromosomes, viruses, DNA and macromolecules.

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References

[1] Pohl H A 1978 *Dielectrophoresis* (Cambridge: Cambridge University Press)
[2] Pethig R 1991 *Automation in Biotechnology* ed I Karube (Amsterdam: Elsevier) pp 159–85
[3] Becker F F, Wang X-B, Huang Y, Pethig R, Vykoukal J and Gascoyne P R C 1995 *Proc. Natl. Acad. Sci. USA* **92** 860–4
[4] Stephens M, Talary M S, Pethig R, Burnett A K and Mills K I 1996 *Bone Marrow Transplantation* **18** 777–82
[5] Burt J P H, Pethig R, Parton A and Dawson D 1995 *Inst. Phys. Conf. Ser.* No 143 pp 381–4
[6] Green N G, Morgan H and Wilkinson C D W 1995 *Proc. St Andrews Meeting, Animal and Cell Abstracts (St Andrews, UK)* **C2.18** 77
[7] Fuhr G 1995 *Proc. St Andrews Meeting, Animal and Cell Abstracts (St Andrews, UK)* **C2.18**
[8] Muller T, Gerardino A, Schnelle Th, Shirley S G, Bordoni F, De Gasperis G, Leoni R and Fuhr G 1996 *J. Phys. D: Appl. Phys.* **29** 340–9
[9] Schnelle Th, Muller T, Fiedler S, Shirely S G, Ludwig K, Hermann A and Fuhr G 1996 *Naturwissenschaften* **83** 172–6
[10] Pethig R, Huang Y, Wang X-B and Burt J P H 1992 *J. Phys. D: Appl. Phys.* **24** 881–8
[11] Wang X-B, Huang Y, Burt J P H, Markx G H and Pethig R 1993 *J. Phys. D: Appl. Phys.* **26** 1278–85
[12] Sasaki S, Ishikawa A and Hanai T 1981 *Biophy. Chem.* **14** 45–53
[13] Washizu M 1992 *J. Electrostat.* **29** 177–88
[14] O'Konski C T 1960 *J. Phys. Chem.* **64** 605–19
[15] Arnold W M, Schwan H P and Zimmerman U 1987 *J. Phys. Chem.* **91** 5093–8
[16] Russel W B, Saville D A and Schoual W R 1991 *Colloidal Dispersions* (Cambridge: Cambridge University Press) ch 7
[17] Grahame D C 1947 *Chem. Rev.* **41** 441–501
[18] Markx G H, Huang Y, Zhou X-F and Pethig R 1994 *Microbiology* **140** 585–91