An evanescent field technique for colloidal particle dielectrophoresis studies.

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Abstract:

Dielectrophoresis (DEP) has **widely** been used to probe the dielectric properties of particles, **recently** including the manipulation and study of colloidal particles. The ability to discriminate between particles collecting on an electrode against those particles in the bulk solution is required for collection rate measurements. In this paper we demonstrate how evanescent imaging methods can be used to observe only those particles in the plane of the electrode array, so that significant improvements in the signal to noise ratio is achieved.

1. Introduction

Dielectrophoresis (DEP) describes the induced motion of polarisable particles suspended in non-uniform electric fields. First described by Pohl [1], it has been widely applied to study the dielectric properties of biological cells [for a review, see 2]. It has also been employed as a separation technology, and has recently been integrated as part of a diagnostics device [3].

The DEP force on a particle depends on a number of factors, including the particle volume and polarisability, which in turn depends on the dielectric properties of the particle together with the applied field frequency. The DEP force scales linearly with particle volume, so that the manipulation of sub-micron particles can be achieved only with relatively high field strengths. Advances in electrode technology have now enabled systems to be developed capable of manipulating macromolecules [4,5], viruses [6-8] and latex spheres [9,10] using relatively low voltage sources.

Observation of colloidal particles are typically made using fluorescence microscopy, a method which illuminates all particles within the aqueous suspending solution. This gives rise to a background fluorescence and a low signal to noise ratio, so that individual particles are difficult to observe. Also, the high fields give rise to electrohydrodynamic (EHD)-induced fluid motion causing particle movement. This makes it difficult to discriminate between DEP and fluid-driven particle dynamics [11].

In this paper we demonstrate the application of an evanescent illumination technique for the study of particles in the electrode plane. This method of observation eliminates interference from the bulk fluorescence and also particles moving above the electrodes due to EHD-induced fluid flow. The applicability of the technique to DEP characterisation of particles is demonstrated through measurements of the collection rate of 557nm diameter latex spheres at electrode edges.

2. Materials and Methods

Fluorescently labelled latex beads were obtained from Molecular Probes (Oregon USA) and suspended in ultra-pure water (conductivity $250\mu\text{Sm}^{-1}$) to a final concentration of 10^{14} particles/ml. Particles were pipetted onto the electrode array. Bead excitation wavelength was 505nm and emission was at 515nm.

Electrodes of polynomial design (see figure 1) were fabricated on quartz slides and consisted of a 100nm Au layer sandwiched between two 10nm Ti layers. The inter-electrode gap was $2\mu\text{m}$ and the distance between opposing electrodes was $6\mu\text{m}$. Electrodes were powered by a **Hewlett-Packard** signal generator providing a $5V_{\text{pk-pk}}$ signal in the range 10kHz-10MHz.

A diagram of the experimental system is shown in figure 2. A 150mW, polarised 488nm Argon ion laser (Cathodeon Ltd) was used as the excitation source with the output limited to 10mW. The beam geometry was as shown in the figure; a polariser was used to control the light, which was coupled into the electrode using a small prism attached to the underside using optically-clear UV-curing glue. The electrodes

were imaged with a Nikon Microphot microscope equipped with dichroic mirror at 510nm and appropriate filters to isolate the laser light from the image. Experiments were observed using a Photonic Science Isis II image-intensified camera and recorded on S-VHS video. Data was processed using a PC and video capture card, and analysed using the Matlab® software suite.

3. Results and discussion

3.1 Observation of particle behaviour

Figure 3a and b are photographs of particles collecting under positive and negative DEP respectively, taken at frequencies of 500kHz and 5MHz. The images show only those particles that are close to the substrate, and lie within the evanescent field. When the electric field was removed, the particles diffused into the solution and the image went dark. This effect was most evident during negative DEP since, **when** the field intensity was increased from $5V_{pk-pk}$ to $10V_{pk-pk}$, the particles disappeared from view as they were levitated above the excitation field.

Since only particles within the penetration depth of the optical field (approximately $0.1\mu m$) will be imaged, this technique allows complicated particle behaviour to be clearly imaged. For example, EHD-induced fluid flow can dominate over DEP forces, particularly at the transition from positive to negative DEP where the DEP forces are low [11]. Using evanescent wave imaging enables particle motion to be examined more clearly. Figure 4 shows particle behaviour at frequencies corresponding to the transition in from positive (1MHz) to negative DEP (2MHz). At 1MHz particles collect at the centre of the electrode array, but as the frequency is increased they are pushed outwards from the electrode forming well-defined “bands”. At a frequency of 2MHz the negative DEP force on the particles increases, the bands disperse, and particles fall into the negative DEP trap at the centre of the array.

3.2 Determination of collection rate

Optically based collection rate measurements have been used to study the frequency-dependent dielectrophoretic behaviour of large particles [12]. In this work, we have used evanescent wave methods to measure the collection rate of sub-micron particles. Particles were collected at different frequencies and the number of particles collected determined by image processing. An example of an experimentally determined collection rate taken at a frequency of 160kHz is shown in figure 5. **In this figure, the total light emitted is plotted as a function of time, taken over 20 seconds at 2-second intervals.** Immediately after the field is applied the number of particles on the electrode increase rapidly with time. After approximately 15 seconds the rate of increase reduces as the trap fills. These trends were analysed further by taking the differential of the data over each 2-second interval, during the first 10 seconds and calculating the mean slope. This method also mathematically eliminated the background emission (which was small, constant and time-invariant). The results are shown in figure 6.

This figure shows a clear maximum in collection in the region of 100 to 200 kHz. Also the dispersion associated with the transition from positive to negative DEP (shown in figure 4), is clearly visible as a change in the collection rate at approximately 0.6MHz . Below 100kHz particle collection was disrupted by electric field driven fluid flow [6], which prevented particles accumulating in the electrode gaps so that the measured collection rate was much reduced.

The dielectrophoretic behaviour of latex particles has been measured by analysis of DEP cross-over frequencies [9]. For the 557nm diameter particles, the low-frequency relaxation associated with the double-layer was found to occur in the frequency range between 100kHz and 330kHz, for 1mM KCl, conductivity = 14mSm^{-1} . Although our measurements were made in a lower conductivity, it is probable that the change in collection rate characteristics which occur at approximately 200kHz (figure 6) are associated with this relaxation.

4. Conclusion

Conventional fluorescence microscopy has been used to study the DEP behaviour of colloidal particles but with a low signal to noise ratio. Evanescent field methods have been shown to give images with very low background noise so that accurate determination of the collection rate of sub-micron fluorescent particles at an electrode is possible.

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1. Figure Legends

1. A photograph of the electrodes used in this experiment. Electrodes are fabricated on quartz slides from a 100nm Au film in between two 10nm layers of Ti. Scale bar: 20 μ m.

2. Schematic diagram of the equipment used in this experiment. All components were mounted on optical tables, using magnetic feet where necessary. (A) laser (B) iris (C) adjustable polariser (D) fixed mirrors (E) adjustable mirror (F) Nikon Microphot fluorescent microscope with FITC filter block (G) Photonic Science Isis II image-intensified camera (H) S-video recorder. The inset section shows how the laser beam is coupled onto the slide beneath the electrodes, creating an evanescent field illuminating a thin volume extending approximately $0.1\mu\text{m}$ into the solution.

3. Photographs showing 557nm-diameter latex particles collecting by dielectrophoresis as observed using evanescent illumination: (a) positive DEP, at 500kHz, $5V_{\text{pk-pk}}$ (b) negative DEP, at 5MHz, $5V_{\text{pk-pk}}$.

4. A sequence of images showing the behaviour of 557nm-diameter latex particles at frequencies in the vicinity of the dielectrophoretic crossover. In all case the applied potential was $5V_{pk-pk}$. Frequencies are (a) 1MHz to (i) 2MHz in approximately 125kHz steps. In images (d) to (f) particles can be seen moving from the centre of the trap under a combination of fluid flow and DEP forces. In (g-h) the particles diffuse into the solution before experiencing negative DEP in (i).

5. A plot showing the total light emitted by particles collecting at a frequency of 160kHz, as a function of time.

6. A plot of the mean frequency-dependent collection rate of particles. A maximum in collection rate occurs at around 100-200kHz and a dispersion can be seen at approximately 0.6MHz. The bars indicate the highest and lowest values of collection rate measured during the first 10 seconds.